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Of nature trusts the Mind that builds for aye"—WORDSWORTH

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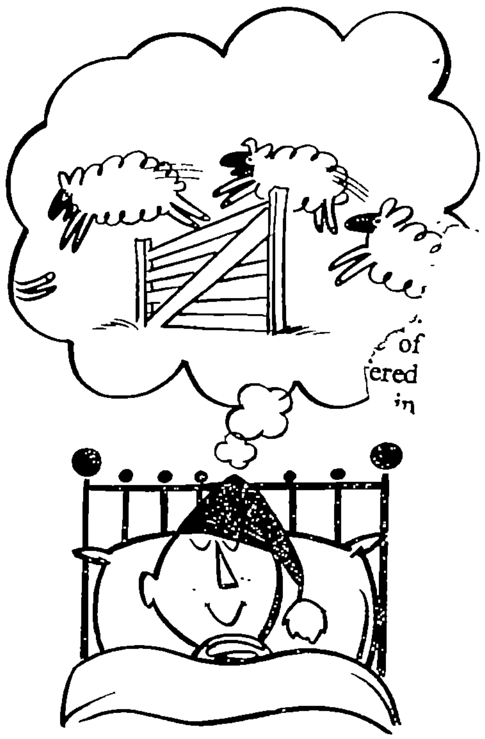

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FUTURE OF BRITISH OVERSEAS RESEARCH AND SCIENTIFIC AID

IN her concluding B.B.C. Reith Lecture, Dr. Margery Perham urged the importance of Britain striving to keep, and indeed to increase, her great knowledge of the peoples of her former empire and the measure of goodwill and understanding which exists between them. To that end, Britain's greatest contribution would be to put all her strength and experience behind the international organs in which alone lie the possibility of peace, order and mutual help. The aid Britain gives must not even bear the moral taint of philanthropy and much will depend on Britain's dealings here with many thousands of African students and, on the other side, on the readiness of our young doctors, lawyers, scientists, and, above all, teachers of every kind, to go out for at least two or three years to serve the great needs of Africa, and to serve in the spirit which Africa now demands. This, in turn, requires that those who hold authority in Britain should facilitate such ventures, perhaps by secondment, and make them a qualification and not a handicap in a career.

Dr. Perham does not overlook the value of the uncounted and rewarding contacts between individuals and groups from each side which have been possible under the official relations or of the service which some 20,000 missionaries from all the churches are quietly rendering in Africa. She recognizes, however, the full force of the new nationalism which may dictate policies from the blood and not from the brain and that it is possible that the lamps of Africa may go out even before they are fully alight, as some of the lamps of Europe have gone out. Whether they do or not will depend very much on the degree of understanding and help which people in the West give to Africa during the next two or three critical years.

In these Reith Lectures, Dr. Perham very fairly assesses both the British achievements in Africa and the rise of African nationalism, particularly the factors which encouraged the development of anti-colonialism. She notes how our tardiness in training Africans for the higher posts in the Civil Service presented serious problems in the transfer of power which remain as grave weaknesses in the new States, so that the African States are strained as on a rack between their political ends and the lack of nearly all the means to achieve them. Unostentatious help and friendship, she reminds us again, might just turn the trembling scale in these States in favour of reason and justice, if not of democracy as we define it, for Britain was their political nurse and tutor, and we bear some responsibility not only for their public virtues but perhaps even more for their political defects. We should therefore show understanding and restraint to the eleventh hour.

Dr. Perham pays a generous and deserved tribute to the men of the Colonial Service and especially to those who served as district commissioners, and while she considers it is a fair count against us that, even after recognizing the growing demands for self-government, we did little to prepare for it, she admits that she herself, like many others involved in Colonial affairs between the Wars, was taken by surprise at the speed with which African nationalism advanced. As late as 1939 the opinion was expressed that in Africa, at any rate, we had unlimited time in which to work, and that attitude may be one factor responsible for the gradualism which in Central Africa has been overtaken by impatient nationalism before generous proposals for educational and other welfare services, including a new multi-racial university, could close the gap between the races and develop a multi-racial community.

It is against this background, and against the vision and urgency which characterize recent reports from the Rockefeller Foundation and the Carnegie Institution of Washington, that the critical importance of the Department of Technical Co-operation is to be seen and the belated report on *Colonial Research** makes such disappointing and even disturbing reading. Beyond a brief reference to the need to ensure continuity of assistance from the United Kingdom to countries evolving from colonial to independent status and to the creation of the Department of Technical Co-operation, to which the functions previously performed by the Colonial Office Research Department and its advisory committees and council have been transferred, there is no reference to the larger issues which give colonial research its significant place in technical assistance and in the development of the emergent territories. Apart from a note in the report of the Colonial Social Science Research Council that a resolution, suggesting that this Council should be reconstituted as an advisory body on matters relating to social science in terms which would enable it to advise on measures required to provide facilities for United Kingdom research in this field which have become independent and to provide liaison between the United Kingdom and independent territories, has been sent to the Overseas Research Council there appears to be no reference to the latter body in this report. The bland announcement that the present volume, which comes from the Colonial Secretary, will be the last to be published, is unaccompanied by any indication whether or not it will be succeeded by any other volume from the Colonial Office or the Department of Technical Co-operation.

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* Colonial Research 1960–61 Reports of the Committee for Colonial Agricultural, Animal Health and Forestry Research; Colonial Economic Research Committee; Colonial Fisheries Advisory Committee, Colonial Medical Research Committee/Tropical Medicine Research Board; Colonial Pesticides Research Committee; Colonial Social Science Research Council; Tsetse Fly and Trypanosomiasis Committee; Director, Anti-Locust Research Centre; and Research Matters not covered by the above Reports of the Specialist Advisory Bodies. Pp. 353. (Cmnd. 1584.) (London: H.M.S.O., 1961.) 18s. net.

This is not good enough. No respect for departmental independence or unwillingness to commit another department can excuse such trifling with issues of this importance. The 350 pages of this report display a record of scientific work of the utmost importance to the dependent and independent territories alike—a record of which the Overseas Research Service and all its collaborators have every right to be proud. It is unthinkable that this work is to be curtailed or suspended. In scale, as the present report itself shows, it is too small and the need is for expansion—a task demanding vision and sound judgment as well as more generous financial support from Britain.

There is a further point here to be noted. Brief as is all the reference possible to any particular piece of research, however significant and far-reaching in its importance, in a report which covers so many fields of work and territories, even such reference can mean much to the isolated research worker in the overseas territories. Both Prof. P. M. S. Blackett and Sir Alexander Carr-Saunders have stressed the bearing of this factor on the recruitment of research workers for overseas service. To suspend the publication of *Colonial Research* without replacement by some equivalent and more effective publication would be a disservice to research that might well involve expenditure many times the trifling saving apparently achieved. Such parsimony is inconceivable except in the light of the Government's own record of lack of imagination and penury in this field.

If, in fact, the United Kingdom is to continue to support research in the dependent territories and to assist the dependent territories in this field, Parliament and the taxpayer are entitled to a full account of the way in which public money is expended. From this point of view, as has been emphasized from time to time in these columns, *Colonial Research* has not been entirely satisfactory. A series of reports from specialist bodies, however adequate and admirable in themselves, is ill-adapted to interpret to Parliament or the general public exactly what is the significance of the work in a wide range of fields. Some general account, picking out, if necessarily merely by way of illustration, some features of the year's work or outstanding advances, would have conveyed far more effectively the value of the research in progress and perhaps enlisted the public understanding and support that are essential if overseas research is to be prosecuted on the scale its importance demands.

This the Colonial Research Council never attempted to provide, and it was hoped that when the Overseas Research Council was constituted the latter would make good some of the defects and omissions of its predecessor. So far, something like a conspiracy of silence appears to surround the Overseas Research Council. No report has yet come from it, and the last two reports on *Colonial Research* seem to be at pains to avoid mentioning it, as is the Minister for Science whenever he discusses the organization of research in Britain. It is understood that, unlike all the other Research Councils, the Overseas Research Council has no funds of its own, and that it now comes under the new Department of Technical Co-operation.

This position is anomalous and in itself indicates a lack of imagination and of judgment that are disturbing. If the opportunities in overseas research are to be seized and the full contributions which Britain could make in this way to the development and welfare of the dependent and emergent territories are to be realized, the Overseas Research Council should be provided with its own appropriate funds and placed alongside the other Research Councils in its proper place—under the Minister for Science. Its demands on Britain's scientific and technological resources are to be assessed in the first place with those of the other Research Councils, and to suggest that the Department of Technical Co-operation would be unable, if this were done, to take care of the demands for other kinds of technical assistance is an unjust reflexion on the ability of the Minister, if not an outright insult to him. The problems of organization are indeed difficult ; but such problems are solved by facing, not running away from, them.

The time is long overdue for a clear explanation of what the Overseas Research Council is doing and what it is intended to do, and of how exactly it fits into the Government's plans for assisting the dependent and the independent territories. There is no reason to doubt the determination with which Mr. Denis Vosper is tackling his vital and responsible task, but there is every reason to believe that he will need the understanding and resolute support of all those, whether scientists or technologists or not, who appreciate the significance of technical assistance in general and research in particular in the world to-day, if he is to secure the resources he needs to discharge his responsibilities and the power to use those resources most effectively. The Overseas Research Council should be one of his most important instruments and a clear statement on its position, its functions and resources would provide a focus for constructive criticism and firm support. Its absence now can only indict the Government's sincerity in the whole field of technical co-operation and overseas aid.

Even now such suspicions are not entirely unjustified. *Colonial Research* provides more than an impressive record of scientific achievement with which Britain may well be proud to be associated. It includes also, in its lists of committees, sub-committees and councils responsible for guiding that work, names of scientists not only distinguished for their own scientific standing and achievements but marked also by the vision and imagination which they have brought to bear on matters of public policy with which science is concerned. Nevertheless, from all the bodies responsible for research overseas has come no report or statement comparable in insight and imagination with, for example, the most recent surveys of the President of the Rockefeller Foundation or of the President of the Carnegie Institution of Washington. Furthermore, the Minister for Science and members of his Advisory

Council on Scientific Policy have repeatedly shown a capacity for exposition and interpretation, for awareness of human issues, in no way inferior to those shown by Dr. Caryl Haskins and Mr. J. C. Harrar. Why is it, where matters of overseas research and technical assistance are concerned, the leadership to which Britain's past efforts and record fully entitle her is allowed to pass tamely into other hands ?

To ask this, or to suggest that the scale of our contribution to technical assistance in general and to overseas research in particular needs to be increased, is not to ignore or disparage what has already been done. Under the Colombo Plan alone the United Kingdom contributed in 1960–61 £35·9 million in capital and technical assistance, making a total of £182·78 million (of which £175·72 million is capital aid); in the current financial year some £180 million was provided in aid to underdeveloped countries, of which about £160 million is for Commonwealth countries, but the significant factor, as the latest report of the Consultative Committee shows, is the urgent need for more rapid development and for more determined efforts to keep pace with the unprecedented growth of population. What is true of South and South-East Asia is no less true of Africa and the West Indies and other areas, and it is what is done in the years immediately ahead that may well be decisive.

It is not belittling what has already been done to urge that more is needed and that because our resources are limited they must even more imperatively be used wisely, imaginatively and boldly. In Government circles it is this that appears to be lacking. We would seem to have established the appropriate instruments but to be unable or unwilling to use them fully or effectively, and it is the great merit of Dr. Perham's Reith Lectures that they reveal something of the measure of that opportunity, as indeed did Mr. John Strachey in his *The End of Empire*, as *Colonial Research* conspicuously fails to do.

Even from our own selfish interest, it is essential that the nation should realize and grasp the new type of opportunities that are ours to-day both at home and abroad. In them we can, as Mr. Strachey truly writes, best show our creative powers and write an enduring name on the roll of history. It is the things of the mind and the spirit that make or mar national fortunes and it will be by serving the peoples of the world that Britain can be great. To serve them by a sustained effort to provide them with both the material means and the technical skills which they desperately need may indeed be the highest and most aspiring ideal we can set ourselves, and it is not in conflict with the continued improvement and perfecting of our own society: rather it is a contribution to that end.

The scientist and technologist will accordingly salute the passing of *Colonial Research* with deep appreciation of the contributions to scientific advance and to human welfare and development made in so many varied fields by the patient and devoted work of many investigators, often working in isolation and deserving of more generous support and professional recognition. That appreciation will be accompanied by regret that those on whom the ultimate responsibility for directing and supporting that work have so seldom seemed aware of its importance or implications, so lacking in any sense of urgency, or consciousness of the opportunities for leadership. Appreciation and regret, however, may fittingly be accompanied by the hope that, under some such title as *Overseas Research*, a succeeding series of volumes will do something more than continue a record of noteworthy achievement in many branches of science of which Britain may continue to be proud. It is to be hoped that the new volumes will display also imaginative insight into the issues and opportunities with which overseas research and technical assistance in general confront us, readiness to make the necessary sacrifices and a determination to offer the service and leadership that are demanded. It is not indeed that the financial contribution needed is likely to be excessive; wise planning, careful timing and a proper sense of proportion and choice of objectives are likely to be the vital factors; but once the projects are selected, adequate support and encouragement and freedom from 'start and stop' practices are essential.

Far more significant is likely to be the demand made on our own limited resources of scientific and technical manpower, and that is why, when full account is taken of the shortcomings of Government policy which are so apparent from *Colonial Research*, it is imperative that neither overseas research nor technical assistance should be regarded as matters for Government alone. Mr. Denis Vosper and Lord Hailsham are entitled to all possible assistance from the universities and colleges of technology and from many professional institutions along lines such as Prof. P. M. S. Blackett, for example, has indicated. It is because of its potentialities in organizing such support that the Overseas Research Council is so important and that it should be given a fitting place alongside the other Research Councils in displaying the nation's scientific and technical resources.

There may well be some difficult administrative problems, but not such as to defeat realistic co-operation between the Minister for Science and the Secretary for Technical Co-operation, and it should be an advantage that the Department of Technical Co-operation has an opportunity to make a fresh start. Given real determination on the part of the Government and a sense both of urgency and the decisive importance of leadership the new Department should be at no disadvantage. There must, however, be no more parsimony and none of the fumbling and shortsightedness that has allowed the Peace Corps of the United States to overshadow so completely the Voluntary Service Overseas launched even earlier by Britain, and leadership in technical assistance to pass from our own research institutions to the great foundations of the United States. The final volume of *Colonial Research* is, above all, a challenge, and the future of Britain itself may well depend in large measure on the vision, the courage and the determination with which that challenge is accepted and faithfully translated into terms of service for the overseas peoples.

GALILEO'S ACHIEVEMENTS

ASKED to name Galileo's greatest scientific achievements, most people would probably point to his discoveries in astronomy and dynamics. The originality of his telescopic observations, their great importance, their revolutionary effect on man's outlook on the universe, are all undoubted. But how great was his achievements in dynamics ? Not nearly so great, at first sight, as has commonly been supposed. Newton, for example, in a celebrated passage in the *Principia*, seems to imply that Galileo was familiar with both the first and second laws of motion. But Galileo, even if he were fully aware of the importance of inertial motion, which is doubtful, only recognized such motion as a 'terrestrial' phenomenon, rectilinear to a first approximation, but in reality circular along the Earth's surface. As for the second law of motion, there is nothing beyond the merest hint of it in any of Galileo's writings. In fact, his two great achievements in dynamics, the derivations of the law of falling bodies and of the parabolic path of a projectile, were largely kinematical rather than dynamical, at least as treated by him. Both these achievements were undoubtedly of great importance. For example, *Lemma X* of Newton's *Principia*, later used in his solution to the problem of Kepler motion, is a direct generalization of Galileo's law of falling bodies. Moreover, the derivation of the parabolic path of a projectile was memorable not only historically, as the first important prediction of modern science, but also for the notion of compounding independent motions on which it was based.

Apart from these two specific achievements, Galileo made another, and perhaps equally important, contribution to dynamics. If one considers his finished work on the subject in the *Discourses on Two New Sciences*, then one is struck by its essentially modern spirit compared with that of his predecessors, or contemporaries, or even some of his successors, such as Wallis in his *De Motu*. Here we have something recognizably in the *genre* of mathematical physics as we know it to-day. In this respect Galileo had only one predecessor, Archimedes, to whom he was undoubtedly indebted for certain aspects of his approach to physics. But Archimedes (and this is one of the most curious and puzzling facts in the history of science) never seems to have given much attention to the problem of motion. Galileo's work in dynamics, such as it was, with all its inevitable limitations, was thus the first modern *De Motu*. This was revolutionary at the time, and still seems recognizably modern to-day, just because it retained so little of Aristotle's influence. It was at this point, in freeing himself*, and dynamics, from a great part of Aristotle's influence, that Galileo made what was probably his greatest contribution both to science and dynamics. He never attained to the true, universal concept of inertial motion, or to any sort of understanding of circular motion: the honour of so doing was reserved for Descartes. But there was no future for the detailed, quantitative development of dynamics on the lines of Descartes' *Principia*, whereas Galileo's work served as the model for Newton's final synthesis of the subject.

It is this which makes Galileo's early, unpublished work *On Motion* of such absorbing interest. For in it we see him in the very act of freeing himself* from two of the most unfortunate Aristotelian presuppositions, the belief in light bodies, and in the impossibility of a vacuum. In the *On Motion* Galileo conclusively explodes the notion of light bodies. All bodies are intrinsically heavy, and tend to move towards the centre of things. The fact that they sometimes do not, but move upward, is contingent only, having a simple hydrostatic explanation in terms of the surrounding medium. Equally conclusive are Galileo's arguments against Aristotle, and in favour of the possibility of a vacuum and motion therein. It is only *in vacuo* that the proper natural motion of heavy bodies can be studied. For example, that of a perfectly smooth sphere on an equally smooth inclined plane.

It is perhaps difficult for anyone unfamiliar with the period to realize the magnitude of Galileo's achievement in freeing himself from these and other peculiar features of Aristotle's physics. The difficulty is rather to understand how any intelligent person, let alone Galileo, could ever seriously have entertained such bizarre notions. But when we recall that Descartes, whose specific contributions to dynamics were equally great, if not greater, than Galileo's, never freed himself from a belief in light bodies, or in a *plenum*, whereas Galileo, unlike Descartes, never escaped from the tyranny of the Platonic or Aristotelian circle, then we begin to realize how plausible and all-pervasive the Aristotelian *weltanschauung* was, and how difficult it was to escape from its influence.

I have mentioned the part played by hydrostatics in Galileo's break with the false, Aristotelian notion of light bodies. His mastery of this subject is evident from the *Discourse on Bodies in Water*. This work was occasioned by a controversy between Galileo and his Aristotelian opponents. The axioms, definitions and theorems at the beginning of the treatise form the necessary propadeutic to Galileo's devastating reply to the criticisms levelled against Archimedes by these opponents, especially Buonamico. First, he disposes of the seeming paradox of the floating of a hollow denser-than-water body; then he refutes Aristotle's doctrine of natural levity as opposed to the belief of Plato and the ancients that levity was due to the action of the surrounding medium. This leaves him with the puzzling question of the floating of thin plates of denser-than-water materials.

Galileo's treatment and discussion of this problem takes up the remainder of the treatise and was for me the most interesting and revealing feature of the whole work. In it we see displayed, one by one, those qualities which together made him, with the possible exception of Kepler, the first true follower of Archimedes, the first mathematical-physicist of modern times. First the unrivalled observer: who before Galileo (and the phenomenon went back at least to Democritus) had looked closely enough to see that the plate actually lay *beneath* the surface of the water ? Next, the capacity to apply a known principle (Archimedes') to a new situation: the true system under consideration was not the plate alone, but the plate together with the little pocket of air above it. It was the upthrust of the water on this

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* Not, however, entirely by his own unaided efforts. Galileo's debt at this point to his master, Benedetti, was very great.

whole volume which balanced the weight of the plate. Lastly, the mathematician able to derive an exact, quantitative, general result applicable to all such situations: to discover, in Salusbury's fine rendering into Jacobean English, "what proportion several Figures of different Matters ought to have, unto the Gravity of the Water, that so they may be able by vertue of the Contiguous Air to stay afloat". To this problem Galileo gives the complete answer in *Theorem VI*, in which he brings out the importance of the maximum possible height of the little 'rampart' of water surrounding the plate.

From this general result he then derives a number of characteristically provocative theorems calculated to confound his adversaries, such as: "All Matters, how heavy soever, even to Gold it self, the heaviest of all Bodies, known to us, may float upon the Water" (*Theorem VII*). The treatise ends with an interesting discussion of the views of Aristotle and Democritus on the same problem.

These two works[1,2], the early one on *On Motion*, and the *Discourse on Bodies in Water*, reveal many of the varied talents which went to make up Galileo's scientific genius. The remaining work, *On Mechanics*, also reminds us of that mechanical bent of mind which he shared with Archimedes and Newton, and which expressed itself in the construction of the telescope and other scientific apparatus. Apart from the section on percussion, a subject in which Galileo made no progress, this work is devoted to a study of the simple machines, and contains nothing original. It is chiefly remarkable for Galileo's consistent use of the principle of virtual velocities. Galileo could scarcely be called the 'originator' of this principle, but his confident manner of using it was novel and very influential.

These three works of Galileo are, finally, important not only as valuable source material in the history of science but for their reminder of the many-sidedness of his interests and abilities. This many-sidedness was something he shared, of course, with Kepler, Descartes and Newton. But although their common breadth of interest could be regarded as a natural outcome of the cultural background of the day, this is not to say that it was an unimportant factor in the birth of dynamics to which each brought his own peculiar gift. For example, in each the philosophical caste of mind seems to have been of crucial importance. Without Kepler's interest and passionate belief in neoplatonic symbolism and world harmony it is hard to believe he would ever have discovered his laws of planetary motion. Galileo, without his complete mastery of Aristotelian philosophy, would never have overthrown Aristotle. If Descartes had been only a pure mathematician and not also a philosopher he might never have been able to formulate the true principle of inertia, or free himself from the tyranny of the platonic circle, any more than Newton would have been able to extract the pure gold from Descartes' *Principia* while demolishing the philosophical system in which it was embedded and replacing it by a better one of his own. One is thus led to speculate: if dynamics sprang from the wonderfully rich intellectual, philosophical and religious background of the Renaissance, it is possible that its most vigorous descendant, fundamental particle physics, will ultimately languish in the over-scientific atmosphere of the twenty-first century? That this should happen is perhaps the secret hope of an increasing number of scientists who have always subscribed to the idea of science as the servant rather than the master, or even destroyer, of life, but who now begin passionately to desire that this should be so, and wonder how it can ever be brought about. A long period of relative stagnation in fundamental scientific advance would certainly give civilization the opportunity of absorbing and integrating to herself the tremendous scientific advances of the past half-century, just as the Middle Ages had time to absorb and synthesize the equally disturbing new *corpus* of Greek science. If this should happen, then one can confidently expect that a new Galileo would ultimately arise, equally master of the total civilization of his day, and equally capable of demolishing its current, over-complicated *De Motu*, and preparing the way for a new dynamics perhaps as obvious, in retrospect, as the dynamics of the seventeenth century to those who understood Newton. For as Galileo himself remarked [Drabkin's translation of *De Motu*, p. 38], "truth hath the property that it is not so deeply concealed as many have thought; indeed, its traces shine brightly in various places and there are many paths by which it is approached. Yet it often happens that we do not see what is quite near at hand and clear".

All those interested in seventeenth century science will be indebted to I. E. Drabkin for his lively and readable translation into English of Galileo's *De Motu*, and for his introduction to that work. Likewise to Stillman Drake for his introduction to the *Le Meccaniche*, and especially for that to the *Discourse on Bodies in Water* in which the setting of the work, its motivation, and its great importance for the remainder of Galileo's scientific career are all clearly displayed. Needless to say, both books maintain the high standards of scholarship and production so long a feature of American publications in the history of science. J. W. HERIVEL

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[1] *On Motion and Mechanics*. By Galileo Galilei. Comprising *De Motu* (*ca*. 1590). Translated with Introduction and Notes by I. E. Drabkin, and *Le Meccaniche* (*ca*. 1600). Translated with Introduction and Notes by Stillman Drake. (University of Wisconsin Publications in Medieval Science, No. 5.) Pp. viii + 193. (Madison: University of Wisconsin Press, 1960.) 5 dollars.

[2] *Discourse on Bodies in Water*. By Galileo Galilei. Translated by Thomas Salusbury. (Illinois Facsimile Reprints in the History of Science, No. 2.) Pp. xxvi + 89. (Urbana, Ill.: University of Illinois Press, 1960.) 5 dollars.

RESTLESS ATOMS IN SOLIDS*

By DAME KATHLEEN LONSDALE, F.R.S.

THE most striking characteristic of a solid is rigidity which, however, is not simply a property of symmetry and periodicity of atomic arrangement, since the atomic arrangement in a glass is not a regular one. Rigidity is dependent rather on the fact that although atoms in every state of matter are moving, yet at sufficiently low temperatures the movements are not, in general, violent enough to cause the relative atomic positions to change. If, however, a crystalline solid contains imperfections, interstitial atoms, vacant sites, dislocations, impurities or grain boundaries, these may well move quite large distances even though local atomic movements are small: and although the mean atomic vibrational displacements are seldom more than 0·1–0·3 Å. at room temperatures, yet occasional movements are much larger, and single atoms sometimes travel large distances in a solid. Different kinds of atoms or ions in a compound will vibrate with different mean amplitudes, and molecules undergo translational vibration, libration and some distortion. All these movements produce or affect so many physical and chemical phenomena that it is not possible to mention more than a selection.

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* Substance of a Friday Evening Discourse delivered at the Royal Institution on December 8, 1961.

Melting, softening and crystal transformations. Theory and experiment both show that atoms are not at rest even at 0° K. They have zero-point energy. As the temperature is raised they also have thermal energy, and this increases until the atomic vibrations are so large that the forces between the atoms can no longer keep the solid rigid in that particular arrangement. If the solid is amorphous it will soften and become a viscous liquid; but if it is crystalline then it takes up latent heat and melts, or it changes its crystal form. One of the more spectacular of such changes is the diamond $\rightleftarrows$ graphite transformation. Diamond begins to change to graphite when heated, in the absence of oxygen, to upwards of 1,500° C. Even at this temperature, however, theory indicates that the vibration displacements of the carbon atoms in diamond have a mean value of less than 0·15 Å.

At room temperatures the Na^+ ions in sodium chloride have a mean vibration displacement of about one-twelfth of the Na^+—Cl^- distance; that of the Cl^- is a little less. But melting takes place when the ratio is about 1 : 6 (ref. 1).

Thermal conduction and thermal expansion. A simple experiment shows that if a copper rod is heated at one end, the increased atomic amplitudes are very quickly transmitted along the rod and the rod simultaneously expands. These two properties do not necessarily go together, however, for diamond conducts heat quite as well as aluminium, but it has a very small expansion coefficient at room temperatures. The expansion of organic crystals such as naphthalene or anthracene is not only anisotropic but many times (up to about × 200) larger than that of diamond.

For purposes of calculation of diffraction effects thermal vibrations in simple crystalline structures may be regarded in either of two ways: (1) as a superposition of a very large number of stationary patterns, in which the atoms are displaced from their mean, regular positions; (2) as a superposition of stationary simple harmonic waves. This only applies, however, when the substance is in a state of thermal equilibrium. If it is expanding then the vibrations must be anharmonic.

Volatility, solubility. At the surface of a crystal the molecules may be subjected to an unbalanced force. If so they will tend to fly off. This may be useful, as when the substance *para*dichlorbenzene, for example, is used to preserve blankets from moths. The molecules fly off the surface so fast that in a few hours exposed crystals will have disappeared completely. But in an X-ray tube, or in an electric light bulb, the emission of atoms from a hot tungsten filament is a real nuisance. In the case of an X-ray tube it contaminates and reduces the characteristic radiation emitted and also increases the absorption of the window by covering it with a tungsten film.

The function of thermal vibration in the process of solution of a solid in a liquid is not so obvious. Atoms or molecules are removed from the surface of the solid and mingle with those of the solvent. In the case of Na^+Cl^- dissolving in water it is not difficult to see that a vibrating ion at the crystal boundary may easily form a temporary attachment to the polar water molecules, strong enough to detach it from the mother crystal. A somewhat similar process may function when anthracene, say, dissolves in an organic solvent; it is while thermal vibration has temporarily weakened its attachment to the main body of the crystal that a temporary attachment to a solvent molecule can suffice to remove it. Such an attachment could well occur most easily at the 9,10-carbon atoms which are the most reactive and also[2] the most mobile.

This would explain why it is that dianthracene, which has a crystal structure very similar to that of anthracene, is nevertheless insoluble in all light organic solvents. The molecules are twice as heavy; and their 9,10-carbon atoms are no longer 'free' to form temporary attachments.

Movements at grain boundaries. The atomic vibrations at grain boundaries or in the neighbourhood of vacant sites will be larger than in the body of the crystal and therefore atoms will tend to make permanent movements of position more easily at these places. Impurities move to vacancies or grain boundaries and, if larger than the atoms of the matrix, they tend to get trapped there. Atoms tend to move across grain boundaries from a grain that is under strain to one that is not. This explains how it is that ice crystals at the bottom of a glacier are found to be very large relative to those at the top. The weight of snow or ice above imposes a strain on the ice crystals which form the glacier. Those in which this strain can be relieved by gliding grow (by transference of molecules across boundaries) at the expense of those less favourably oriented. The large crystals at the foot of the glacier are, therefore, so orientated that the basal (0001) layers, which define the directions of easy glide, lie along the direction of flow of the glacier[3].

Annealing of radiation damage. Heat treatment will heal radiation damage in diamond; and it is probable that self-annealing is going on, during neutron irradiation, simultaneously with the damage imposed. Laue photographs of diamonds which have had their density reduced by 4 per cent as the result of irradiation by fast neutrons show diffraction effects which are entirely similar to those produced by thermal vibration, except that they correspond to very much larger atomic displacements and that they are not temperature-sensitive. The random-rotation photographs of such irradiated diamonds show that all the unit cells are enlarged; it is not a case of a partly undistorted diamond containing regions of intense damage. There is no trace of diffraction spots at normal diamond spacings, and the spots due to the enlarged structure are sharp enough to show copper $K\alpha_1\alpha_2$ separation (Milledge, H. J., unpublished).

Nature of disorder in crystals. Any form of disorder which is completely random must give a diffraction effect similar to that of a gas; or if there are regions in the crystal where the disorder is essentially close-packed but amorphous, then its diffraction effect will be that of a liquid. Such effects are observed. But when diffraction effects are found which are not temperature-sensitive but which nevertheless show some symmetry and periodicity (related to that of the crystal) then the disorder cannot be random. It must affect every unit cell, or a definite proportion

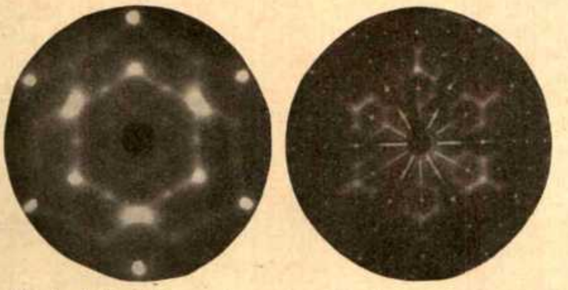


Fig. 1. Left, diffuse background on benzil photograph due to thermal vibration; temperature-dependent; right, diffuse background on trimethylsulphonylmethane photograph (Abrahams and Speakman) due to ordered disorder; temperature-independent

of the unit cells, in a partially ordered way. Such disorder may simulate thermal-vibration and cause an apparent increase of the Debye coefficients, but it will also cause a real variation of the thermal vibration, as compared with that in an ideal crystal, just as holes, impurities and other imperfections must do (Fig. 1).

Solid-state diffusion. Ionic conductivity. Gold placed in close contact with lead diffuses into it very easily. Near to the melting-point of lead, the diffusion may be as fast as that of salt through water at room temperatures. Such diffusion can occur partly because gold atoms are smaller than those of lead, partly because of the holes and dislocations in the lead structure and partly because of the large thermal vibration in the lead crystal, which allows rapid movement of vacant sites towards the common boundary where gold atoms are then trapped.

Sodium ions can easily migrate through glass. If an evacuated glass bulb is immersed in molten sodium nitrate and the evacuated space in the bulb is made conducting by emission of electrons from the hot tungsten filament; and if a voltage is imposed between a copper anode in the molten sodium nitrate and the tungsten filament acting as cathode, then a current will pass. The fact that the current is conducted through the glass by means of Na^+ diffusion is proved by the deposition of a layer of sodium on the inside of the glass bulb.

Similarly, it is stated that when yellow β-Ag_2HgI_4, which is tetragonal, changes at 46° C. to red α-Ag_2HgI_4, which is cubic, the electrical conductivity is greatly increased. In the cubic form, the three $(2Ag + Hg)$ ions randomly occupy four equivalent positions. There is therefore a random arrangement of one vacant site per unit cell and current passes mainly by means of the passage of Ag^+ ions one way and of vacant sites carrying an effective negative charge the other way.

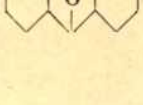
Generation of lightning by movements of electric charge through ice subject to a thermal gradient. J. Latham and B. J. Mason[4] have succeeded in explaining how it is that a sufficient quantity of charge separated by a large distance is generated, in a cumulo-nimbus thunder cloud, for a series of lightning flashes to occur. Soft hail pellets, falling at several metres per second through the cloud, will meet a fairly high concentration of small (say, 0·1 mm. diameter) supercooled water droplets. These freeze on to the surface of the hail, and afterwards, for each such droplet, about a dozen positively charged tiny ice fragments are thrown off and are carried upwards taking their positive charge with them. The negatively charged hail pellets continue to fall. Ingeniously contrived laboratory experiments have shown that this process actually occurs, and that the charge separation is such that within 20 min. about 1,000 coulombs could be generated in a volume of 50 km.[3].

The supercooled water droplets, in freezing on to the hail, give up latent heat and there is, therefore, a temperature gradient at the surface layers of the ice. The body of the hail may be, say, 10 deg. warmer than the exterior and where the ice is warmer the thermal vibrations will permit the formation of large numbers of H^+ and OH^- ions. These diffuse to the colder surface, but the velocity of migration of the proton is perhaps ten times as great as that of the hydroxyl group. The actual proton movement is, in general, by transfer only along a single OH—O bond, with the formation of temporary $(H_3O)^+$ groups. This differential diffusion results in an accumulation of positive charge at the surface and the ejection of the positively charged ice splinters.

Change of polarity of certain ferrite magnets as their temperature is changed. Ferromagnetism results from the interaction of the magnetic moments of atoms which have unbalanced spin orbitals; it is confined to crystals where there is, within certain limits, a definite ratio of interatomic distance to spin-orbital diameter. Sometimes the interaction is direct, sometimes it occurs through an intermediate anion such as O^{2-} (super-exchange), in which case both interatomic distances and metal–oxygen–metal angle may be important. Either of these may change with temperature and do, in fact, change continuously with thermal vibration, which opposes perfect lining up of the 'atomic magnets' and which, above the Curie (or Néel) point is sufficient to prevent it altogether, leaving only paramagnetism.

In the spinels, there are two (or more) different kinds of metallic ions, which distribute themselves on two kinds of sub-lattices within the oxygen ion framework. One sub-lattice (A) consists of small tetrahedrally surrounded sites; the other (B) of larger octahedrally surrounded sites. Magnetic atoms on A sites will line up with each other (AA^+ interaction) as far as thermal vibration will allow. So do those on B sites (BB^+ interaction). But there is a negative interaction between magnetic ions on A sites and those (which may be of the same or another element) on B sites (AB^- interaction). There is, in general, a resultant ferrimagnetism, that is, a preponderance of magnetic moment in one direction. Since, however, these interactions vary differently with temperature there may be a 'compensation temperature' at which the magnetic spinel becomes an antiferromagnet and then, with further change of temperature, changes its polarity.

This occurs for a number of ferrites of which $Li_{0·5}Fe_{1·25}Cr_{1·25}O_4$ is typical[5]. At room temperatures the distribution of ions is as follows: on A sites $Fe^{3+}_{0·91}$, $Li^{+}_{0·09}$; on B sites $Li^{+}_{0·41}$, $Fe^{3+}_{0·34}$, $Cr^{3+}_{1·25}$, and it is probable that there is some local ordering of the metal ions within the respective sub-lattices. The compensation temperature is 38° C. and the Curie point is 214° C. The rather large non-magnetic Li^+ ions, which also have relatively large thermal vibrations, will certainly be a disturbing factor, especially in the small A sites.

Solid-state chemical reactions. When the photo-oxide of anthracene  is heated to 160° C.

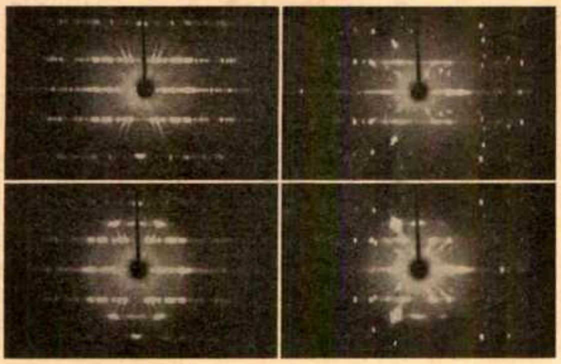


Fig. 2. Top left, initial rotation photograph of the photo-oxide of anthracene (*b* axis); top right, initial Laue photograph of the photo-oxide of anthracene, showing disorder layer lines corresponding to independent strings of molecules parallel to the *b* axis; bottom left, repetition of top left after 50 hr. X-irradiation. Layer lines due to orientated anthraquinone appearing strongly; original layer lines weaker; bottom right, repetition of top right. Laue pattern and layer lines of photo-oxide of anthracene disappearing. New Laue spots and layer lines appearing, due to anthraquinone

it explodes[6]. If a single crystal is irradiated by X-rays at room temperatures, or even at low temperatures, it changes slowly, but in the end completely, into a single crystal of anthraquinone. Laue photographs show that the photo-oxide, even when freshly crystallized, does contain some chains of molecules, parallel to the unique monoclinic axis, which are detached from their neighbours and randomly orientated about that axis. The thermal vibrations of the outer phenyl groups and of the oxygen bridge are very large and the bridge easily breaks; the hydrogen atoms are flung off and the molecules straighten out into anthraquinone, but without breaking up the structure entirely. Both structures are present in parallel at an intermediate stage (Fig. 2).

The photo-oxide of 9.10-diphenylanthracene behaves differently when heated. At 200° C. it gives off all its oxygen and reverts to the pure hydrocarbon[7]. It is evident that in this case the thermal vibrations, in breaking the bridge, cause the loss of oxygen rather than hydrogen, simply because the phenyl side-groups are too large and heavy to be thrown off and diffused out of the crystal. Diffraction experiments will be undertaken to see whether X-irradiation has a similar effect.

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A POSSIBLE ORIGIN OF TEKTITES BY SOIL FUSION AT IMPACT SITES

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TEKTITES are glassy objects found scattered on the Earth's surface in discrete arrays up to tens of thousands of kilometres across. They are composed of a highly siliceous and a luminous glass, devoid of crystalline phases except rare coesite[1] and nickel–iron[2] and containing particles of lechatelierite, pure silica glass.

A terrestrial versus extra-terrestrial origin for these objects has long been debated. As Viste and Anders[3] have shown, neither the presence of aluminium-26 nor any other distinctly extra-terrestrial property of tektites has yet been demonstrated. The chemical composition of tektites has been compared by various workers to various terrestrial rocks, especially sedimentary rocks, and contrasted with the compositions of meteorites and most igneous rocks. Spencer[4] has drawn analogies with impact-produced glass at known meteorite craters formed from the rocks of the impact site. Urey[5] and Barnes[6] have suggested that some impact process could produce the observed areal distribution of tektites.

I shall assume here, with Spencer[4], Urey[5] and Cohen[1], that tektites are formed on the Earth by the impact of some extra-terrestrial object. Tektites are presumably formed by the rapid fusion of rock during this impact. In this case the compositions of tektites should range widely over the possible range of terrestrial rocks, since the likelihood of (continental) impact on any given rock type should be proportional to the areal extent of the rock type. On the contrary, tektites are of restricted compositional range suggesting that the parental rock which has been fused to form them has a similar limited range of composition within which its components vary in some consistent fashion[7]. In a petrological sense they form a 'clan'.

A suitable mixture of shale and quartz sandstone[8] or an appropriate selection of igneous rocks[9] could account for the observed compositions; but it remains to be shown why just such a mixture or selection of rock types should be chosen as impact-site material to the exclusion of limestone, basalt, granite, etc.

A possible widely occurring rock type that may be the parental material of tektites is soil. It is worth while to see to what extent a soil parent rock will explain the observed properties of tektites. Soils formed within a given climatic zone on a diversity of bedrock types have remarkably similar composition[10]. The relations between the abundance of the chemical components of soil is largely a function of the weathering, dissolution and accumulation processes in the soil modified by the abundance ratios in the source bedrock. Therefore soils have a characteristic field of compositions and also form a clan, distinct from that of the parent rocks.

An average soil composition taken from analyses of temperate and humid zone soils is given in Table 1, column *A*. The data are from Glinka[11] and Bear[12].

Table 1

	A	*A′*	*B*	*C*	*D*
SiO_2	75·81	76·18	74·43	44·58	74·60
Al_2O_3	12·86	12·92	13·13	2·93	12·49
Fe_2O_3	4·18	—	—	—	—
FeO	—	3·77	4·27	14·57	4·11
MnO	0·13	0·13	0·12	0·29	0·13
TiO_2	0·93	0·94	0·83	0·13	0·90
CaO	1·83	1·83	1·76	2·28	2·04
MgO	0·88	0·89	2·01	33·76	2·01
K_2O	2·11	2·12	2·24	0·11	2·03
Na_2O	1·41	1·41	1·23	1·15	1·39
P_2O_5	0·30	0·30	nd	0·25	0·30

A, average of 11 soil analyses taken from Glinka (ref. 11) and Bear (ref. 12).

A′, same average recalculated with all Fe^{3+} reduced to Fe^{2+}.

B, average of 13 analyses of tektites taken from Barnes (ref. 25). Average includes 4 moldavites, 2 indochinites, 2 australites, 3 Ivory Coast tektites, 2 bediasites.

C, average chondrite excluding metal and sulphide (modified from average by Urey and Craig (ref. 14).

D, average soil plus 3·5 per cent chondrite. Proportion chosen to set MgO content equal to that in tektites

For comparison I have constructed an average of 13 analyses from Barnes taken in roughly equal numbers from the 4 main areal (and age) groups, Ivory Coast, Central Europe, Australia–Asia and Texas. Each of these groups presumably represents a single impact site and parent material. This average is shown in column *B*. The similarity between these two averages is striking. Although one could construct a mixture of shale and sand or igneous rock and more closely approximate a tektitic composition this comparison with soil compositions is all the more striking because such a homogeneous rock-type does exist and is accessible to metamorphism into tektites by terrestrial impact processes. However certain discrepancies are noted between these averages:

(1) As in the sedimentary rock mixtures[8] iron is present in the starting material mostly as Fe^{3+} while it is mostly as Fe^{2+} in tektites. This point has been discussed by Friedman, Thorpe and Senftle[13], who conclude that the heating process alone could account for most of the reduction. The presence of lechatelierite particles indicates that temperatures as high as 1,700° C. have been reached during formation. For the reaction:

$$Fe_2O_3 + SiO_2 = Fe_2SiO_4 + \tfrac{1}{2}O_2 \qquad (1)$$

$\Delta F = -7{\cdot}8$ kcal. at 1,527° C. and decreases at higher temperatures so that even in the absence of any reducing agent the reduction of iron is favoured. No doubt the 2–8 per cent humic matter present in most soils would also contribute to the reduction of the iron.

(2) Magnesia and equivalent FeO (column *A′*) are lower in soils than in tektites. The magnesium deficiencies are such that addition of a mixture of MgO and FeO to soil in the ratio of MgO : FeO = 1 : 0·45 would produce an average tektite composition. The ratio of these two oxides in stony meteorites is 1 : 0·43 (ref. 14). In column *C* is shown a mixture of soil and 3·5 per cent chondrite. The proportions are chosen to give the appropriate MgO content (2·01 per cent). Such a mixture could occur in Nature if the tektites were produced by impact of a stony meteorite and there occurred some mixing of the silicate phases of the meteorite with the soil of the impact site. However, no appreciable mixing of the metal or sulphide components appears to have occurred. This is also indicated by the low nickel content in tektites observed by Ehmann[15].

Taylor, Sachs and Cherry[7] observed that of all major element abundances studied in tektites only that of calcium showed no significant correlation with any other element. Presumably the source of the parent tektite material contains varying amounts of a phase containing only CaO (or $CaCO_3$). This is probably material from the almost ubiquitous zone of calcium enrichment found at various depths in temperate-zone soil profiles. The varying CaO contents would correspond to varying depths of sampling by the impact process.

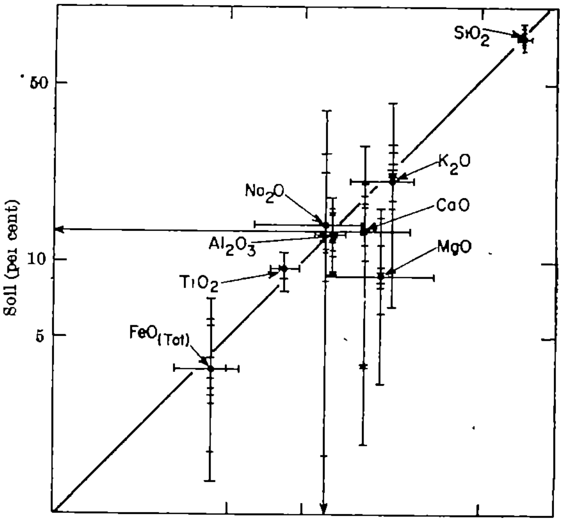

Fig. 1. Major element composition of eleven soils versus mean composition of each of four major tektite groups (moldavites, Asia–Australia, bediasites and Ivory Coast). K_2O, Na_2O, CaO, MgO and TiO_2 × 10. Logarithmic plot

The trace-element abundances of tektites have been studied by Preuss[16], and more recently some individual elements have been measured by various workers (nickel, Ehmann[15]; rubidium, strontium, Schnetzler and Pinson[17]; lead, uranium, thorium, Tilton[18]; gallium, germanium, Cohen[19]; lithium, cæsium, Taylor and Sachs[7]). Using Preuss's data for the sake of its completeness I have compared the rank of the abundance-levels for tektites and two possible source rocks, soil and shale (Fig. 2). The abundance ranking for tektites agrees for most elements with that of soils, and less closely with that of shales, which are of course of related origin. Other possible source materials, such as igneous rocks and sandstones, agree more poorly.

Gases included in bubbles within tektites have been studied by F. E. Suess[20], who found that these cavities contained carbon dioxide, carbon monoxide and water. More recently H. Suess[21] observed that the gas pressure in these cavities was very low, but that on heating crushed tektites they did evolve carbon dioxide, carbon monoxide, hydrogen and water. If the tektites were produced by fusion of soil these gases were presumably formed by the thermal decomposition of humic matter plus the adsorbed water of the soil. Note, however, that carbonaceous matter is also present in shales: an average of 44 shales compiled by Clarke[22] contained 0·8 per cent carbon.

Studies of lead, uranium and thorium in tektites by Tilton[18] showed that tektites contain modern lead such as would be derived from a typical crustal rock-system in which $^{238}U/^{204}Pb = 9{\cdot}8$ and $^{232}Th/^{204}Pb = 3{\cdot}9$. However, the observed values in tektites were 38·5 and 5·4 respectively. If the parent material of the tektites had had such high uranium and thorium contents for more than 50–100 million years a measur-

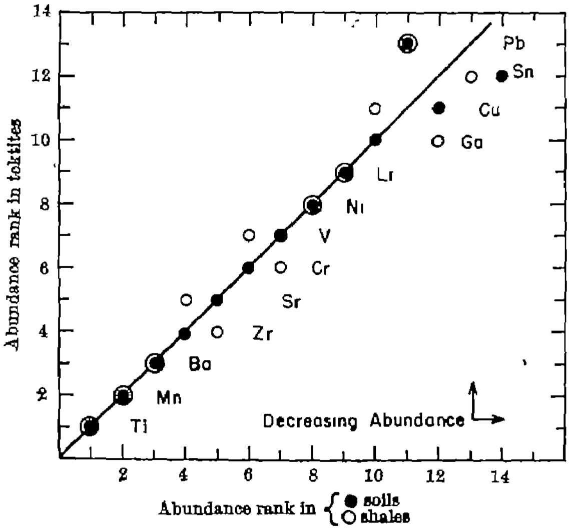

Fig. 2. Relative abundance rank of trace elements in tektites (Preuss) versus rank of same elements in shales (●) (Turekian, K. K., and Wedepohl, K. M., *G.S.A. Bull.*, 72, 175, Table 2; 1961) and soils (○) (ref. 12)

able deviation in the ^{206}Pb/^{207}Pb and ^{206}Pb/^{204}Pb ratios should have been observed. It is unlikely that thorium and uranium are enriched with respect to lead by the heating process alone. Therefore the tektites must have been derived from a rock of less than 100 m.y. age, that is, Cretaceous or Tertiary. In view of the limited surficial extent of Tertiary and Cretaceous rocks it seems an improbable coincidence that four (or more) random impacts, producing the four main families of tektites, should all have fallen on such young rocks. Soils, on the other hand, are known to be enriched in uranium and thorium, adsorbed into humic matter[23]. Such adsorbates would be short-lived since the humic matter is constantly being oxidized and carried out of the soil with a half-life of a few thousand years. The small amount of radiogenic lead produced in this interval by the decay of the adsorbed uranium is presumably carried away with the oxidized humic acid and would always be in low levels compared with the common and radiogenic lead of the mineral components of the soil.

With respect to the impact process itself, Bjork[24] has shown that on "hypervelocity" impact of a large iron meteorite the highest upward velocities are obtained by material originally located at and near the ground surface of the impact site. Material with such high velocities oriented at high enough angles to the horizon to follow intercontinental trajectories will therefore of necessity be near-surface material.

In conclusion, I have demonstrated that a possible parent rock for tektites is terrestrial soil. Soil forms a discrete, geochemically well-defined family of rock types which, with some *ad hoc* modifications, match the family of tektite compositions. Unlike the various mixtures of sedimentary or igneous rock types which have been proposed, soil is truly ubiquitous, covering all land surface except high mountains and deserts. Barnes[25] and Hawkins[26] have drawn comparisons between tektites and fulgurites. These latter would also be formed by fusion of soil, though by lightning rather than impact. I believe such a process is ruled out by the geographical distribution and the recently described occurrence of nickel–iron spherules in some tektites.

A test of the soil hypothesis would be some correlation between the local soil-chemistry at an impact site and the chemical composition of the resultant tektites. It must be assumed that climates have not changed appreciably since the time of impact since soil compositions would have changed accordingly. The Ivory Coast tektites are thought to have originated in an impact at Bosumtwi crater in Ghana[1]. If we can assume this area to have had a tropical or subtropical climate at the time of impact the soil of the impact site should have been enriched in Al_2O_3 and Fe_2O_3 and depleted in alkalis and CaO. Table 2 shows a comparison of these components with the average values. The alumina and iron oxide values are the highest for any tektites. The alkalis are not greatly depleted however. A second such comparison between source area and tektite is perhaps the Libyan desert glass for which no impact-site origin has been as yet proposed. The glass is close to pure silica in composition. This is the composition one would expect for a mature dune-sand such as that which forms the surficial cover over large parts of northern Africa. The rapid movement of these dunes may have buried and obliterated the impact site.

Table. 2. PARTIAL ANALYSIS OF IVORY COAST TEKTITES AND COMPARISON WITH AVERAGE TEKTITE

	A	*B*
Al_2O_3	14·60	13·13
FeO	5·61	4·27
K_2O	1·53	2·12
Na_2O	1·71	1·41
CaO	1·67	1·76

A, partial analysis (average) of Ivory Coast tektites, data from Barnes.

B, average tektite. Average described in Table 1.

I thank Dr. R. N. Clayton and Dr. E. Anders for their advice.

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THE BOTANIC GARDEN AND ITS FUNCTION IN MODERN SOCIETY

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HAVING lived for most of my life in close proximity to a botanic garden, and having recently moved to a city with no public botanic garden and with its University Botanic Garden at an early stage of development, I have been forced to think about the functions and future of botanic gardens. The gardens I have known have played a very large part in my life and I should like to think that any young person with botanical leanings could gain access to a well-run botanic garden.

There was a time, when medicine was concerned with plants, and later, when taxonomy and the exploration of the world for economic plants were in their heyday, that the making of a botanic garden was the proper work of a botanist. Later still, and in our own day, many botanists have found their interest in the functions of plants and in experimental work. The botanic garden lost active favour with these botanists and became the home of specialists—the taxonomist and horticultural plantsman. We have a situation to-day where the established botanic gardens flourish but where no new botanic gardens are being created unless we class as botanic gardens the large publicly supported horticultural gardens. I wish to suggest now that we are entering a new phase in the development of botanic gardens, that they have an ever-increasing part to play in the education of the public, of the professional botanist and in university teaching, and that there is a case for the establishment of more botanic gardens.

The great botanic gardens of the country need no justification. Expensive to maintain, they are visited and enjoyed by millions every year. In addition, they are great centres of research and form a training ground for horticultural plantsmen. It would not be sensible to try to recreate the Royal Botanic Gardens, Kew, or the Royal Botanic Garden, Edinburgh, in each of our great cities, but there is a real need for dynamic, named collections of plants in many of these cities. This need arises from the innate desire of people to determine the boundaries of their ignorance and to find some names as landmarks in the unknown field that interests them. It also arises from the needs of young men and women on the threshold of their careers. They may be born botanists, but the latent interest may not develop without opportunity and encouragement such as can be given by a good botanic garden. It is fashionable to decry the 'natural order bed'; certainly an attempt to cram the plants of the garden into an ordered arrangement without consideration of their horticultural or ecological needs is disastrous. On the other hand, it is a great revelation to the young botanist to see, for example, the range of variation in the Ranunculaceae, the repetition of leaf shape, the flux of genera, as *Thalictrum*, *Clematis*, *Anemone*, *Ranunculus*; the range of species in a genus and the ecological differentiation within species.

Apart from stimulating the young and aspiring botanist, the garden forms a reference collection for horticulturists, pharmacists, biochemists and other interested persons, and is one of the major tools of the taxonomist. It should also be æsthetically pleasing and a source of enjoyment for those who, while not experts, derive pleasure from the form and variety of plants. If this were all that a botanic garden did it would be as easy to justify as an art gallery or technical library in any sizeable town. One might well ask if the public parks do not already fulfil a part of the function of a botanic garden and inquire if this function could not be extended? In fact, some of the larger towns do run botanic gardens as part of the activities of the Parks Department, for example, Glasgow and Bath; other towns run specialized gardens which have part of the function of a botanic garden, for example, the Golden Acre at Leeds. These municipal efforts can play a very useful part in the provision of the facilities we desire. Parks superintendents of the smaller towns would be the first to admit, however, that they could, in attempting to develop a botanic garden, come up against formidable difficulties of lack of finance, of lack of support by the local authorities, and of lack of informed help from knowledgeable people. Good botanic gardens need constant care from botanical enthusiasts to keep them viable. Not every local authority parks department could provide the necessary botanical enthusiasm. Even where every portent is good, a botanic garden may not flourish under the supervision of the parks department because of the intrinsically different aims of park superintendents and curators of botanic gardens. The atmosphere of colour and interest produced in the park is quite different from the scholarly calm of the true botanic garden.

One of the strengths of the botanic garden is its association with active research work on taxonomic and other problems. As workers in different groups pass through the garden so collections will be increased and better documented, and the garden will appear always to contain novelties to the casual visitor. It is the scientific slant too which makes for much of the æsthetic quality of the garden. The practical ends of science, which should be the first concern of the garden, prevent it from having an immediate popular appeal, and so preserve some of the peace and informality which are an attraction to visitors. It could be said that when the botanic garden puts its visitors first and its plants second it loses much of its attraction.

If only a limited accretion of botanic gardens can be expected from developments of public parks, then the further developments must come in part from the universities. As new universities spring up, with departments of botany, they are the obvious places to nurture botanic gardens. If we can admit that such gardens should be established, we must inquire how big they shall be, how they shall be financed, who shall control them, and what services they shall give to the town they are in? To the first of these questions there is no answer; but I feel personally that the garden should be small, not large. Once a garden grows beyond a certain size, and some of the great gardens must, it loses the encompassed feeling which should be one of its pleasant features. Quite small gardens of an acre or two can show a remarkable range of plants. Where trees are to be included—and they should be—10–20 acres is better. Once past that size the garden becomes a showplace, and greater skill is required to ensure that the needs of the plantsman are not overlooked in the desire for display.

To the second and third questions I would answer that where possible the universities should finance and control the garden through their departments of botany. Having said that, one is faced with a number of problems. Botanic gardens, even small ones, cost a great deal of money to run. A small garden may easily use up one-quarter to one-half of the annual grant of the department of botany which it serves. This is a deterrent only in the sense that the garden should not be allowed to outgrow what the annual grant can accommodate. The actual sum spent on the garden will not be large in comparison with the sums spent on, for example, technological equipment, and the educational and research yield should be as great, if not greater.

If the university garden is to be open to the public the costs rise, if only by the provision of a policeman or garden ranger; questions of safety arise from such hazards as falling trees and poisonous plants, and it may be that the department of botany assumes a burden which may become intolerable financially and administratively. To overcome this difficulty it may be sufficient to arrange that the garden, while not open to the public, is open to a limited number of persons as ticket holders. The ticket holder would accept personal responsibility for any hazards which might befall him in the garden. If, in time, the garden grows to be a major cultural attraction in the town, then the town should share in the administrative expenses of opening the garden.

In new and developing institutions the policy decisions on the apportionment of land for the botanic garden must be firmly pressed home. The aspiring botanist is only too ready to accept the use of land at any price. This land may soon become the target of a take-over bid by the university building committee, with consequent upheaval for the department and discouragement for the garden staff. Another danger is that a specific area of land is not allocated but the botanists are given *carte blanche* to use the university grounds for planting purposes. This is a highly unsatisfactory procedure, for the botanists then become the prey of all the æsthetic pressure groups in the university. It is of the greatest importance that some knowledgeable member of the botany department should be in liaison with the horticultural and building staff of the university to make use of all the botanical and horticultural opportunities which present themselves. At the same time, the greatest evil that can befall a professor of botany is to be charged with the administrative care of the grounds of the university. The nearest analogy would be if the professor of physics were responsible for the supply of electricity to the university.

Very often a university garden will consist of an intensively cultivated small garden, exclusive to the department of botany, and a larger area which contains specimen trees and shrubs and which is part of, or which merges with, the grounds of a university hostel. This arrangement makes the opening of the garden to the public extremely difficult without an adequate police force because wardens of hostels are naturally averse to public access. It is possible to cite the freedom of access to the Cambridge and Oxford colleges; but there a very large police force is maintained by the discreet use of porters. If the university botanic garden is to play its part in providing an educational and æsthetic service to the town, then in new universities, and when changes take place in old universities, the department of botany should have a piece of land of adequate size under its sole control. Moreover, this should be associated with a policy of adequate provision of essential services for the future garden. Much money is wasted by the piecemeal extension of facilities, for example, electricity, water and heat for glasshouses and great savings can be achieved by planning large from the beginning.

If the botanic garden is to be a university-sponsored garden, and if, because of administrative and financial difficulties, it is to be of a semi-private nature, we have to consider the part the garden is to play in the educational policy of the department of botany. Unless the department and the garden are closely integrated in the teaching, the garden can become a 'white elephant'. If the taxonomic teaching in the department is limited, then the calls on the garden for teaching material will be limited and could be met by the provision of a service garden run cheaply for this limited purpose. Where, in the modern department of botany, there is the need for the growing of large numbers of plants for experimental work, then the additional cost of maintaining a collection of botanical interest is small and can well be justified. The student who comes from a department with a well-run botanic garden has an enormous advantage in that familiarity with a well-organized collection helps him to an ordered familiarity with the range of the plant kingdom. But the collection must be well organized and well displayed.

Few botanic gardens have tackled the problem of display. They still maintain the indigestible collections similar to those shown by the museums of thirty years ago. Nowadays, museums are careful to select material and to signpost it in such a way that a walk around a museum is an education. Botanic gardens must learn to select and to label too. This is particularly important in departments where formal taxonomic teaching is reduced to a minimum. In such departments the function of teaching must be undertaken by the well-documented garden. Moreover, the garden must display not only the taxonomic collection but where possible the ecological collection, the geographical and historical collection as well. The botanic garden should reflect the vitality of the department it serves, and one might almost say that the department can be made active by a well-run garden. In this age of specialization, when semantic barriers arise within the subject, the garden should be the integrating factor in the department of plant sciences, for such the modern department of botany is becoming. The biochemist should find the plant which interests him displayed in its taxonomic position, the taxonomist should find on the label an indication of the biochemical nature of its colour, its smell, its poisonous properties or a plant-physiological explanation of its ecological range, and so on. All will then achieve some appreciation of the point of view of other workers, and thus the garden would have a unifying influence.

Such a concept of the botanic garden demands skilled labelling, skilled growing, skilled display. It calls for a high degree of scientific ability on the part of the horticultural staff, and it demands a botanist who will devote time to the development of the garden. These are not easy things to achieve in short-staffed departments, but needs must first be formulated before they will be satisfied.

There have been two threads running through this article. The problem of the provision of more botanic gardens in general and the problems of the university botanic garden in particular. The conclusion is that we must encourage the public park to develop some sort of educational display of plants, that this may be better to have a horticultural bias because in this way it will command the enthusiasm of the parks staff. The second conclusion is that where possible the new (and sometimes the old) universities should develop a university botanic garden which can be open to the public but that where that is not possible the university should make its garden as freely available as possible to organized parties and should, by example and encouragement, help the parks department of the local authority to develop educational collections. Active botanic gardens should play an ever-increasing part in our cultural life.

OBITUARY

Sir James Wordie, C.B.E.

By the death of Sir James Wordie on January 16 both the University of Cambridge and the world of polar affairs have lost a valuable and experienced adviser. James Mann Wordie was born on April 26, 1889, in Glasgow, where he afterwards attended the Glasgow Academy and the University before moving on to the University of Cambridge in 1910 to continue his geological studies. From this time on, his life was centred about Cambridge for more than fifty years although his interests and contacts in Scotland continued.

He was elected Fellow of St. John's College in 1921 and was in turn supervisor, tutor, senior tutor, president and Master. Many men who passed through his hands during these years have since made their mark in various fields, including that of his greatest interest—polar research.

Wordie's interest in polar regions was stimulated by a visit to Alaska in 1913 and by contact in Cambridge with the geologists who had just returned from Scott's last expedition. He joined Shackleton's Imperial Trans-Antarctic Expedition of 1914–16 as geologist and senior scientist. After a year spent drifting in the ice, first in the ship, then living in tents on an ice floe after it was crushed, the party reached Elephant Island, where they spent the winter under their upturned boats while Shackleton made his remarkable boat journey to organize their relief. After return to England he served in the Royal Artillery until he was wounded and invalided home.

In 1919, Wordie joined the late Dr. W. S. Bruce as second-in-command of an expedition to Spitsbergen, which started a pattern, that has grown considerably in recent years, of university undergraduates taking an active part in summer expeditions to polar regions. In 1921 he led a small expedition to Jan Mayen Island, and in addition to geological studies he led the first ascent of Beerenberg (8,090 ft.). During the following ten years he led three more expeditions to East Greenland, which in addition to the immediate scientific results stimulated an interest in polar regions of men such as Sir Vivian Fuchs, the late Augustine Courtauld, P. D. Baird, of Canada, Prof. Gordon Manley and M. A. F. Barnett, of New Zealand. He continued with similar expeditions in 1934 and in 1937, travelling via West Greenland to Baffin and Ellesmere Islands. His last major trip was to the antarctic in the southern summer of 1946–47, when he visited the South Orkneys, the South Shetlands, where he had wintered in 1916, and Graham Land on behalf of the Colonial Office.

Even before the Second World War, but especially during and since the War, his advice and influence played a large part in the development of polar activities from the United Kingdom. He was a member of the "Discovery" Investigations Committee of the Colonial Office from its inception in 1923 until it handed over its responsibilities to the newly formed National Institute of Oceanography in 1949. He was a founder member of the Committee of Management of the Scott Polar Research Institute in 1926, and was its chairman during 1937–55. His lifelong interest in glaciers and sea ice led to his appointment as reporter for glaciology on the main planning committee for the International Geophysical Year as well as the vice-presidency of the International Commission of Snow and Ice and of the British Glaciological Society. He was chairman of the British Committee of the Norwegian–British–Swedish Antarctic Expedition 1949–52, and vice-chairman of the Committee of the Trans-Antarctic Expedition 1955–58. This expedition was initiated during his term as president of the Royal Geographical Society. It was also during this presidency that the first ascent of Mount Everest was made, and Wordie had played a part in the important decisions made by a joint committee of the Royal Geographical Society and the Alpine Club which led to this success. His last major task was that of chairman of the British National Committee for the International Geophysical Year and its various Antarctic Sub-Committees. His long-held belief of the accessibility of the eastern side of the Weddell Sea was well proved by the successful establishment of the Halley Bay Station by the Royal Society International Geophysical Year Antarctic Expedition, as well as the establishment of the Shackleton Base of the Trans-Antarctic Expedition.

For his own exploratory activities, Wordie was awarded the Back Grant (1920) and the Founder's Medal (1933) of the Royal Geographical Society, the Bruce Medal of the Royal Society of Edinburgh (1926) and the Gold Medal (1944) of the Royal Scottish Geographical Society. His administrative activities in polar affairs resulted in his award of the C.B.E. in 1947 and a knighthood in 1957.

James Wordie was a quiet and modest man, whose strength of character and shrewd judgment caused many responsibilities to be thrust on his shoulders. Throughout his life the support and interest of his wife as well as that of his three sons and two daughters provided an atmosphere against which he could give of his best in public responsibilities.

G. de Q. Robin

NEWS and VIEWS

Industrial Chemistry at Liverpool :
Prof. C. H. Bamford

Dr. C. H. Bamford, who has been appointed to the Campbell Brown chair of industrial chemistry in the University of Liverpool, is forty-nine years of age and was educated at St. Patrick's and King Edward VI Schools, and at Trinity College, Cambridge, where he graduated with first-class honours in 1934, and was awarded a Ph.D. in 1938 and an Sc.D. in 1954. He was elected a Fellow of Trinity College in 1937 and served as a director of studies in chemistry, Emmanuel College, Cambridge, during 1937–43. In 1941 he was awarded the Meldola Medal of the Royal Institute of Chemistry. During the War he was employed by Interservices Research Bureau with the Ministry of Economic Warfare. After the War he joined Courtaulds, Ltd., and was responsible for the fundamental research undertaken at the Maidenhead Laboratory, and in 1947 he was appointed head of the Laboratory. Dr. Bamford has served on committees of the Faraday Society and the Society of Chemical Industry. He has made visits abroad to Australia, the United States and the U.S.S.R. He has published three books and a large number of papers in chemical journals, mainly on photochemistry, polymerization and synthetic polypeptides and proteins.

Organic Chemistry at Liverpool :
Prof. A. R. Battersby

Dr. A. R. Battersby, who has been appointed to the newly instituted second chair of organic chemistry in the University of Liverpool, is lecturer in organic chemistry in the University of Bristol. He is thirty-seven years of age and was educated at Leigh Grammar School and the University of Manchester, where he graduated B.Sc. with first-class honours in 1946 and M.Sc. in 1947. He was awarded a Ph.D. of the University of St. Andrews in 1949. In October 1948 he was appointed lecturer in chemistry at the University of St. Andrews. He was awarded a Commonwealth Fund Fellowship in 1950 and studied for one year at the Rockefeller Institute, New York. The Fellowship was extended for a second year, which he spent at the University of Illinois undertaking research in biochemistry. He returned to the University of St. Andrews as lecturer in chemistry in June 1952, and in January 1954 was appointed lecturer in chemistry in the University of Bristol. He was awarded the Corday–Morgan Medal and Prize for 1959 by the Chemical Society.

Veterinary Research at the Wellcome Foundation, Ltd.:
Dr. R. F. Montgomerie

Dr. R. F. Montgomerie, director of veterinary research in the Wellcome Foundation, Ltd., has retired. Born on the Island of Bute in 1898, Dr. Montgomerie graduated from the University of Edinburgh and the Royal (Dick) Veterinary College in 1923. He was one of the first three veterinary surgeons appointed in the old Agricultural Advisory Service and was located at the School of Agriculture, University College of North Wales, Bangor, from 1924 until he joined the staff of the Wellcome Research Laboratories at Beckenham in 1937. In North Wales he quickly established a reputation by his work on diseases of sheep, and his introduction of carbon tetrachloride for the treatment of liver fluke disease earned him a world-wide reputation. This was enhanced by his study of the cause of sudden deaths in thriving lambs which had hitherto been ascribed to 'wool ball', but which he showed was due to enterotoxæmia, the condition thereafter being called pulpy kidney disease.

At the Wellcome Research Laboratories he led the team which has done so much to provide important sera and vaccines for the control of the common diseases of sheep. In his direction of the Wellcome Veterinary Research Station, Frant, he built up a research centre now widely known for its work on infertility in farm animals, the discovery of anthelmintics for the treatment and prevention of nematodirus disease in young lambs and the treatment of lungworm in sheep and cattle. His work was recognized by the National Sheep Breeders Association when, six months ago, he was made the first recipient of the George Hedley Memorial Medal for outstanding service to the sheep industry.

Mr. S. L. Hignett

Dr. Montgomerie has been succeeded by Mr. S. L. Hignett, head of the Wellcome Veterinary Research Station at Frant since it was established in 1943. Mr. S. L. Hignett has, like Dr. Montgomerie, been president of the British Veterinary Association (1959–60). He is probably best known for his work on bovine mastitis and infertility in farm animals.

The Australian Association of Clinical Biochemists

The following are the officers and State members of Council of the newly formed Australian Association of Clinical Biochemists: *President*, Dr. J. A. Owen; *Honorary Treasurer*, Dr. J. E. O'Hagan; *Honorary Secretary*, Dr. D. H. Curnow (Royal Perth Hospital. Perth, Western Australia); *Members*, Mr. M. J. Thomas (Queensland), Dr. F. C. Neale (New South Wales), Miss M. Bick (Victoria), Dr. R. S. Parsons (Tasmania), Dr. W. Roman (South Australia), Dr. G. A. Sarfaty (West Australia). The first annual general meeting and scientific meeting of the Association will be held in Sydney on August 21.

Laboratory of the Government Chemist Steering Committee

The Council for Scientific and Industrial Research has set up a Steering Committee for the Laboratory of the Government Chemist. The Committee will be under the chairmanship of Dr. B. K. Blount, deputy secretary of the Department of Scientific and Industrial Research. The members of the Committee will be: Prof. R. Belcher, professor of analytical chemistry, University of Birmingham; Mr. R. C. Chirnside, chief chemist and head of the Chemistry and Technical Services Division of the General Electric Co., Ltd.; Dr. D. T. Lewis, the Government Chemist; Mr. H. Wooldridge, deputy director (Stations Division), Department of Scientific and Industrial Research Headquarters.

The Laboratory of the Government Chemist is the oldest Government chemical laboratory; its origin dates back to 1842. It provides a wide variety of analytical and advisory services for Government

Departments. In addition, the Government Chemist has a statutory function as official analyst or referee in cases of disputed analyses under various Acts of Parliament. Responsibility for the Laboratory was transferred from H.M. Treasury to the Department of Scientific and Industrial Research in July 1959.

Exchange Programme in Nuclear Science

A BROCHURE, *The Exchange Programme in Nuclear Science*, issued by the International Atomic Energy Agency outlines the opportunities offered by the Agency for visiting professors and scientists, the courses organized and the research grants available (Pp. 36. Vienna: International Atomic Energy Agency, 1962). Information is included on the sponsorship of visits of scientists to foreign centres, the use of the mobile radioisotope laboratories and the financial arrangements. Lists of visiting professors, research grants made and training courses already organized and of the activities of the two mobile radioisotope laboratories are appended.

Glass Manufacturers' Federation Fellowship

THE importance of fundamental research to the modern glass industry is underlined by the endowment of a new research fellowship in the University of Sheffield by the Glass Manufacturers' Federation. Contributions towards the five-year fellowship have been subscribed by thirty-four firms in, or connected with, the British glass industry. Companies supporting the fellowship range in size from those employing more than 10,000 people, to small tradition firms of 100 or so craftsmen. The British glass industry ranks as the third largest glass producer in the world, but is probably one of Britain's smallest industries to support a distinct technology. The British industry played a direct part in the foundation, in 1917, of the Department of Glass Technology in the University of Sheffield, the first University school to undertake the study of glass technology as a separate applied science. This intimate connexion between the University and the industry has been maintained since that date, and Sheffield is still the source of the many glass technologists needed to-day by the highly mechanized glass industry. A feature of the Department is a strong postgraduate school, and the value of fundamental research is fully recognized by the industry. Further information concerning this fellowship can be obtained from Mr. J. Small, education officer, Glass Manufacturers' Federation, 19 Portland Place, London, W.1.

World Inventory of Historic Scientific Apparatus

A MEETING of the Commission de l'Inventaire Mondial des Appareils Scientifiques Historiques (president, A. Léveillé) was held in the United Nations Educational, Scientific and Cultural Organization Building, Paris, on January 24, and was attended by representatives of scientific organizations in Austria, Belgium, Czechoslovakia, France, Germany, Holland, Israel, Italy, Luxembourg, Poland, the United Kingdom, the United States and the U.S.S.R. The Commission is supported by the United Nations Educational, Scientific and Cultural Organization, and has also received financial aid from many Governments and national institutions. Research into the use and whereabouts of historic scientific apparatus is now being carried out in twenty-eight countries, and national inventories are being made. A working group has been set up to examine possible methods for the presentation of the results. At the next meeting of the Commission, to be held on October 1, the contributions from the various countries will be discussed with the view of incorporating the most important into a world inventory of historic scientific apparatus. Further information can be obtained from the Inventaire Mondial des Appareils Scientifiques Historiques, Palais de la Découverte, avenue Franklin D. Roosevelt, Paris 8e.

The Field Studies Council

THE annual report of the Field Studies Council, 1960–61 (Pp. 29. London: Field Studies Council, 9 Devereux Court, W.C.2, 1962), presented at the annual general meeting of the Council on February 16, includes the report of the Executive Committee and the reports of wardens at the field stations of Dale Fort, Flatford Mill, Juniper Hall, Melham Tarn, Preston Montford, Slapton Ley and Skokholm Bird Observatory. The steady progress of the previous 10 years was maintained and bookings increased again to 9,334, from 8,539 in 1960. Of these 5,183 were in biological and 3,006 in geographical and geological subjects. More than 2,000 applications had to be refused, and the executive committee is concerned at the number of last-minute cancellations and some failures to turn up for the courses booked. Of the individual attendances, 5,781 were from sixth forms, 782 from training colleges, 1,134 from universities and 1,249 amateurs. The membership is now approximately 4,000. comprising 1,050 corporate members and 2,950 individual members. Purchase of Dale Fort was completed in 1961, and in discussions with the Ministry of Education it has been agreed that the Ministry's grant should in future be for capital purposes and that the standard weekly fee should be raised by 10*s*. to £8 10*s*. It is hoped that the settlement will permit an adequate programme of capital improvements at the centres and that this will include a scheme for converting the High Stables at Melham Tarn. The opening of an additional centre is under consideration and during the year about £4,000 was spent on improvements to buildings and equipment.

British Technology Index

IN *British Technology Index* the Library Association has issued a valuable current subject guide to articles in British technical journals. The term 'technical' is interpreted generously, the 402 periodicals covered in the first issue (January 1962) including a number which ostensibly are scientific rather than technical (1, No. 1; January 1962. A Current Subject Guide to Articles in British Technical Journals. Pp. 70. Published monthly. London: The Library Association. Annual subscription for 11 monthly issues and bound annual volume, £15 15*s*. post free; 50 dollars, U.S.A.). Delays through the postal dispute excluded a further 120 periodicals which it was intended to cover in this issue. Entries are arranged alphabetically by subject, with adequate and common-sense cross-references, and are limited to title and bibliographical details. Among the features that should commend the *Index* to the industrial and technological user is the marked reduction in time-lag between publication of an article and its inclusion in the *Index*. In this connexion it is worth directing attention to the effective reply of Mr. L. L. Ardern in the *Library Association Record* for February to some questions asked about the response to the *Index* from industrial libraries in the United Kingdom.

Mental Health throughout the World

During June 1961 an International Study Group met at the Roffey Park Institute, Horsham, to discuss international aspects of mental health. Its primary function was to make a broad survey of the progress being made in the many aspects of mental health field throughout the world. An attempt was made to cover the spectrum of interests from research and care of the mentally sick at one end, to international relationships at the other (*Mental Health in International Perspective*. Pp. 84. London and New York: World Federation for Mental Health, 1961).

Causes of Deafness in Schoolchildren

A total of 270 children born in Northumberland and Durham during the years 1945–54 are receiving education in schools for the deaf. This represents an incidence of severe deafness of 0·7 per 1,000, the same as the national incidence. Dr. M. E. Barton, Dr. S. D. Court and Dr. W. Walker of the Department of Child Health, Durham University Medical School, King's College, Newcastle upon Tyne, have studied the early medical history and family background of these children (*Brit. Med. J.*, 5275; February 10, 1962). Twenty-five per cent were classified as having hereditary deafness, in 25 per cent deafness followed meningitis or some other infection, but in 50 per cent the cause of deafness was unknown. Of the unexplained cases there was a history of maternal rubella during the first three months of pregnancy in two instances, while two more of the children had suffered from severe hæmolytic disease of the newly born. Of the remainder, 21 per cent were 'premature' infants, 7·6 per cent having a birth weight of 4 lb. or less. Only 18 per cent of the premature infants gave a history of severe jaundice, and it would appear that prematurity even in the absence of jaundice may be an important cause of deafness.

Influence of Diet on the Incidence of Atherosclerosis

Dr. M. Toor, Prof. A. Katzir and Dr. I. Agmon have carried out an investigation of the socio-economic conditions, diet and serum cholesterol-levels of 2,680 immigrants to Israel from Eastern and Western countries belonging to different social groups (*Dapim Refuiim*, 11, No. 1; January 1962). The lowest serum cholesterol values were found among 'recent' Yemenites, the least fortunate economically and with an imbalanced intake and the lowest fat content. Higher cholesterol values were found among the early Yemenites and European manual workers whose diet was richer in calories and a percentage of calories derived from fats. In middle class Europeans the serum cholesterol-levels were still higher. A positive correlation was found between these serum cholesterol-levels and morbidity and mortality from atherosclerosis for the different groups investigated. The results support the assumption that atherosclerosis is a disease of lipid metabolism in which diet is a dominant factor.

Schistosomiasis

The latest issue of *Phoenix* is concerned with schistosomiasis or bilharziasis (1, No. 3; December 1961. *Bilharziasis/Schistosomiasis*. Pp. 16. Ann Arbor, Michigan: Michigan Memorial–Phoenix Project, The University, 1961). Estimates of the number of people afflicted with schistosomiasis range from 114 million to 200 million. These infested people live principally in agricultural communities in Asia, Africa and South America. In many areas, particularly Asia, schistosomiasis is also a disease of animals, thus adding detrimental economic effects to its human toll. Because of the growing number of countries in which the disease is known to exist, and because of the extension of the snail habitats in these countries and the growing number of infested people, schistosomiasis has become one of the worst diseases in the world. The interest of the Phoenix Project in schistosomiasis stems from a research programme supported by the Project and conducted in the Phoenix Memorial Laboratory, University of Michigan. The investigators are using radiation from a cobalt-60 source in an attempt to create a vaccine that will provide immunity to the disease.

Paralytic Rabies in Greenland

For at least a hundred years epizootics of a disease resembling rabies have raged among the sledge dogs of Northern Greenland. In 1859 Dr. N. Rudolph, medical supervisor of the Upernavik district, described the clinical picture in dogs of extreme exhaustion, hydrophobia, abundant secretion from the mouth, and weakness of the hind legs. In the initial stage the disease was clearly epidemic. Between 1864 and 1865 a similar outbreak occurred in the northern districts. In 1908 Mr. S. R. Hjortlund described a highly contagious and deadly epizootic in dogs: in most cases the disease ran a quiet paralytic course, but a few dogs were aggressive. The cause of the disease was never established, but it was noted that often the disease broke out a few days or weeks after fights between dogs and 'mad' polar foxes. Because rabies is widespread among arctic foxes and other animals in Canada, it was assumed that Canadian foxes traversing the icebound Davis Strait had transmitted the disease to Greenland foxes, which in turn had transferred it to the dogs. This, however, was never proved, and only recently have the combined efforts of American and Danish laboratories shown that the canine disease is true rabies. In recent years canine epizootics have been numerous. Four outbreaks between 1957 and 1960 played havoc among the dogs in northern Greenland, and nearly caused famine among the Eskimos, to whom the dogs are indispensable. All the affected dogs died—a fact suggestive of rabies—and extensive vaccination of the dogs against rabies rapidly stopped the spread. Despite the recurrent canine epizootics, not a single human case of rabies has previously been reported from Greenland, where adults, and especially children, are often attacked and severely bitten by aggressive dogs. Prof. H. C. A. Lassen, of the University of Copenhagen, now describes an unequivocal case of human rabies from Greenland (*The Lancet*, 7223; February 3, 1962). The disease ran an exclusively paralytic course.

New Zealand Barrier Island Study

The Little Barrier Island (Hauturu to the Maoris) is a nature reserve lying some 50 miles north-east of Auckland. Bulletin 137 of the New Zealand Department of Scientific and Industrial Research provides an admirable and detailed account of the Island accompanied by clear, well-drawn and relevant maps (*Little Barrier Island* (*Hauturu*). Compiled by W. M. Hamilton. Second edition. Pp. 198+4 maps. Wellington: Government Printer, 1961. 30*s*.). The story of the nature reserve is quite interesting. By 1896, the year the reserve was established, about one-third of the natural forest had been modified by burning, tree-felling or grazing and there was considerable danger that certain birds adapted to the forest habitat, already rare, might become extinct. The Island is now one of the few remaining large areas of native forest in New Zealand and one bird, the stitchbird (*Notiomystis cincta*), is believed to be unique to the area. The Bulletin provides an admirable account of the various elements of the environment—geology, physiography, soils, fauna and flora together with an introductory section on the Maori occupation. Hauturu is a dissected volcanic cone, probably Mid-Pleistocene in age, rising to a height of 2,370 ft. Andesites and andesitic breccias are the predominant rock types although there are small areas of Holocene (Flandrian) deposition. The vegetation shows a vertical zonation modified by the destruction that took place before 1896. These areas are rapidly reverting to Kauri forest (and other broad leaf communities) through transition phases of heptospermum forest. The forest areas provide the habitat for many native (and some introduced) birds. The main disturbing factor is the presence of cats although every attempt is being made to eliminate these. Their effect on the bird population is, however, considered to be slight. Some species have been introduced to the Island, one of which, the North Island Kiwi, seems to have been successful. There are also several curiosities including the tuatara and *Spenceriella gigantea*, a 4½-ft. long earthworm. The Bulletin, naturally, is mainly of local interest but it should have a wider appeal to geomorphologists and ecologists for the accounts of the dissected volcano and the vegetation pattern.

Cosmology and Meteorology in the U.S.S.R.

A NUMBER of articles dealing with the various aspects of recent cosmic flights was published during 1961 in the Soviet popular scientific journal *Priroda*. These include: 8, 7, 15, 16; 9, 9, 24, 54, 75; 10, 23, 32; 11, 9. According to V. P. Petrov and A. A. Sochivko (*Priroda*, 7, 25; 1961), future development of cosmic flights may help to map out the cosmic background responsible for the changes in terrestrial weather. Special space stations, regularly distributed around the Earth and to be called 'geophysical sputniks', will carry special equipment suitable for such a task. The recorded data will be transmitted continuously to the terrestrial receiving stations. In a short article, B. I. Vronsky (*Priroda*, 9, 90; 1961) describes the five meteorites (locality marked on a sketch map) which have recently been found in the north-east Siberian territory. They are: (1) Susuman, 1937; (2) Mal'dyak, 1939; (3) Burgavli, 1941; (4) Yudoma, 1946; (5) El'ga, 1959. The last-mentioned meteorite, El'ga, found deep down in auriferous gravels (see also *Nature*, 187, 648; 1960), consisted essentially of nickel–iron with 10–15 per dispersed stony chondrules, the main constituents of which are alkali feldspar and clinopyroxene, with small amounts of glass and maskelinite. The metallic part, the chemical analysis of which is given, is composed essentially of kamacite and tænite. On February 18, 1961, Soviet and visiting astronomers had the chance of observing a full eclipse of the Sun, and this event gave the physicists the opportunity of trying a new method of insemination of clouds by granular 'dry ice'. These experiments, carried out in the Crimea, were very successful. The details of the process of artificial cloud dispersion are described by V. V. Vyal'tsev (*Priroda*, 11, 79; 1961.

Hydrology and Water-power in the U.S.S.R.

A PROJECT of linking some of the rivers flowing into the Arctic Ocean with the River Volga is briefly outlined by G. L. Sarukhanov (*Priroda*, 7, 53; 1961) and also by N. S. Buyakovsky and M. I. L'vovich (*Priroda*, 6, 58; 1961). The main necessity is the steadily lowering level of the Caspian Sea, producing a crisis in navigation and fisheries. It is suggested that the upper reaches of the Rivers Pechora and Vychigda (a tributary of Northern Dwina) be dammed so as to transfer 40 milliards cubic feet of water annually into the Rivers Kama and Volga. This would make possible the formation of large water reservoirs and improved river navigation. Ya. A. Mazover and V. K. Saveliev give a summary account of the prospects for the wider utilization of water and fossil fuel powers in the U.S.S.R. (*Priroda*, 10, 49; 1961). The important feature in the problems of energetics in the European part of the U.S.S.R. is that consumption is above reserves, while in the Asiatic part consumption is well below reserves. Gigantic hydroelectric installations, like those of the Angara–Yenisey, will in time enormously increase the output of energy. Transport of fuel and transmission of energy from regions of potentially low consumption to regions of high consumption may solve this discrepancy. Local development of industries will also tend to restore the balance of energetics. In any event, it is anticipated that there will be a considerable flow of energy westwards from central Asia and western Siberia.

Non-Pressure Methods of applying Wood Preservatives

RECORD No. 31 of Forest Products Research, issued by the Department of Scientific and Industrial Research, includes concise descriptions of applying preservatives to timber by brushing, spraying, dipping, steeping, and the hot and cold open-tank and diffusion processes (Record No. 31. *Non-pressure Methods of applying Wood Preservatives*. By J. Bryan and D. F. Purslow. Pp. iv+14+8 plates. London: H.M.S.O., 1961. 2*s*. 6*d*. net). Immersion treatments ensure more complete contact between the wood and preservative, and thus can be more effective than other non-pressure methods. Their effectiveness depends to a certain extent on the period of immersion, but better results can be obtained by the well-known open-tank process. The plates illustrate the varying degrees of penetration obtained and two examples of an open-tank. Simple and readily understood graphs show rates of absorption for different preservatives by steeping and by double heating open-tank process. This useful booklet will be appreciated by those requiring an understanding and a practical guide to the subject.

Resinification of Knots in Radiata Pine

IN New Zealand it has been found that during the kiln drying of radiata pine, the checking of intergrown knots is much worse in those which have a low content of resin and are large. Therefore, in order to try to improve the quality of kiln-dried radiata pine so far as knots affect it, two practical possibilities are considered in *Forestry Research Notes*, No. 28, issued by the New Zealand Forest Service, namely, the increased resinification of knots and artificial pruning treatments (*The Resinification of Knots in Radiata Pine*. By J. Maddern Harris. Pp. 10. Wellington: Government Printer, 1961). Experiments dealing with the latter are being carried out, but this research note deals mainly with an examination of all the variables which might affect the results of pruning experiments and other treatments and an investigation of the resinification of knots as a process associated with the ageing of the stem. The results of the study show that the normal course of resinification can be regarded as beginning in one of two ways, depending on whether a branch dies before heartwood starts to form in the stem or heartwood formation has begun. The observations and analysis of the results are well described and discussed. They form a practical basis to take the subject a step further, that is, to relate it to time and height of pruning and, of course, to economics. This paper is a very useful contribution to the questions of when, how and to what extent resinification of a knot takes place.

Enrico Fermi School of Physics: Summer Courses

The International School of Physics "Enrico Fermi" is organizing five summer courses in physics at the Villa Monastero, Varenna sul Lago di Como. The subjects of the courses will be: (1) space exploration and the solar system, June 4–16; (2) advanced plasma theory, July 9–21; (3) elementary particles, July 23–August 4; (4) dispersion and absorption of sound by molecular processes, August 6–18; (5) star evolution, August 20–September 1. Further information can be obtained from Prof. S. Olbert, Massachusetts Institute of Technology, Cambridge 39, Massachusetts (course 1); Prof. M. Rosenbluth, General Atomic Division, General Dynamics Corporation, P.O. Box 608, San Diego 12, California (course 2); Prof. M. Conversi, N.P. Division, CERN, Geneva 23 (course 3); Prof. D. Sette, Cattedra di Fisica, Facoltà di Ingegneria, Via Eudossiana 18, Rome (course 4); Prof. L. Gratton, Laboratori Nazionali di Frascati, Casella Postale N. 65, Frascati, Rome (course 5).

University News: London

The title of professor of experimental physics has been conferred on Dr. W. Ehrenberg and that of professor of electrochemistry on Dr. D. J. G. Ives, in respect of their posts at Birkbeck College.

Dr. A. Cameron has been appointed to a readership in mechanical engineering, tenable at the Imperial College of Science and Technology, and Dr. K. Fotherby has been appointed to a readership in chemical pathology, tenable at the Postgraduate Medical School. The title of professor has been conferred on Dr. W. Bowen Bonnor (mathematics) in respect of the post held by him at Queen Elizabeth College. The titles of reader have been conferred on: Dr. P. G. Walker (chemical pathology) in respect of the post held by him at the Institute of Orthopædics; Dr. A. F. Cobbold (experimental physiology) in respect of his post at St. Thomas's Hospital Medical School; Dr. A. E. Kellie (biochemistry) in respect of his post at the Middlesex Hospital Medical School. Dr. J. F. Fowler has been appointed reader in physics at St. Bartholomew's Hospital Medical College.

Announcements

Among the newly appointed life peers announced on March 29 is Sir Alexander Todd, professor of organic chemistry in the University of Cambridge, and chairman of the Advisory Council on Scientific Policy.

Dr. Jean-Pierre Barrat, of the University of Caen, has been awarded the Adolph Lomb Medal of the Optical Society of America. The Medal is awarded to a person under thirty years of age who has made "a noteworthy contribution to optics".

The Zoological Society of London has awarded the Edward Forbes Medal for scientific merit to Dr. Arthur James Cain, of the Department of Zoology, University of Oxford, and to Dr. Robert Aubrey Hinde, of the Department of Zoology, University of Cambridge. The Medals—awarded to persons under forty years of age for distinguished work in zoology—will be presented by H.R.H. the Duke of Edinburgh, president of the Zoological Society of London, immediately following the annual general meeting of the Society, which will be held on April 27.

The office of the International Society for Tropical Ecology has moved from Allahabad to Varanasi (India). All communications should be addressed to Prof. R. Misra, Banares Hindu University, Varanasi, India.

A meeting of the British Society of Soil Science will be held at the London School of Economics, Houghton Street, W.C.2, during April 16–18. There will be three sessions: (*a*) productivity and soil type; (*b*) microbiology of podzols; (*c*) micro-organisms in relation to nutrient availability in soil. Further information can be obtained from Dr. D. V. Crawford, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough.

The National Engineering Laboratory, East Kilbride, is holding open days during May 2–4. Developments on show in the field of mechanical engineering will include: the application of air-lubricated bearings to machine tools; research on the production of new materials by the application of very high pressures; facilities for research on creep of metals; new automatic measuring techniques; further data on the cold extrusion of steel; advances in pump and fan design. Further information can be obtained from the Director, National Engineering Laboratory, East Kilbride, Glasgow.

A symposium on "The Protection of Structural Steel" will be held in the University of Birmingham on April 18. The programme will include: the hot-dip galvanizing of structural steel; the properties of hot-galvanized steel; galvanized structures for power transmission; the use of hot-galvanized steel in building; the design and protection of tubular steel structures; the application and uses of zinc spraying; economic aspects of protection. Further information can be obtained from the Zinc Development Association, 34 Berkeley Square, London, W.1.

Errata. In the article entitled "Fatty Acids in Recent and Ancient Sediments and Petroleum Reservoir Waters" (*Nature*, 193, 744; 1962), by J. E. Cooper, the fifth sentence in the second complete paragraph on p. 746 should read: "Intermediates of this type derived from even-numbered acids could react to give a mixture of odd-numbered acids and paraffins as a product".

In Table 1 of the communication entitled "Inhibition of Dopamine β Oxidase by Adrenalone", by M. Goldstein and J. F. Contrera (*Nature*, 192, 1081; 1961), cols. 1, 2 and 3 should be headed Dopamine, Adrenalone and Epinine, respectively; the last insertion in col. 2 should read 1·2, and the second in col. 3 should read 3·2.

SCIENCE IN PARLIAMENT

On February 27 the Parliamentary Secretary for Science, Mr. D. Freeth, answered a series of 22 questions in the House of Commons dealing with a wide range of scientific matters.

Concerning the 15 research stations and 52 research associations (47 of which have research stations) to which the Department of Scientific and Industrial Research made grants, Mr. Freeth stated that while the siting of co-operative industrial research associations and laboratories was primarily a matter for the industrial members of the associations he would look into the matter of location.

As regards research into the Turner system for constant-flow traffic signals, it was expected that a pilot scheme for this or a similar system would be tested later in the year on the Road Research Laboratory's research tract at Crowthorne.

The distillation of sea-water was an established industrial process, and British-made equipment was among the best in the world. Compared with normal sources of supply in Western Europe, however, distilled water was expensive, and any extensive use for industrial purposes in Britain was unlikely, except in very special circumstances where it would be economic. A considerable amount of research was being undertaken by industrial companies.

To questions regarding the establishment of a social sciences research council, Mr. Freeth said that the Minister for Science was consulting the Research Councils and Government Departments and the University Grants Committee on the views of the Council for Scientific and Industrial Research. Contributions from the latter in one sector of this field had nearly doubled during the past two years, and he did not agree that the establishment of a further research council was the only way to secure a substantial increase in research in the social sciences.

The Minister for Science had asked the Department of Scientific and Industrial Research for its views on the proposals in the Toothill report on research and development contracts for Scottish industry.

About 15 scientists and engineers from the Atomic Energy Authority will participate in the British experimental nuclear test in Nevada, but no decision had yet been taken about holding tests at Christmas Island.

As to the use of operational research and social survey methods in administration, Mr. Freeth said that the Industrial Operations Unit of the Department of Scientific and Industrial Research had recently co-operated with the Ministry of Health, the Royal Mint and the War Department. Social survey methods were mainly used by the Social Survey Division of the Central Office of Information.

As regards the exhaust from Diesel-powered vehicles, Mr. Freeth said that the Warren Spring Laboratory of the Department of Scientific and Industrial Research had not found any suitable means of recovering smoke from such exhausts, but would examine any device for this purpose that came to its notice. Mr. Freeth repeated an earlier statement that the real remedy lay in correct operation and maintenance of the engines. Concerning the future programme of the Economics Section in the headquarters office of the Department of Scientific and Industrial Research, Mr. Freeth stated that this was currently under discussion; 374 students graduated in Scotland with honours degrees in science and 105 with honours degrees in technology in 1959–60.

Research was proceeding at the National Physical Laboratory on noise in industry from motor-vehicles and from aircraft, including jet aircraft; at the Building Research Station, on noise in buildings; and at the National Engineering Laboratory, on noise from machines. Extensive experiments had been made on a subjective assessment of motor-vehicle and aircraft noise, and surveys were in progress on noise in communities and in industry. The report of the Wilson Committee on the problem of noise was expected before the end of the year.

In a written answer on February 27, Mr. Freeth stated that research on the structure, function and metabolism of normal and diseased muscle, which might assist in elucidating the cause of, and the most effective methods of treating, muscular dystrophy, was supported from public funds by the Medical Research Council and by university and hospital departments.

The British Commonwealth Scientific Committee met in the United Kingdom in 1960 and would meet in India during November–December, 1962.

British Satellite Research

Replying to a question in the House of Commons on February 26, the Minister of Aviation, Mr. P. Thorneycroft, said that the cost of continuing the development of the *Blue Streak* missile as the first stage of a satellite launcher to the end of January 1962 was approximately £6·1 million, and the total cost of the proposed three-stage launcher had been estimated at about £70 million, of which the United Kingdom would pay £26 million. The objects of the project were to develop a heavy launcher capable of putting satellites into orbit for scientific and commercial purposes to disseminate associated technical knowledge, and by pooling scientific and technical resources to permit Europe and Australia to play a full part in the peaceful exploitation of space. On February 27 the Parliamentary Secretary for Science said the space research programmes, for which the Minister for Science was responsible, employed 17 qualified staff early in 1960, 27 in January 1961 and 25 in January 1962, while 20, 32 and 40, respectively, suitably qualified staff in Government departments were engaged in similar experiments or supporting work, or the observation and tracking of satellites.

Expanding the Field of Education

In a written answer in the House of Commons on February 27, the Minister of Education, Sir David Eccles, announced that from April 1, 1962, all the colleges of advanced technology would be administered by independent governing bodies and would receive direct grants from the Ministry. The colleges now had nearly 9,000 full-time students and had arrangements in hand to increase this number to 15,000 by the mid-1960's. He was now authorizing the colleges to plan for an expansion to 21,000 places, the date depending on decisions still to be taken regarding the rate of public service capital investment after 1963–64. Capital expenditure on educational building in 1963–64 would rise to £130 million as against £125 million in 1962–63, including starts of £55 million for major school-building projects, with a special priority for the provision of science accommodation at secondary schools, amounting to £7·5 million during 1963–64. The expansion of teacher training colleges by 24,000 places would proceed as planned, and the further education programme, calling for £17 million in 1963–64, also remained unchanged. The Youth Service was authorized to start £7 million of work in the first three years after the Albemarle Report and the programme for 1963–64 would be £3 million. On March 1, Sir David said that he understood that conversations were about to commence between the principals of the colleges of advanced technology and the Central Council for University Admissions. Of 55,000 full-time overseas students in the United Kingdom, 12,000 were in universities, 13,000 in technical colleges and 1,400 in teacher training institutes.

Education of Commonwealth Scholars in Britain

In reply to a question in the House of Commons on February 15, the Joint Under-Secretary of State for Commonwealth Relations, Mr. B. Braine, said that the Government was considering various means by which the Commonwealth Scholarship Commission could extend a substantial number of scholarships for a third year without reducing much below the present level the number of new scholarships to be offered annually. Last year, Britain spent £122,000 on the scholarships plan, and it was estimated that this year £330,000 would be spent, and next year £490,000; the Government was unable to increase its total financial contribution to Commonwealth educational co-operation at present. Asked as to progress with the scheme for increased residential accommodation for overseas students, Mr. Braine said that, up to February 12, twenty-six projects, involving 1,113 additional beds, had been approved in principle; but in spite of this response from voluntary organizations, the need for new hostels was still very great, and the British Council would be pleased to advise and help other organizations which contemplated providing them. The approved scheme, designed to supplement the efforts of voluntary bodies, provided for 557 beds in London and 556 beds outside London. A total of 153,000 copies of university text-books and 155,000 copies of other books had been printed to date for distribution through the publishers to Commonwealth and other countries in Asia, and a further 241,500 text-books and 469,500 other books were in production.

Higher Education in Colleges of Technology in Britain

In a written reply in the House of Commons on February 12, the Minister of Education, Sir David Eccles, gave the number of students enrolled in January 1962 in the 108 courses leading to the Diploma in Technology as 6,201; of these, 1,624 were in electrical and 1,441 in mechanical and production engineering; 798 in chemical technology; 558 in physics; 332 in aeronautical engineering; 304 in civil engineering; 268 in mathematics; and 224 in chemical engineering. Of the 2,360 first-year students, 1,729 qualified for entry by the General Certificate of Education and 561 by the Ordinary National Certificate. On February 14, Sir David gave the number of full-time teachers in technical colleges in 1960–61 as 19,847, with 52,435 part-time teachers; these figures compare with targets of 18,615 and 46,939 in the Willis Jackson Report. As regards action on the White Paper on technical education of January 1961, Sir David stated that the whole pattern of part-time courses in engineering had already been approved and a new general engineering course and an important new engineering technician course started last autumn. Agreement had been reached on the common Ordinary National Certificate and Diploma courses in engineering which would form a basis for later specialization, for example, in mechanical, electrical, chemical or aeronautical engineering, and discussions were proceeding about possible new courses in mining, textiles and applied science.

Road Safety in Britain

In moving the second reading of the Road Traffic Bill on February 28, the Minister of Transport, Mr. E. Marples, said that the Bill made a small contribution to road safety. During 1960, 6,970 people were killed and 347,551 injured on the roads and the cost to the nation was estimated at £229 million. Mr. Marples referred to an experiment to be tried at three places in London with a light signal of a new design and also to measures in education, especially with adults, which were being discussed with the Road Research Laboratory.

As regards provisions for enforcement, the first part of the Bill dealt with driving offences and with drink, and was intended to reduce the number of accidents which could be attributed to alcohol.

In replying on the debate the Parliamentary Secretary to the Ministry, Mr. John Hay, said it was proposed to tackle the problem of the education of adults in road safety by appointing a special research team, consisting of technical experts, and including some with experience in psychology and related matters. The team would study first the human factors involved in road-user behaviour and accident situations and, secondly, explore the means of influencing this behaviour most effectively. Its task would be to ascertain what causes accidents and to examine the matter from a personal point of view. The details of these proposals were being explored by the Ministry and the Road Research Laboratory.

It was proposed to set up two or three experimental road-safety teams in selected areas to take an overall view of road accidents and propose combinations of road-safety measures in their areas. The teams would familiarize themselves with the accident pattern of their areas and make specific proposals to the authorities concerned. They would not be executive bodies.

Mr. Hay reiterated that the overall objective of the Bill was to try to save lives and to prevent accidents, and he was emphatic that in dealing with dangerous or negligent driving the fact that disqualification for a professional driver involved deprivation of livelihood should not be allowed to affect the penalty imposed.

Control of Toxic Chemicals

In a written answer in the House of Commons on March 8, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that the Research Councils were concerned in general with those recommendations of the Research Study Group on toxic chemicals in agriculture which related to fundamental research: those relating to surveys and inquiries would mostly be the concern of the Agricultural Departments. The Research Councils proposed to continue their already substantial research in the fields mentioned in the report and to extend it, so far as resources permitted, where fruitful results appeared likely. The various tasks were shared between them in accordance with their normal interests. Research into problems bearing on the toxicity of agricultural chemicals to human beings fell to the Medical Research Council while the Agricultural Research Council was responsible for basic research on chemical hazards concerning farm crops, animals and soil, and the Nature Conservancy was responsible for research relating to the effects of agricultural chemicals on wild life and the natural environment. Research into improved methods of determining residues of toxic chemicals in foodstuffs and animal tissues was the responsibility of the Government Chemist in the Department of Scientific and Industrial Research. Certain problems were of concern to two or more research councils, and the Agricultural Research Council was setting up a scientific committee with Prof. A. C. Frazer as chairman to keep all relevant research under review and report progress.

Civil Science by Government Agencies

In answer to a question in the House of Lords on March 7, the Minister for Science, Lord Hailsham, said that the Government had decided to review the existing organization for the promotion of civil science by Government agencies. This review, which would be concerned mainly with issues of administrative organization and the machinery of Government, would take full account of the views expressed by the Advisory Council on Scientific Policy, and advice would also be sought from scientists both inside and outside the public service. Lord Hailsham undertook to inquire into the consultation of representatives of industry also.

THE COLOMBO PLAN

TENTH ANNUAL REPORT

THE tenth annual report of the Consultative Committee on the Colombo Plan for Co-operative Economic Development in South and South-East Asia* includes a short chapter on technical co-operation, which has been an integral part of Colombo Plan operations since 1950 and provides a framework for exchanging technical assistance between participating countries. Excluding some 6,840 who went to the United States in 1950–58 before the United States became a formal participant, technical co-operation under the Plan enabled 19,533 men and women of the region to visit other countries to acquire specialized knowledge, aptitudes and experience enabling them to increase their contribution to the development of their own countries and has filled gaps in the technical knowledge in the region by providing experts whose influence extends far beyond the technological field. Despite the achievements of the past ten years, however, the importance of technical co-operation in overcoming the shortage of trained manpower has in no way diminished. The needs of countries in the area for trained men and women remain very great and frequently the progress of development has brought new requirements for the expansion of technical skill.

Total expenditure on technical assistance by all members of the Plan in 1960–61 exceeded £20 million, that on the provision of training places substantially increasing while that on equipment almost doubled, to reach £5·6 million. New training awards increased from 4,268 to 4,417, and the number of new experts provided from 535 to 786. Training capacity is now much greater than it was in 1950, particularly at the higher professional levels, and thirteen of the twenty-one member countries are now among those who in the past ten years have provided 1,852 training places within the area. A survey during the year revealed a serious gap in trained manpower between professional technologists and skilled craftsmen but also indicated that facilities for many specialized types of training at the latter level are not fully utilized, and the need for constant endeavour to find improved methods and procedures of rendering technical assistance.

Even in this chapter of the report reference is made to the urgency which the growth of population in the area over the past decade has brought to the area, and this is one of the dominant notes of the review of economic progress during the past ten years and of the task ahead which are probably the two chapters of most outstanding interest in the report. Since the Colombo Plan commenced, the population of South and South-East Asia has increased by 150 million, and by 1980 it is expected to approach 1,000 million as against the 720 million expected for 1970 in 1950—a figure which was reached in 1960. The central fact of this situation is a sharp rise in the rate of natural increase, partly due to the very success of the Colombo Plan in such fields as health and medicine. The difficulties faced by these countries exceed any experienced in this field by the industrially advanced countries, and against this background the growing pressures of the peoples of the region for higher living standards and the extent of the progress which can be achieved can affect political stability.

It is not surprising, therefore, that the report singles as the dominant achievement of the past ten years under the Colombo Plan the awareness growing throughout the area and among the countries outside of the urgent need for more rapid development and for efforts to be directed towards that end. National income has indeed grown almost continuously, particularly since 1953, and most countries have recorded substantial gains in overall output as well as in important sectors, but frequently the rapid increases in population diminished the measurement of gains on a *per capita* basis. Invariably, the area-wide attack on poverty and low living standards through economic development has been pressed, and the forward movement of the economics of the area is clearly evident.

Agricultural production—the mainstay of the area's economy—has significantly increased both in terms of total production and in terms of yield per unit of cultivated land, and the index of agricultural output increased by about 12·5 per cent, compared with an average world increase during the same period of about 15·5 per cent. The production of rice, the staple food of the area's population, increased by about 40 per cent to more than 108 million tons in 1960, and that of maize by more than 30 per cent. Output of petroleum increased from 10·8 million tons to 26·5 million tons, of coal from 35 million tons to more than 54 million tons; steel production from about 1·3 million tons to more than 3 million tons; and of cement from less than 6 million tons to 10·8 million tons, while diversification is gradually being achieved both in agriculture and industry. Illiteracy is being reduced, and most countries aim at establishing universal primary education in the foreseeable future, while secondary and higher education are being rapidly promoted. Malaria is being eradicated, and health programmes have helped to mitigate the ravages of influenza, tuberculosis and other epidemics

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* The Colombo Plan for Co-operative Economic Development in South and South-East Asia. Tenth Annual Report of the Consultative Committee, Kuala Lumpur, October–November 1961. (Cmnd. 1600.) Pp. 238. (London: H.M.S.O., 1962.) 13s. net.

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Looking at the task ahead, particularly in the light of the increase in population during the decade 1950–60 from 595 million to 743 million, or 1·5 times that of the increase during 1920–50, the report points out that while the objectives set out in 1950 have been shown to be correct, the task has proved even more formidable than was expected. The peoples of the region are aware that progress will continue to depend above all on their own efforts and determination and that external assistance, however massive, cannot replace those efforts. Apart, however, from this continuing need for popular understanding and support, the Governments of the region will continue to be faced with the need for resolute action to raise the standards of living, and in this priorities present special difficulty. The precise balance between investment in agriculture and in industry, of priorities within the agricultural and industrial sectors, between investment and consumption, between a lower level of education and training for the many and a higher level for the comparatively few will never be easy to determine. Beyond this there remains a challenge to the more developed countries within the limits of their resources to help the less-developed countries to help themselves. In doing so they will make the greatest possible contribution to the development and welfare of the region, but while external aid will remain an important factor in determining the degree of economic progress, the more-advanced countries must continue to adapt their methods and approach to changing circumstances. There are no simple solutions to the many problems that arise and progress can only be made as difficulties are tackled in a continued spirit of goodwill and co-operation and on the basis of growing appreciation and understanding of each other's difficulties. The second, and largest, part of the report outlines the progress made in the individual member countries of the Plan in 1960 and during the decade 1950–60, while in Part 3 are outlined some contributions to economic development in South and South-East Asia made by individual countries. United Kingdom Government expenditure on capital and technical assistance for development in South and South-East Asia during 1960–61 totalled £35·9 million, bringing the total of such expenditure under the Plan to £182·78 million, with outstanding commitments at June 30, 1961, of £84·13 million (including the whole of the £20·8 million contribution to the Indus Basin Development Fund) to be paid over the period 1960–72. Expenditure of £1·14 million on technical assistance brought the total since 1951 to £7·06 million, and the 565 students in the United Kingdom undergoing training on June 30, 1961, brought the total of Asian students who have received instruction in the United Kingdom under the Plan to 3,892, and total expenditure for this purpose to £3·12 million. The 42 experts provided during the year brought the total to 427, and the £235,988 provided for equipment for training and research purposes brought the total under this head to £2·01 million. Capital aid expenditure during the year amounted to £34·76 million, bringing the grand total under the Plan to £175·72 million, with outstanding commitments at June 30, 1961, of £82·55 million.

PLANT TISSUE AND ORGAN CULTURE

A SYMPOSIUM on the above subject was organized under the joint auspices of the University of Delhi and of the South Asia Science Co-operation Office of Unesco, which is also at Delhi. These arrangements were made by Prof. P. Maheshwari on behalf of the University of Delhi and by Dr. J. Swarbrick on behalf of Unesco. The 38 active participants in the symposium are greatly indebted to its organizers for the opportunity so presented to discuss various aspects of this interesting and rapidly developing field of work, which was covered in 36 different papers presented to the symposium. These papers will be made more widely available in a symposium volume which is to be printed as a separate issue of the journal *Phytomorphology*.

Seven foreign delegates were able to participate in the conference under the arrangements made by Unesco. These delegates were: Dr. J. P. Nitsch (from Laboratoire de Phytotron, Gif-sur-Yvette), Prof. J. Reinert (Pflanzenphysiologisches Institut der Freien Universität, Berlin-Dahlem); Prof. H. E. Street (Department of Botany, University College of Swansea), Prof. F. C. Steward (Cornell University, Ithaca, New York), Dr. A. N. Rao (University of Malaya), Prof. B. L. T. de Silva (University of Colombo), Dr. Tun (University of Mandalay). The remainder of the delegates to the symposium were from different parts of India.

Four of the foreign delegates (Dr. Nitsch, Profs. Reinert, Steward and Street) delivered informative general lectures under the following titles: "The *in vitro* Culture of Flowers and Fruits" (Dr. J. P. Nitsch); "The Experimental Modification of Growth and Differentiation in Plant Tissue Cultures" (Prof. J. Reinert); "Carrots and Coconuts: Some Investigations on Growth" (Prof. F. C. Steward); "Nutritional Problems Raised by Work with Root Cultures" and "Studies on the Hormonal Control of Root Growth" (Prof. H. E. Street).

In addition, these delegates delivered other papers on more specialized subjects such as: carbohydrate nutrition of excised tomato roots (Prof. H. E. Street); naturally occurring growth substances in relation to the growth of tissue cultures (Dr. J. P. Nitsch); and totipotency and variation in cultured plant cells (Prof. F. C. Steward). In this way these four foreign delegates presented to the symposium a broad picture of work in their respective fields and laboratories. This was further supplemented by two films in sound and colour, which had previously been recorded by Prof. F. C. Steward, and were shown under titles of "The Requirements for Growth in Plants" and "Growth and Development of Plants".

The remaining papers fell into four main groups: (1) nine papers dealt with the culture of reproductive organs such as the nucellus (as in citrus), embryos, ovules, ovaries, flowers or pieces of inflorescence; (2) four papers on the culture of cryptogams, particularly fern gametophytes, the thalli of *Marchantia*, and on the culture of a moss (*Physcomitrium coorgense*); (3) four papers on the culture, mostly the technique of culture, of grown gall tumour tissue from the hollyhock (*Althaea rosea*); (4) four papers dealing with aspects of morphogenesis in tissue cultures of horseradish (*Armoracia lapathifolia*), of carrot root (*Daucus carota*), in orchid seedlings, and in *Bryophyllum*.

The conference drew heavily on the wealth of experimental morphological material which was available in the Department of Botany at Delhi, through the work of students working largely under the direction of Prof. P. Maheshwari and Dr. B. M. Johri as well as of other members of the Delhi staff. This Department, already well known for its contributions to plant morphology, has turned increasingly in recent years to an experimental approach to morphological problems. The visitors to the conference were impressed with the range of interesting plant material under investigation by these means, especially the examples of the culture of nucellus, ovules, ovaries, etc., and of other parts of the reproductive system. It is a healthy sign for plant morphology, and one from which physiology will also profit, that those already well trained in morphology are now bringing their skills to bear on the culture of selected and interesting plant parts. The published volume that will recount these various investigations may thus be anticipated as one which will significantly advance knowledge in this field. The hope also is that the conference will have stimulated interest in this subject in South-East Asia, even as it furnished the foreign delegates with a valued opportunity to see what is going on in India.

In fact, at the end of the Delhi symposium, Dr. Nitsch and Profs. Reinert, Steward, and Street went to the Indian Science Congress, held at Cuttack, where they were again received as foreign delegates and guests of the Congress. Dr. Nitsch and Profs. Street and Steward delivered special lectures to the Indian Science Congress on topics similar to those presented to the Delhi symposium; Prof. Steward also addressed the annual meeting of the Indian Society of Biological Chemists. on the "Soluble Nitrogen Compounds of Plants", and Profs. Street and Steward participated with the Indian Society of Plant Physiologists in a meeting specially devoted to honouring Prof. F. G. Gregory, whose recent death was especially regretted in India where there are so many plant physiologists who were trained by him.

The foreign delegates especially are deeply grateful to the University of Delhi, to Unesco, and to the organizers of the Indian Science Congress for the opportunity to present an account of their own work and also to understand more about the range of experimental botanical work in progress in the various centres which they were able to visit. This left an impression of experimental plant morphology as a thriving branch of experimental science, which poses unique problems of growth and development for botanists to solve. In doing this work, classically trained morphologists and biochemically trained physiologists can now usefully combine forces and so benefit their respective disciplines.

F. C. Steward

TEXTILE RESEARCH AND COLOUR CHEMISTRY AT LEEDS

THE eighty-fifth and eighty-sixth reports of the Advisory Committee on Textile Industries and Colour Chemistry and Dyeing in the University of Leeds to the Worshipful Company of Clothworkers, covering the sessions 1958–59 and 1959–60, record considerable progress*. In the Department of Textile Industries the number of students remained about 400, but in 1958–59 extra-mural activities were an outstanding feature. In textile physics considerable progress was made in electron microscopic studies of various fibres. The uranyl group has been found to give selective staining of the endo-cuticle, and it has also been shown that two intercellular layers of the cell-membrane complex are attacked by keratinolytic reagents and enzymes. The structural changes brought out by variations in the conditions under which filaments of polyacrylonitrile are spun have also been studied.

In textile chemistry, cross-linking and polymerization reactions continued to receive much attention. The physical properties of wool fibres (elastic modulus, swelling ratio) have been correlated with theoretical predictions. It has also been shown that an oxidized and alkali-treated wool fibre shows reversible spontaneous contraction and extension as the *p*H of an ambient aqueous solution is altered, the thermodynamic and X-ray diffraction results corresponding closely to those observed with muscle preparations. New methods of forming anchored and unanchored polymers on and in wool fibres have been evolved, and promising results have been obtained with insoluble organic pigments in the low-temperature, solvent-assisted method of dyeing. The constitution of suint has been established as essentially a polymerized dihydroxyphenol carrying a long-chain acid which can form peptide links with amino-compounds. The heat, free energy and entropy of sorption of methacrylic acid from aqueous solution by nylon have been determined, and the polymerization of methacrylic acid in nylon and the properties of nylon fabrics containing this polymer are being examined. The thermodynamic functions of β-keratose fibres are being examined and also the setting properties of wool, and the plasticity of wool has been related inversely to the sulphur content of the β-keratose obtained from the oxidized fibres. The principles underlying the use of tetrakis (hydroxymethyl) phosphonium chloride in flame-proofing wool have been examined, and it has been found that presence of this substance enhances the ability of fibres to take a set in aqueous media.

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* Reports to the Worshipful Company of Clothworkers of the City of London of the Advisory Committee on the Departments of Textile Industries and Colour Chemistry and Dyeing in the University of Leeds. Session 1958–59: Pp. 50; Session 1959–60. Pp. 52. (Leeds: The University.)

In the Department of Colour Chemistry and Dyeing there were 48 undergraduate students in the eightieth (1958–59) and 45 in the eighty-first (1959–60) session, with 17 and 23, respectively, working for higher degrees. A study of the influence of the acetyl value of acetate rayon on the rate of dyeing and affinity of disperse dyes was completed. Work continued on the kinetic and thermodynamic aspects of the dyeing of acetate rayon with sulphated dyes and on the influence of the chemical constitution of non-ionic azo dyes on their affinity for cellulose. Other work has been concerned with the effect of benzyl alcohol and *n*-butyl alcohol on the absorption by wool of dyes containing metal and the chemistry of ketones related to *meso*benzanthrone, particularly their behaviour towards substituting agents and their ability to undergo self-union in alkaline media with formation of vat dyes. An investigation of the status of sulphinic acids as substituting agents yielded some interesting and potentially useful sulphones and some new reactions, and provided an explanation of the course of several applications of sulphinic acids in organic chemistry. Work was completed on derivatives of 2-methylanthraquinone and 2-alkylquinizarins and the influence of the chemical constitution of non-ionic azo dyes on their affinity for cellulose. Lists of publications during the year are included in both reports.

THE BRITISH LEATHER MANUFACTURERS' RESEARCH ASSOCIATION

The annual report of the British Leather Manufacturers' Research Association for the year October 1960–September 1961*, includes the reports of the Council and of the Director of Research, the latter including a list of publications, together with details of membership of the Council, its committees and of staff. Renewed attention was given to the problem of finding new outlets for leather and emphasis was placed in a report to the Council on the overwhelming importance of using the raw hides and skins to the best advantage.

The Director's report outlines the programme of research for 1961–62 and notes some features of the current year's work. In background research, work on the composition of skin proteins centred on the separation of collagenous and non-collagenous protein in acetic acid extracts of calf-skin. Investigations of the mechanical and elastic properties of collagen and leather have been extended to combination-tanned fibres, both semichrome and chrome re-tan. In studying the deterioration of leather by heat, moisture and perspiration it has been shown that exposure to dry heat or to high humidities at normal temperatures causes little deterioration and that it is the combined action of heat and moisture which is deleterious, involving breakdown of polypeptide chains both by hydrolysis and an oxidative process involving loss of amino-acids.

Experiments in which sheep-skins were deliberately allowed to stale before using have shown that while staling reduced the quality of the skins, as judged by thinness, pits and holes and also the strength of the leather, it did not appear to lower rot-resistance. Examination of the effect of adding antiseptics to the salt used for curing sheep-skins indicated that processes within the control of the fellmonger or tanner have at least as big an effect. Progress in reducing the cost of unhairing by enzymes is reported through the demonstration that the enzyme liquor can be re-used several times. It has also been found that salt concentrations of 10 per cent or more reduce enzyme activity in de-woolling sheep-skins and a 2 per cent solution of enzyme is just as effective as a 20 per cent solution.

Other work has been concerned with control of the thickness of vegetable-tanned sole leather, vegetable tan sludges, laminated leathers and solvent-assisted dyeing, in which a technique was developed for topping suèdes by spraying by emulsifying in water a solution of the pigment dyestuff in benzyl alcohol. More recently, anionic dyestuffs have been dissolved in a single-phase solvent system of water, isopropyl-alcohol and benzyl alcohol and sprayed direct on to the leather. Work has been started on new impregnating finishes for upper leather and considerable progress made in waterproofing chrome upper leather with silicones. The outstanding problem remaining is to improve the retention of silicones by the leather over long periods of use.

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* British Leather Manufacturers' Research Association. Annual Report, 1960–61. Pp. 58. (Egham: Surrey: British Leather Manufacturers' Research Association, 1961.)

NEW DIRECTIONS IN PETROLEUM EXPLORATION, PRODUCTION AND TRANSPORTATION

Writing in the November–December 1961 issue of the *Battelle Technical Review* published by the Battelle Memorial Institute, Columbus, Ohio, Dr. Frank C. Croxton propounds some novel, if not revolutionary, ideas for explorations of oil-pools, techniques for crude-oil production and transportation.

In the field of exploration location of future oil deposits envisages extensive use of gas surveys, microbiological indicators, radio-geochemical methods and advanced electronic instruments. The aim is development and use of geophysical methods of greater sensitivity and portability, thus utilizing more direct methods of oil-finding, especially in "vast new areas which are conceivably as rich as the rest of the world in oil reserves". In the realm of production, conventional drilling operations may well be superseded by processes designed to eliminate altogether the rig as we know it to-day; in this direction the author relies on the rapidly advancing knowledge of more efficient application of energy, for example, effects of high temperature, explosives, sound and supersonics. "Production research of the future will, therefore, give much attention to faster and more economical ways of making, in rock, holes a few inches in diameter, many thousands of feet long, and aimed with precision." This ideal implies removal of torsional and tensile stresses from the drill-string, replacing drill-heads by electrical currents, use of plasma techniques, and even underground nuclear explosions.

Thermal drive, that is, heating the underground crude-oil deposit, is forecast as a secondary recovery procedure, since at elevated temperatures viscosity would be reduced and thereby flow to wells accelerated. As a corollary to this the possibility of attaining cracking temperatures, "*in situ* vis-breaking operation of the crude", is seriously considered.

Two noteworthy advances in petroleum transportation are the flexible oil barge and the flexible pipeline. The former, made of nylon-reinforced rubber, is towed practically submerged to its destination, thereafter collapsed into a comparatively small package and shipped back to point of origin. Flexible pipe-line, of proved value in submarine transportation of oil during the Second World War and since, is destined to be of far greater importance in this connexion in the future. "It has not been given to previous generations to see an area of technology born, develop to maturity, and then experience a substantial evolution, all during a human lifetime." Such has indeed happened in the petroleum industry, and because of this Dr. Croxton's speculations in this paper cannot be dismissed lightly.

SYNTHESIS OF PROTEIN AND RIBOSOMES BY BACTERIA

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THE antibiotic chloromycetin (chloramphenicol) inhibits protein synthesis in bacteria but allows synthesis of ribonucleic acid (RNA) to continue[1]. Although there is evidence for a type of RNA termed 'messenger'[2,3] which is rapidly synthesized and degraded in *Escherichia coli*, most bacterial RNA exists as ribosomes which are particles containing protein and stable RNA. This 'normal RNA' differs from that accumulated during chloromycetin inhibition: the latter may be rapidly degraded by the cells during a phase of recovery from the antibiotic and some of the bases or nucleosides may pass from the cells into the surrounding medium from whence they are reabsorbed when growth resumes[4].

This contrast in stability has given rise to the view that 'chloromycetin RNA' is abnormal or non-functional despite the fact that its base composition is the same as that of normal RNA (ref. 5). It may be noted, however, that the kinetics of degradation are not those to be expected for a detoxication process by which useless or harmful material is ejected: that is, degradation does not begin some time after the new material has accumulated; instead, synthesis and degradation proceed simultaneously with the rate of the former initially greater, so that 'chloromycetin RNA' is observed in radiotracer experiments to be 'turning over' during the whole period of its accumulation[5]. There is, in fact, no direct experimental evidence that this RNA causes protein synthesis to cease; on the other hand, it has been shown that chloromycetin itself inhibits biochemical reactions preceding peptide-bond synthesis, namely, those concerned with transfer of amino-acids from 'soluble RNA' to ribosomes[6].

Our results, and those of other workers, are readily interpreted on the view that: (1) most of the RNA synthesized by bacteria is labile unless it combines with protein; (2) 'chloromycetin RNA' is exceptional only in so far as it occurs in particles with a lower ratio of protein : RNA than that normally found, one of the effects of the antibiotic being to favour their stabilization. Such particles may normally exist as precursors of ribosomes.

When the cell synthesizes protein at the normal rate only a few of them may exist at any given time since they would be removed by the completion of stable ribosome structures; and when protein synthesis is suppressed by chloromycetin they would also be rapidly degraded by enzymes unless combination with the antibiotic, in addition to combination with protein, could reduce the rate of breakdown. Horowitz, Lombard and Chargaff[5] found that the RNA accumulating early in chloromycetin treatment was more stable than that accumulating later: thus, a factor opposing breakdown, probably protein, appears to have been less readily available the longer protein synthesis was inhibited. In agreement with this view it has been shown that 'chloromycetin RNA' occurs in particles which have a lower ratio of protein : RNA than 50*S* ribosomes and give rise to additional peaks in schlieren diagrams at 14–18*S* [7–9]. We find that extracts of puromycin-treated cells also show additional peaks sedimenting at this speed on examination in the ultracentrifuge. Now puromycin appears to inhibit the same steps in protein synthesis as chloromycetin[10], but in contrast the RNA which accumulates is stable[11]. Accordingly, the argument that 'chloromycetin RNA' is abnormal because it is labile is greatly weakened: the ribonucleoprotein (RNP) particles which accumulate during inhibition by puromycin are similar physically to those from chloromycetin treatment, but they contain stable RNA. It is simpler to suggest that puromycin stabil-

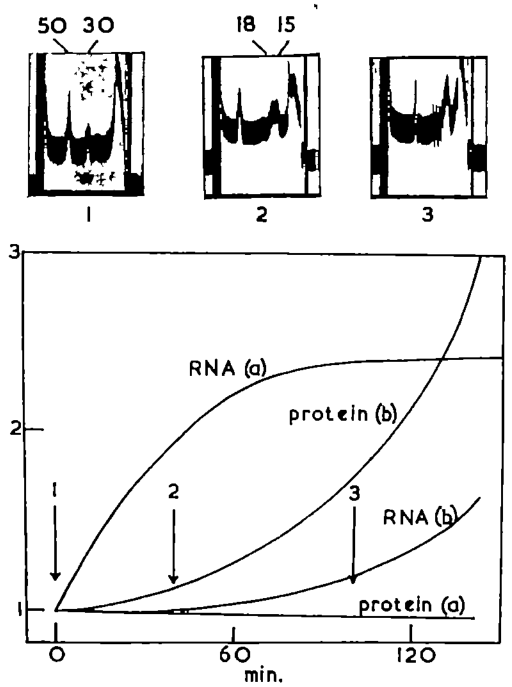

Fig. 1. Action of puromycin on *E coli* Bacteria were incubated at 37° with 0·5 mgm. of puromycin/ml. of growth medium Changes in amounts of intracellular RNA and protein are expressed as multiples of the initial values: curves (*a*). Cell extracts, each containing 10 mgm. of protein/ml., were prepared at times shown by arrows, and the corresponding Schlieren diagrams are given above the graphs. Sedimentation at 187,000*g* is to the left, and photographs were taken 16 min. after reaching full speed. Each leading peak is due to 50*S* ribosomes and in (1) this peak and that for 30*S* ribosomes are numbered. In (2), peaks for 'puromycin RNA' are numbered 18 and 15; in (3), some convection has occurred on account of the high concentration of RNA. In a second experiment, bacteria were incubated for 100 min. with puromycin as above and then washed and incubated in growth medium without antibiotic. Increases in intracellular RNA and protein are expressed as multiples of initial values on resuspension curves (*b*)

izes ribosome precursors more effectively than chloromycetin for reasons that will be discussed.

The strain of *E. coli* used in previous investigations with chloromycetin[8,9] was collected from a similar growth medium, and the cells were re-suspended in an equal volume of fresh medium at 37° C. containing 0·5 mgm. of puromycin/ml. This concentration, which gave 80 per cent inhibition of growth measured by turbidity, is greater than that used in experiments with chloromycetin since the strain is more sensitive to the latter than to puromycin. At various times during the incubation, cells were centrifuged, disrupted[12] and extracted with 0·015 *M* phosphate buffer, *p*H 7, and the clarified cell extracts were examined in the ultracentrifuge. There was no net synthesis of protein, but the amount of RNA increased by 250 per cent during incubation with puromycin (Fig. 1); also, ultracentrifuge patterns showed additional peaks at 15*S* and 18*S*, in the same region as those observed for chloromycetin.

As seen in Fig. 1, when puromycin-treated cells were washed and incubated in growth medium without antibiotic there was a gradual resumption of protein synthesis but little increase in RNA for 120 min. During this phase of metabolic recovery the 15–18*S* peaks were gradually lost from Schlieren diagrams, and there was a reversion to the characteristic pattern for untreated cells. These changes were much slower than those found for chloromycetin-treated cells when 30 min. after release from the antibiotic, the 14–18*S* peaks of 'chloromycetin RNP' disappeared and were replaced by a large single peak due to 30*S* ribosomes. The slower resumption of protein synthesis after puromycin treatment and the stability of the additional RNA may both be due to stronger binding of puromycin to ribosomal RNP. The structural formula of the antibiotic resembles that of aminoacyl RNA[13], and ribosomes are irreversibly poisoned for *in vitro* experiments concerned with protein synthesis[10]. If puromycin is strongly bound to sites it may be displaced by metabolites only on resumption of protein synthesis and this would therefore be a gradually accelerating process, as in Fig. 1. Incorporation of 'additional RNA' into ribosomes from their precursors would also be slow.

Borek and Ryan[14] showed that a mutant of *E. coli* requiring methionine for growth continued to synthesize RNA when protein synthesis ceased due to lack of methionine. Dr. W. Hayes kindly provided us with a similar strain of *E. coli* which showed this effect. This mutant synthesized little or no protein when cells were suspended in a methionine-free medium and after 180 min., synthesis of additional RNA also ceased. Cell-free extracts made at this time showed additional peaks in the same region of the ultracentrifuge pattern as those arising from the action of chloromycetin and puromycin, namely, at 14*S* and 16*S* (Fig. 2). When methionine was added and protein synthesis resumed, these peaks diminished and there was a simultaneous increase in area of the peak due to 50*S* ribosomes: these changes took place over a time-interval for which the increase in turbidity corresponded to less than one-third of a generation. The peaks at 14*S* and 16*S* may thus be due to ribosome precursors stabilized by protein which becomes available by 'turnover' of pre-existing material, known to occur in starved bacteria[15]; how-

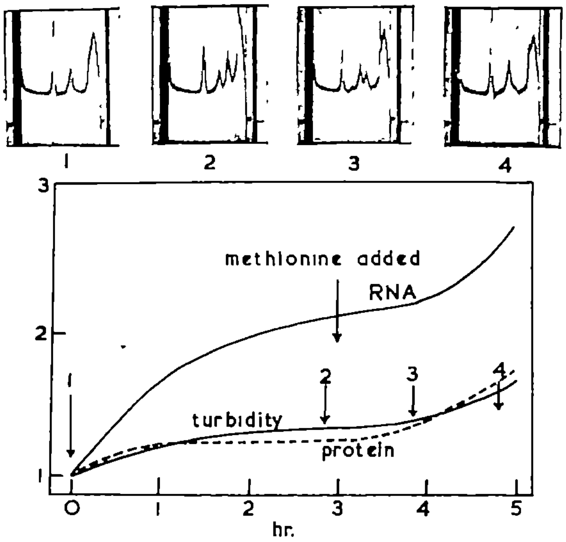

Fig. 2. Ribonucleic acid content of a methionine-requiring mutant of *E. coli*. A fully grown culture was collected and the bacteria resuspended in glucose and mineral salts medium lacking methionine. Curves show the increases in turbidity of the culture and intracellular concentrations of RNA and protein, all expressed as multiples of initial values. *dl*-Methionine (130 μgm /ml.) was added after 3 hr. Cell extracts were prepared at times indicated by arrows and the corresponding Schlieren diagrams, which are shown above the graphs, differ in appearance from those of Fig. 1 because a phase-plate was used. Sedimentation at 187,000*g* is to the left and photographs were at 16 min. The leading peaks in every photograph are for 50*S* and 30*S* ribosomes: the additional third peak which appears in photographs (2) and (3) resolved into two peaks (14*S* and 16*S*) on further sedimentation

ever, before it can be finally accepted, this view must be tested by experiments using radioisotopes in order to decide whether there is a direct incorporation of 14–18*S* RNA into 50*S* ribosomes. The additional RNP of the mutant resembles 'puromycin RNP' in metabolic stability, and both resemble 'chloromycetin RNP' in being broken down by short exposures to sonic vibrations which leave 50*S* ribosomes unaffected.

The purine analogue 8-azaguanine resembles chloromycetin in its action on *Bacillus cereus* because the synthesis of cytoplasmic protein is suppressed in both cases, but not the synthesis of RNA or cell-wall material[16]. 'Azaguanine RNA' is certainly abnormal since the analogue is incorporated into polynucleotide chains[17], and it may be argued from the similarity in action of the two inhibitors that 'chloromycetin RNA' is also abnormal. However, experiments like those of Fig. 1 were performed for *B. cereus* treated with 8-azaguanine and for *E. coli* with 8-azaadenine: protein synthesis was inhibited and RNA accumulated, but there were no additional peaks in ultracentrifuge patterns such as those shown for chloromycetin and puromycin. In this respect, therefore, the antibiotics differ from the purine analogues: 'azaguanine RNA' evidently exists as much smaller units than 'chloromycetin RNP'. Mandel and Markham[18] found that polynucleotides built during inhibition with azaguanine had relatively short chains; while experiments of Aronson and Spiegelman[19] suggested that 'chloromycetin RNA' occurs in large units, probably precursors of ribosomes, which are not degraded when the bacteria were supplied with amino-acids but were converted directly into ribosomes. Since these important experiments were rather briefly reported, we have confirmed that when ^{14}C-uracil is incorporated into 'chloromycetin RNA' it leaves the cells when they are washed and then incubated with mineral salts medium, but it remains inside them if peptone is present during the phase of recovery.

Very recently a complete account of the work of Aronson and Spiegelman has appeared[20] which supports the view that 'chloromycetin RNA' is a normal intermediate in the fabrication of ribonucleoprotein. They present a formal scheme in which stable free RNA undergoes conversion to an unstable form before RNP is synthesized and suggest that the action of chloromycetin is to block this conversion.

Several features of our work have a wider interest than the elucidation of antibiotic action. First, when peaks due to 'chloromycetin RNP' are lost from ultracentrifuge patterns their place is taken by a single peak at 30*S*: there is little or no change in area of the 50*S* peak at this time. The reverse is true for the 'mutant RNP': a precursor-product relationship seems to exist between 14–16*S* material and 50*S* ribosomes but not with 30*S* ribosomes. Although the two types or ribosomes may have common precursors at an early stage in synthesis, they thus appear to be formed from them by separate routes. Secondly, 'mutant RNP' sediments at 14*S* and 16*S*; it is much more sensitive to ribonuclease than are 50*S* ribosomes; and when the Mg^{++} ion concentration of extracts is raised to 10^{-2} *M* so that 70*S* ribosomes are formed, the 14*S* and 16*S* peaks are lost and a larger and broader peak than in normal extracts appears in the 30*S* region of the pattern. 'Chloromycetin RNP' also sediments at 30*S* when the Mg^{++} ion concentration is raised[7,9]. A similar set of properties is claimed for the 'messenger RNA' of uninfected bacteria[3]; and since ribosome precursors may also incorporate radiotracers very rapidly it is clearly impossible to distinguish between the two forms of RNA by sedimentation and tracer studies alone. Thirdly, the practice of preparing extracts in 10^{-2} *M* magnesium acetate solution for ultracentrifuge analysis has been criticized in a previous communication[21]. Had we used this solvent, the changes we have described would not have been observed since most of the RNP would have sedimented at 30*S* or at higher speeds.

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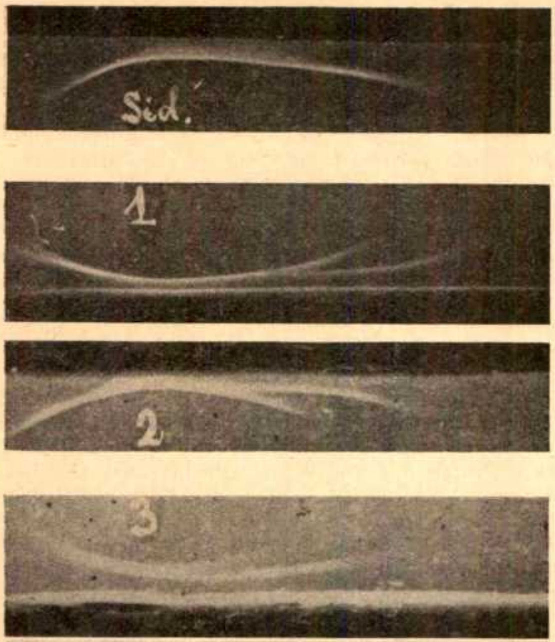
IMMUNOCHEMICAL STUDIES ON HUMAN SIDEROPHILIN

Enzymatic Splitting of the Antigenic Structure of Siderophilin

PURIFIED human siderophilin was submitted to enzymatic proteolysis in order to obtain fragments carrying parts of its antigenic structure[1,2]. (The starting material was Cohn fraction IV, 4, which was obtained through the courtesy of Dr. K. Walton, Department of Experimental Pathology, the Medical School, Birmingham; the purification was achieved by 'Rivanol' precipitation followed by DEAE chromatography in acetate buffer *p*H 6, 0·01 *M* and *p*H 5·4, 0·02 *M*. The material obtained showed traces of γ-globulin impurity when tested by immunoelectrophoresis with a powerful anti-whole human serum antiserum. For our study, we had an anti-siderophilin serum which gave no precipitation with other constituents of human serum obtained through the courtesy of Dr. J. F. Soothill, Department of Experimental Pathology, the Medical School, Birm-



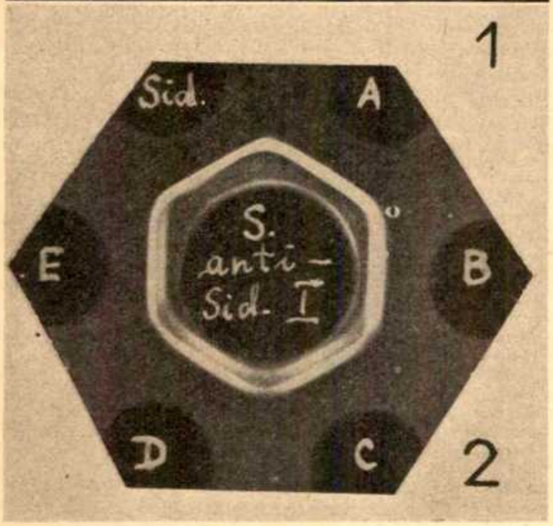


Fig. 1. The immunoelectrophoretic pattern of the control siderophilin and three degraded samples (enzyme: chymotrypsin; 45·64 and 75 per cent of material non-precipitating with 5 per cent trichloroacetic acid)

Fig. 2. Comparison of the control siderophilin, one trypsin-degraded (*A*) and two chymotrypsin-degraded samples of plasma siderophilin (*B*, *C*); two chymotrypsin-degraded samples of urinary siderophilin (*D*, *E*)

ingham. The siderophilin preparation used was saturated with iron and was pink-coloured—other preparations, containing a little albumin, were yellow-red.)

The enzymes chosen for the proteolysis were papain, trypsin and chymotrypsin, all acting in neutral or alkaline *p*H in order to avoid the splitting of the iron occurring at acid *p*H.

The papain digestion did not produce a visible change of immunological properties. Trypsin and chymotrypsin were used at *p*H 8·1 either in borate or phosphate buffer or without buffer. The amounts of enzymes used varied between 1 and 10 per cent for 1–5 days at 37° C.

The observed changes were less in borate buffer and the best conditions were without buffer.

The tryptic and chymotryptic digestions result in the splitting of a considerable amount of material: after dialysis the remaining protein (as measured by biuret reagent) is about 25 per cent of the original material. After chymotryptic digestion the protein loses its colour; for example, when comparing three degraded samples, 1 (45·6 per cent of material non-precipitating with trichloroacetic acid at 5 per cent), 2 (64 per cent) and 3 (75 per cent), the first was pink-coloured, the second very pale and the third colourless (all three were compared at the same concentration of biuret-reacting material). Tryptic digestion did not produce such changes. It was impossible to fix iron on those colourless samples.

The sedimentation constants of the first two samples were very close to that of the control siderophilin (4·68 and 4·18); the third sample showed 2·37, but the concentration of the material was very low (0·1 per cent biuret-reacting material).

The immunochemical changes are seen in Figs. 1 and 2. By immunoelectrophoretic analysis in barbiturate buffer, *p*H 8·2, two lines are shown by the degraded samples; they seem not to be separate lines, but they may be superimposed for the first two samples; the third gives two lines of very similar electrophoretic mobility. By double diffusion in agar, two separate lines can be obtained when the most degraded samples are tested. The patterns obtained vary with the antiserum used, which means that the proportions of different antibodies may be widely different.

Thus the antigenic structure of siderophilin is split by trypsin and chymotrypsin and the digestion results in two separately precipitating components.

A mixture of a series of degraded samples was separated by DEAE cellulose chromatography into four fractions: with acetate buffer *p*H 6, molarity 0·01, the first fraction was a pink-coloured material which showed no splitting when tested by immunoelectrophoresis and resembled the original siderophilin.

The second fraction eluted with the same buffer was very slightly coloured and showed no definite splitting by immunoelectrophoretic analysis.

The third fraction was eluted with a buffer of *p*H 5·4 and molarity 0·02; it was coloured, and by immunoelectrophoresis showed a pattern of two lines separated at the anodic extremity. Washing the column with the same buffer removed a final product which was colourless (after concentration it was greenish) and showed two separate lines by immunoelectrophoresis.

The sedimentation constants were 5·14 and 3·29 (50 per cent of each constituent) for the third fraction and 4·38 (100 per cent) for the last.

A preparation of urinary siderophilin from patients with proteinuria (obtained through the courtesy of Dr. J. Hardwicke, Department of Experimental Pathology, the Medical School, Birmingham) was submitted to proteolysis in the same conditions. Immunoelectrophoresis showed splitting of the line, while, by double diffusion in agar, comparison of the degraded samples of plasma and urinary siderophilins the analogous lines fused (Fig. 2). Thus, with respect to the anti-plasma siderophilin serum used, both degraded products seem identical.

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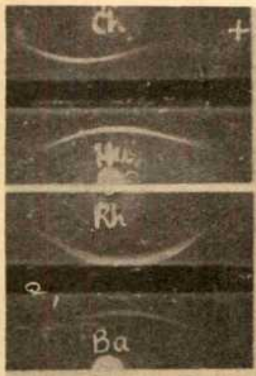
Cross-reactions of Human and Monkey Siderophilin

When tested by immunoelectrophoresis chimpanzee siderophilin has not the same mobility as human, rhesus or baboon siderophilins (Fig. 1). (The antigens used in this work were: for the human siderophilin either the purified preparation[1] or the whole serum, and for monkeys the whole individual sera: 6 chimpanzee sera, 6 rhesus sera and 2 baboon sera. The antisera were three pools of sera from rabbits immunized with a purified siderophilin preparation which were obtained through the courtesy of Dr. J. F. Soothill.)

By double diffusion in agar with the antiserum I (the same as used previously[1]) no cross-reaction (spur formation) was observed between human and any monkey siderophilins. With sera II or III, spurs are seen between human or chimpanzee siderophilin lines and rhesus or baboon siderophilin lines.



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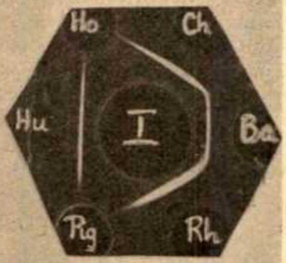

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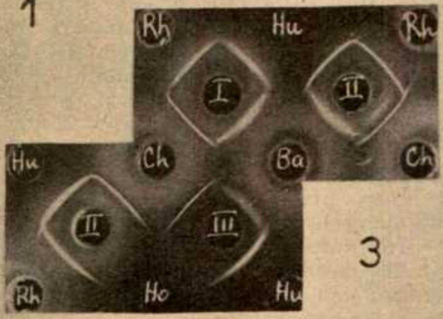

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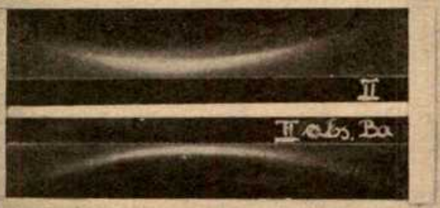

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Fig. 1. Immunoelectrophoretic patterns of chimpanzee, human rhesus and baboon siderophilins as revealed by anti-human siderophilin serum I

Fig. 2. Reactions of horse, chimpanzee, baboon, rhesus, pig and human siderophilins with the anti-human siderophilin serum I

Fig. 3. Reactions of human, chimpanzee, rhesus, baboon and horse siderophilins with anti-human siderophilin sera I, II and III

Fig. 4. Immunoelectrophoretic pattern of a chymotrypsin degraded sample of human siderophilin revealed by anti-human siderophilin serum II: unabsorbed and absorbed by baboon siderophilin

The lines of human and chimpanzee siderophilins and of baboon and rhesus siderophilins fuse completely, thus showing their immunological identity with respect to those two antisera (Figs. 2 and 3).

None of the antisera tested reacts with horse, pig or goat siderophilins (Figs. 2 and 3).

When comparing several spurs formed by those cross-reacting systems it seems that the difference between chimpanzee and rhesus is greater than between human and rhesus siderophilins.

All observed spurs are shorter than those formed, for example, by horse serum albumin and bovine serum albumin with anti-horse serum albumin serum, or hen and duck ovalbumins with anti-hen ovalbumin, and they are often very weak.

Since the length of a spur seems independent of the density of the line it means that the length of the spur is probably related to the number of determinant groups in the two antigens being compared using a particular antiserum[2], and the density of the spur line reflects the quantitative relationship between the amount of antibody reacting only with the homologous antigen and antibody reacting with both homologous and heterologous antigens.

It was not possible to absorb all antibodies reacting with the homologous antigen in sera II and III with the heterologous antigen.

When the antiserum II or III absorbed by rhesus or baboon serum is used for the immunoelectrophoresis of a digested sample of human siderophilin which gives two lines with the unabsorbed serum I only one line can be seen (Fig. 4). When the degraded siderophilin gives only a line split at one extremity with the unabsorbed antiserum, there is a single line with the absorbed antiserum.

These facts resemble the observation on the reaction of papain-degraded human γ-globulins with the corresponding antiserum absorbed by rhesus γ-globulin[3], but they differ from the observation of chymotrypsin-degraded hen ovalbumin with the corresponding antiserum absorbed by duck ovalbumin[2].

When the same sort of experiment is performed using antiserum I which does not give any spur with monkey siderophilins, absorption by those heterologous antigens is capable of removing all antibodies precipitating with the homologous antigen, but more heterologous antigen is required to remove antibodies producing one line than the other.

Thus it seems that the antibodies present in antisera II and III which react only with the homologous antigen are related to antibodies precipitating with one of the components of digested human siderophilin while the antibodies precipitating with the other component are related to antibodies precipitating with both homologous and heterologous antigens.

I thank Prof. J. R. Squire and P. G. H. Gell and the staff of the Department of Experimental Pathology, the Medical School, Birmingham, for their hospitality during my stay in that laboratory.

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SEPARATION OF AMINO-ACIDS BY CIRCULAR PAPER CHROMATOGRAPHY

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CIRCULAR paper chromatography is said to have advantages over other methods of separating mixtures of amino-acids. The techniques of Krishnamurthy and Swaminathan[1] gave striking results but they have not come into general use. In this communication we summarize a practical method of separating amino-acid mixtures by circular paper chromatography in a modified Kawerau apparatus.

The starting point was Krishnamurthy's method which separated the 16 amino-acids of casein on circular paper 28 cm. in diameter. The papers were supported on a glass stand and the wick was a piece of filter paper 2 × 3 cm., rolled and cut at one end to form a brush. The whole was covered by a circular glass trough 30 cm. in diameter. Five solvents were used: (*a*) phenol/butanol/acetic acid/water 20 : 20 : 8 : 40; (*b*) phenol saturated with *p*H 12·0 buffer; (*c*) *m*-cresol saturated with *p*H 8·4 buffer; (*d*) 1-butanol/acetic acid/water, 40 : 10 : 50; (*e*) benzyl alcohol/tertiary amyl alcohol/water, 50 : 50 : 100. The papers were multiple developed.

We experienced considerable difficulty in repeating Krishnamurthy's method in a Kawerau apparatus. The method which follows proved satisfactory for the study of amino-acids in schistosomes and their hosts.

Whatman No. 1, 26·5 cm., slotted (5 section) circular papers were used, buffered when necessary by running them in a Kawerau dish until fully saturated. The solvents, prepared as described here, were shaken vigorously for 15–20 min. and allowed to stand for 2–6 days before use. Amino-acid samples were streaked on the paper at a distance of 1·5 cm. from the centre. After multiple development, the chromatograms were dipped in 0·25 per cent ninhydrin in acetone to detect the amino-acids.

Initially, the aim was to obtain good separations in standard 26-cm. Kawerau dishes, but the dishes were too small. When the solvent front was kept within the border of the dish the separation was poor, but if the solvent were allowed to travel beyond the ground glass surfaces of the base and lid, its movement was irregular and the amino-acid bands on the chromatograms were often diffuse and uneven. Evaporation of volatile solvents, such as benzyl alcohol/tertiary amyl alcohol/water, occurred at the edge of the paper and prevented identification of the outer bands.

The following modification was made to allow longer development. The Kawerau dish, without the lid, was placed in a pneumatic trough, 30 cm. in diameter by 12 cm. deep. Four pieces of fine drawn capillary tubing were placed across the Kawerau dish to support the paper. The solvent was placed in the outer dish as well as in the Kawerau base to ensure a saturated atmosphere. After equilibration at 70° F. the paper was inserted, the lid of the trough replaced and development started.

Methods for Different Solvents

(1) *Phenol/1-butanol/acetic acid/water*. 20 vol. of hot phenol were stirred into a mixture of 20 vol. of 1-butanol, 8 vol. of glacial acetic acid and 40 vol. of water. After shaking for 20 min. and standing for three days, the top layer was used as a solvent. Papers used with this solvent were buffered at *p*H 2·0 with potassium chloride/hydrochloric acid buffer of McFarren[2] before being developed three times for 5½–6½ hr. Development was always stopped within 1 hr. from the time the solvent front reached the edge of the paper.

(2) *Phenol p*H 12·0. An excess of sodium hydroxide/disodium hydrogen phosphate *p*H 12·0 buffer[2] was stirred into hot phenol, shaken and allowed to stand. The lower layer was used as a solvent with papers buffered at *p*H 12·0. Double development for 6–7 hr. gave satisfactory results.

(3) *Cresol p*H 8·4. Cresol (mixed isomers) was mixed up with an excess of boric acid/potassium

Table 1. CHROMATOGRAPHIC BANDS SEPARATED BY THE FIVE SOLVENTS

	Butanol–acetic acid–water	Phenol–butanol–acetic acid–water	Cresol	Phenol	Tert. amyl alcohol–benzyl alcohol
1.	Isoleucine, leucine	Phenylalanine, isoleucine, leucine	Phenylalanine	Arginine, proline, phenylalanine, leucine, isoleucine, lysine	Phenylalanine
2.	Phenylalanine	Tryptophan, methionine, valine, proline	Proline	Methionine, tryptophan	Leucine
3.	Tryptophan	Tyrosine	Isoleucine, leucine, tryptophan	Valine, histidine,	Isoleucine
4.	Methionine, valine	Alanine	Methionine	Tyrosine	Methionine
5.	Tyrosine	Threonine	Valine	Alanine	Tyrosine
6.	Proline	Glutamic acid	Tyrosine, histidine, arginine	Threonine	Valine
7.	Alanine	Glycine	Alanine	Glycine	Proline
8.	Threonine, glutamic acid	Serine, arginine, aspartic acid	Threonine	serine, cystine	Alanine, threonine, histidine
9.	Serine, glycine, aspartic acid	Histidine	Lysine	Glutamic acid	Arginine, cystine, glycine, serine, lysine
10.	Arginine	Lysine	Glycine	Aspartic acid	Aspartic acid, glutamic acid
11.	Histidine, lysine	Cystine	Serine		
12.	Cystine		Glutamic acid, cystine		
13.			Aspartic acid		

Table 2. Single Amino-acids separated by the Five Solvents

	Butanol/acetic acid/water		Phenol/butanol/acetic acid		Cresol/pH 8·4		Phenol/pH 12·0		Tert. amyl alc./benzyl alc./water	
	1	2	1	2	1	2	1	2	1	2
Phenylalanine	+	+			+	+			+	+
Leucine									+	+
Isoleucine									+	+
Tryptophan	+									
Methionine					+	+			+	+
Valine					+	+			+	
Tyrosine	+	+	+	+		+	+		+	
Proline	+	+			+				+	
Alanine	+	+	+	+	+	+	+	+		
Threonine			+	+	+		+	+		
Glutamic acid			+	+			+	+		
Serine					+			+		
Glycine			+		+		+	+		
Aspartic acid					+		+	+		
Arginine	+	+				+				
Histidine			+	+		+				
Lysine			+	+	+					
Cystine	+	+	+	+						

1, Our results; 2, results of Krishnamurthy and Swaminathan (ref. 1).

chloride/sodium hydroxide pH 8·4 buffer[2]. After shaking and standing, the bottom layer was used as a solvent. The papers, which were treated with the same buffer at pH 8·4, were developed three times for 9–10 hr.

(4) *Butanol/acetic acid/water*. This was prepared by adding 40 vol. of 1-butanol and 10 vol. of acetic acid to 50 vol. of water. The mixture was shaken and allowed to stand; the top layer was used as a solvent. Unbuffered papers were used and developed three times for 5–7 hr.

(5) *Tertiary amyl alcohol/benzyl alcohol/water*. 50 vol. of tertiary amyl alcohol and 50 vol. of benzyl alcohol were shaken with 100 vol. of water. After shaking and standing, the top layer was used as a solvent. Krishnamurthy *et al.*[1] say that this solvent can be used both with unbuffered paper or with paper buffered at pH 8·4; we have found that buffered papers give a better result. The papers were developed twice for 15–16 hr.

The results are shown in Tables 1 and 2. Table 1 lists the amino-acid bands formed by each solvent, arranged in order beginning with the outside band. Table 2 shows the amino-acids that are clearly separated by each solvent, and gives the results of Krishnamurthy for comparison.

As can be seen from Tables 1 and 2, R_F values have not been quoted. Although Krishnamurthy *et al.*[1] gave the relative distances travelled by the amino-acids, we found it more reliable to express the result as bands containing one or more amino-acids.

Chromatograms developed with tertiary amyl alcohol/benzyl alcohol/water gave a good deal of trouble, and we were unable to move the amino-acids to the edge of the paper. After three developments, the inner bands were separated, but at the expense of the outer, which became very diffuse. Since the main use of this solvent was to separate the outer bands, phenylalanine, isoleucine and leucine, the papers were only developed twice and gave good separation of these components.

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MOLECULAR AND FINE STRUCTURE OF ALPHA-KERATIN

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IT has been difficult to reconcile the results obtained from the X-ray diffraction studies of metal-treated keratin fibres with the evidence revealed in the electron micrographs of their thin sections and fragmentation products[1,2]. Satisfactory, qualitative, agreement has long been evident between the transverse dimensions (d = 60–80 Å.) and packing of the individual ('cylindrical') microfibrils and the equatorial X-ray data. Yet, it has proved impossible to derive a quantitative expression which would account for the interference effects involved in such a system of cylinders, separated by distances of the same order of magnitude as their own dimensions[3]. The discrepancy between the values of the axial repeats: (1) 90 Å. observed directly in the intermicrofibrillar matrix of the longitudinal sections and fragmentation products of keratin, treated with silver nitrate, but not in the microfibrils themselves; (2) 25 Å. derived from the one-dimensional Patterson function, had to be resolved by our own suggestion that the metal atoms are deposited not only in the intermicrofibrillar matrix but also in the microfibrils themselves[2]. Indeed, Filshie and Rogers have found[4], in the paracortex of transverse sections of merino wool fibres, evidence of fine detail within the microfibrils which they regard as representing a system of nine peripheral sub-units (d = 20 Å.) with two similar subunits situated in the centre[5]. They stress a resemblance between this system and that found on a higher level of dimensions, in animal cilia[6] and bacterial flagella[7]. The sub-unit, designated as protofibril, was identified with the 3-strand rope (d = 20 Å.) of α-helices, which was shown, by Fourier transforms[8] and more recent work on optical transforms[5,9], to conform reasonably well with the X-ray data (there remains, however, the unexplained equatorial reflexion at 27 Å. and the low calculated density[8]).

We have obtained evidence of fine detail inside the microfibrils, in thin transverse sections of porcupine quill, reduced and treated only in osmium tetroxide (cf. ref. 10), but Filshie and Rogers found that a treatment with osmium tetroxide in bulk, followed by lead hydroxide after sectioning, was necessary to obtain adequate contrast in the electron micrographs of wool fibres[4]. We believe that it is not possible to consider the observed sub-units ($d = 20$ Å.) simply as end-on projections of unstained 3-strand ropes, surrounded by metal-containing inter- and intrafibrillar matrix, for the following reasons. First, because the orientation of the long α-helices ($d = 10$ Å.) in relation to the axis of the transverse sections, about 200–400 Å. in thickness, and tilting imposed by the morphology of cortical cells[11], the space-filling polyhedra[12], will affect the microscope image. Secondly, because of a periodicity of scattering sites along the loci of α-helices, revealed particularly well by an enhancement of the meridional reflexions of keratin containing one of the following elements, mercury[13,15,16], osmium[14,16], silver[15,16] or uranium[16], we would expect electron scattering of a type associated with some regularity of atomic arrays in a specimen, though much lower than in a crystal. Thirdly, the dimensions involved approach the resolving power of the microscope.

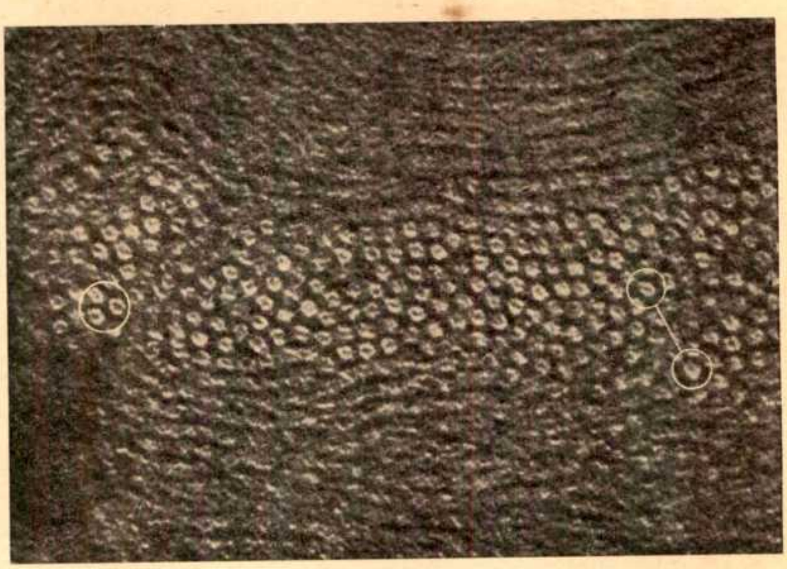


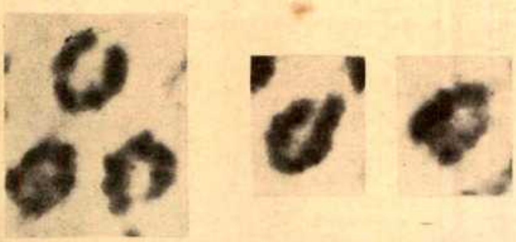
Fig. 1. African porcupine quill-tip. Reduced and treated in osmium tetroxide. Transverse section. (× c. 350,000)

Porcupine quill tips were treated in 0·5 *M* sodium thioglycollate at 35° C. and *p*H 4·6 for 18 hr. and, after washing, in 1 per cent unbuffered aqueous solution of osmium tetroxide for 10 days, a standard procedure was used for embedding[17] (in 'Araldite') and sections were obtained on the Cambridge Huxley-type ultramicrotome with a diamond knife. The sections were examined in a Siemens Elmiskop I electron microscope, using the double condenser, a 50-μ objective aperture, an accelerating potential of 80 kV. and instrumental magnification × 82,500. The astigmatism was corrected and the usual precautions were taken to reduce the effect of contamination. Fig. 1 is a typical electron micrograph considered to be nearest to true focus, in a particular 'through-focus' series, showing in the central portion clearly defined microfibrils the diameter (D) of which (see Fig. 4) and separation (S) were determined from about a hundred measurements, by two observers, as 70 Å. ($\sigma = 5·0$ Å.) and 83 Å. ($\sigma = 7·6$ Å.), respectively. Fig. 1 is a direct print from the original electron micrograph; therefore, the dense areas would be usually considered as corresponding to a metal-containing, thus electron-opaque, matrix[18]. The amount and disposition of the fine detail, now observed in the microfibrils, which are, in any event, clearly resolved only in a relatively small fraction of the total cross-sectional area, varies considerably, as seen from Figs. 2 and 3 which represent, at × 1,650,000, the two encircled areas of Fig. 1. To obtain better contrast, Figs. 2 and 3 were printed from an intermediate plate, consequently the regions containing metal would be usually identified as white areas. The separation (a) of the sub-units, in this case, was estimated to be lower than 20 Å., but it has not been possible to determine the exact number of the sub-units within an individual microfibril or to observe any typical form of their packing in other areas of the transverse section.

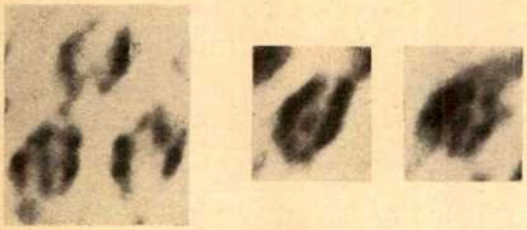
However, if a particular arrangement of one type of sub-units could be accepted as possible, it should be compatible with the X-ray diffraction results in both the low- and large-angle regions, and not in the latter only[8]. Indeed, it is not necessary to insist on the presence of a fixed number of aggregates of the α-helices in the microfibrils, as packing would not be very regular in such fibrous tactoids, formed from long molecules of unequal lengths[19]; thus, imperfections of fine detail could be considered as due to some local discontinuity in the sub-units of α-helices, as distinct from an amorphous segment in the microfibril as a whole[20,21]. However, the origin of effects observed in our electron micrographs must rather be sought in the regularity of distribution of heavy elements in the specimen[22]. The consideration of such possibilities directed our attention to the origin of contrast in periodic structures which is known to change in a through-focus series of electron micrographs[23], and it is, perhaps, not surprising that similar effects were observed in the present case of the paracrystalline lattice of keratin.

Figs. 2*b* and 3*b* correspond to a change in focus $\Delta f = 0·1\mu$ (the smallest instrumental step available) from that in Figs. 2*a* and 3*a*, respectively. It can be shown that de-focusing by twice this amount should not affect the resolution of an extended object of about 10 Å. in diameter, but only its contrast[24,25]. The following typical changes are evident, after de-focusing, in the central region (C) of the microfibrils: (1) a small increase in contrast between Figs. 2*a* and 2*b*; (2) the appearance of the fine detail in Fig. 3*b*, which was absent in Fig. 3*a*. In the peripheral regions there is some loss in contrast between the sub-units (P), and this effect can be seen more clearly in the original prints of Figs. 2*a* and 3*a* and Figs. 2*b* and 3*b*, respectively. Both these effects were recorded in numerous microfibrils.

Observations of this kind stress again the necessity for the great care required in the interpretation of electron micrographs. For example, attention has been directed to the fact that observation of a periodic repeat, of dimensions comparable with the resolving power of the electron microscope, cannot be considered as conclusive evidence of a periodic structure



Figs. 2*a* and 3*a*. Detail of Fig. 1. See text. (× 1,650,000)



Figs. 2*b* and 3*b*. As Figs. 2*a* and 3*a*, de-focused by $\triangle f = 0{\cdot}1\mu$. See text. (× 1,650,000)

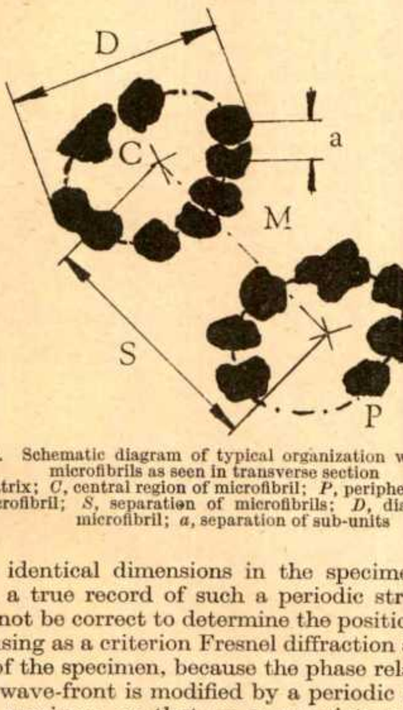


Fig. 4. Schematic diagram of typical organization within the microfibrils as seen in transverse section
M, matrix; *C*, central region of microfibril; *P*, peripheral region of microfibril; *S*, separation of microfibrils; *D*, diameter of microfibril; *a*, separation of sub-units

of the identical dimensions in the specimen[26]. To obtain a true record of such a periodic structure it would not be correct to determine the position of true focus using as a criterion Fresnel diffraction along the edges of the specimen, because the phase relationship in the wave-front is modified by a periodic structure of the specimen, so that an appropriate amount of de-focusing is required[27]. It must, of course, be remembered that out-of-focus images of a non-periodic fine detail in the specimen are affected by Fresnel diffraction, which introduces greater contrast[28]. However, it would appear that the changes described by us are, at least in part, due to phase effects enhanced by some regularity in the arrangement of metal in the microfibrils. Indeed, it has been suggested that a row of *n* atoms, parallel to the direction of the electron beam and contained within a cylinder of a diameter smaller than the resolving power of the electron microscope, would produce contrast directly proportional to their number (*n*) (ref. 24).

We are not able to decide at this stage to what extent the variable fine detail in the microfibrils could be due to the structural heterogeneity of keratin or other factors such as orientation of the microfibrils with respect to the direction of the electron beam. Similarly, although we know that the layered structures, originally observed in our early fragmentation experiments, are endowed with strong cross-linkages which had to be broken before the individual microfibrils could be isolated[29], certain features of the images of these regions now revealed in the transverse sections could well be due to other causes[30]. We are, therefore, carrying out a series of experiments which have in the past been applied extensively to solve similar problems in the case of electron microscope images of crystals.

Whatever the outcome of this work, some important conclusions can be drawn from a comparison between electron micrographs of porcupine quill and the para-cortex of Australian merino wool. Though distinct microfibrils appear in greater numbers in the latter, only a relatively small fraction of the total area of the cross-section of porcupine quill shows this type of structure. Since the X-ray diffraction evidence is interpreted to indicate greater regularity of organization in porcupine quill than in merino wools, it could be considered as indirect evidence that the layered regions are endowed with greater regularity than those with arrays of isolated microfibrils.

We have originally suggested[29] that there are three distinct stages in the fragmentation of keratin by the combined chemical and mechanical methods: (1) separation of ribbons or sheets of microfibrils; (2) isolation of microfibrils; (3) breakdown of the latter into 'globular' units. Globular debris observed at the second stage of fragmentation was regarded as originating from the intermicrofibrillar matrix and we tentatively identified it with the fraction rich in sulphur, which can be isolated from keratin following disulphide bond breakdown by oxidation[29]. The evidence presented here and that obtained earlier for wool[4] and feather-keratin (Rogers, G. E., private communication) do not favour a recent suggestion that some feather-like elements could be present around each microfibril of α-keratin[31]. If it were so, changes in contrast in the fine detail should be observed in the matrix, in the out-of-focus position, similarly to those visible in the periodic elements. There is at present no direct conclusive evidence to indicate the type of structure in the matrix. However, we would like to recall that from experiments involving detailed analysis of supercontraction phenomena, we have concluded that the matrix must necessarily possess a certain degree of orientation of its chains to be capable of contraction, in parallel with the microfibrils[32]. Thus, it is not possible to accept conclusions, from the experiments even less direct than the electron microscope evidence, which are considered to imply that microfibrils and matrix possess two distinct structural characteristics and functions[20,21].

It may be of some interest to add that calculations, based on a consideration of the elastic scattering of electrons in extended non-periodic objects containing heavy elements[28,33], have yielded results of the correct order of magnitude for the maximum thickness of the transverse sections of keratin containing osmium[14] and silver[11]. However, some guesswork was unavoidable to allow for absorption by the cuticle cells, membrance complexes, nuclear remnants, etc.

It has been suggested that any attempts to determine the amino-acid composition of various fractionation products of keratin are of little value because of uncertainty as to their histological origin[34]. Yet there is still a tendency to regard 'α'-keratose as derived from the microfibrils and γ-keratose, rich in cysteic acid, from the surrounding matrix. While agreeing that there has been considerable concord between the electron microscope and chemical evidence on this latter point, we would like to direct attention to disconcerting conclusions which emerged from the analysis of data of Corfield *et al.*[35], showing that there is a greater proportion of the bulky amino-acid residues[36] in 'α'-keratose than in γ-keratose; the opposite result to that which would be expected from the general assumption of greater regularity and better packing of the chains inside the microfibrils and in the layered regions.

Finally, we have already stated that it was not possible to determine the exact number of sub-units within a microfibril of porcupine quill. However, even if a 3-strand rope could be identified with the sub-unit, the diameter of our microfibril is too small to accommodate a '9 + 2' system of ropes. Furthermore, other systems of packing of the latter units would result in an inordinately low value of the calculated density. It would appear that the required density could be obtained by accepting the recently suggested packing of the coiled-coils without rope twist[37]; a concept akin to the idea of a discontinuous helix[38].

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A SYSTEM FOR CONTINUOUS MEASUREMENT OF RADIOACTIVITY IN FLOWING STREAMS

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AN apparatus for the determination of β- or α-radioactivity in either circulating liquid or gas streams has been developed. The well-known principles of coincidence type liquid scintillation spectrometry offer an attractive approach to such determination in that backgrounds are generally low and counting efficiencies high. By now the electronics are well established and reliable and are available in many laboratories.

Steinberg[1,2] noted that solid anthracene crystals (blue-violet fluorescence) suspended in aqueous solution containing radioactivity gave relatively high counting efficiencies for carbon-14 and low but usable efficiencies for tritium measurement. He proposed that his method be used whenever it was difficult or inconvenient to solubilize the solution to be counted in one of the more usual liquid scintillation solvents. Afterwards, Karmen and Tritch[3] described a detector filled with silicone-coated anthracene for use in the observation of radioactive components in the effluents from gas chromatography columns. The counting efficiencies obtained for carbon-14 are high and the lower counting efficiencies observed for tritium are attributed to the low tritium β energies and absorption of tritium β's by the silicone coating.

Rapkin and Packard[4] confirmed Steinberg's original report and offered some preliminary results on determination of both carbon-14 and tritium activity in flowing streams wherein these streams were passed over anthracene crystals placed between two photomultipliers and observed in the fashion which is by now well known. Recently, in independent investigations, Scram and Lombaert[5-7] have likewise examined the continuous measurement of β-activity using microcrystalline anthracene as the scintillator. We would like to extend the earlier observations of Rapkin and Packard and to report not only on the use of solid anthracene crystals for the measurement

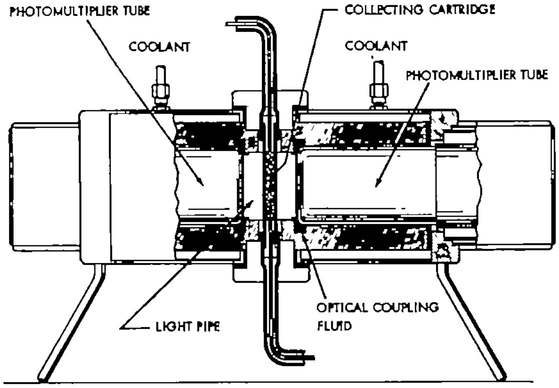

Fig 1. The continuous flow detector a simplified drawing

of activity in flowing liquid streams, but also the measurement of activity in gas streams.

A detector was constructed which is shown in simplified form in Fig. 1. A 'Lucite' light pipe coated the titanium dioxide, which was made according to the suggestions of Horrocks and Studier[6], was coupled to two photomultipliers which were in turn fed to appropriate coincidence type circuitry. The detection cell, consisting of a glass tube 9 mm. outer diameter with appropriate fittings on either end, was packed with anthracene crystals. A glass frit, or glass wool, was incorporated in the cell on the downstream side to contain the anthracene and a pledget of glass wool was placed above the anthracene on the upstream side.

The electronics took one of two forms. Initially, a Tri-Carb spectrometer was used and was operated on a pre-set time basis automatically recording counts which were accumulated during the counting period. For graphical presentation a special rate-meter was constructed. It is essentially identical to the circuitry of the Tri-Carb spectrometer except that the scalers and timers have been removed and replaced by two rate-meters. Two channels of analysis are possible. They may both be observing identical portions of the energy spectrum, or widely differing parts may be monitored by each. Separate amplifiers are used on each channel with continuous gain controls, so that for dual-label experimentation one channel may be operated at maximum gain to observe the less energetic isotope, while the second channel is operated at reduced gain to observe the more energetic.

In order to determine counting efficiency, it is always necessary to know the amount of material being measured. In a device such as that which we described, the determination *in situ* of the volume of voids in the sensitive zone necessitates indirect measurement such as that advocated by Schram and Lombaert[7]. To circumvent indirect measurements and to afford standardization of technique, our preliminary studies were made using sealed tubes approximating the size of the detection cell which were filled with anthracene crystals and a radioactive chemical containing a known number of disintegrations per minute (d.p.m.). Fig. 2 shows the excellent straight-line relationship which was observed between total activity and counts observed with varying amounts of both $^{14}CO_2$ and tritiated methane. Carbon-14 counting efficiency was of the order of 50 per cent with a coincident background from 100 divisions to infinity of less than 40 c.p.m. at 900 V. Tritium counting efficiency was in the range of 7 per cent and the integral background from 100 divisions to infinity was about 90 c.p.m. at 1,130 V. Using a plastic scintillator, Boyce *et al.*[9] report a counting efficiency of tritium gas of 2·2 per cent with a coincident background of 300 c.p.m.

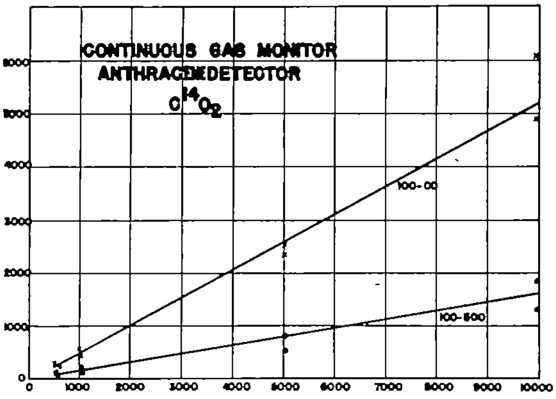

Fig. 2. A linear plot illustrating the continuous measurement of radioactive carbon dioxide using crystalline anthracene as the scintillator

The approach used to test the system for application to liquids was the same as that used with bases. For aqueous solutions of carbon-14 benzoic acid integral counting efficiencies in excess of 50 per cent were observed; but it was necessary to operate at higher voltages and with somewhat higher backgrounds. For tritium measurement, using tritiated water as a reference material, performance was somewhat erratic and under the best conditions efficiencies were between 1 and 3 per cent over a wide range of activity.

The detector described here has now been further tested in closed flowing systems. For liquid-flow determinations when used in conjunction with commercial amino-acid analysers of the Spackman[10] or Piez[11] design, carbon-14 differential counting efficiencies of 20–30 per cent have been experienced with a background of about 20 c.p.m. With discriminator settings of 100–500 divisions, peaks containing as little as 300 c.p.m. (less than $1\cdot5 \times 10^{-4}$ μc.) are readily observed and once the peak has passed through the detector, absolutely no activity remains in the detection zone. Even with sharp peaks containing as much as 20,000 d.p.m. there was no residual activity evident and background immediately returned to base-line. Resolution of the

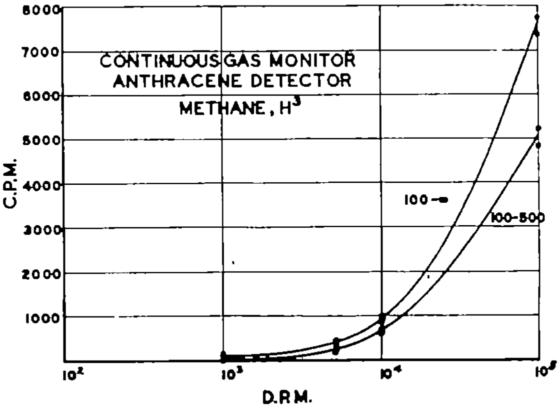

Fig. 3. A semi-log plot illustrating the continuous measurement of tritiated methane using crystalline anthracene as the scintillator

amino-acid analyser was unimpaired by insertion into the system of the small volume detector described. The optimal volume which we have determined for the detection zone when the detector is operated in conjunction with an amino-acid analyser is approximately 1 ml. This observation is in agreement with the results of Schram and Lombaert[7].

For gas analysis we have used the 1-ml. cell, and for breath analysis we have also constructed a cell with a free space after packing of about 10 ml. We have begun some preliminary work in which the atmosphere about a fermentation broth is continuously circulated through the detector and back into the fermenter. A record of evolved activity is obtained.

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SCATTERING OF UNDERWATER SOUND IN SAANICH INLET, BRITISH COLUMBIA

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BACK-SCATTERING of the ultrasonic pulses of echo-sounders is usually attributed to reflexion of sound-waves from planktonic or nektonic organisms[1,2]. It is recorded as 'blips' on the echogram, and is a common phenomenon in the sea. Single animals, such as fish, or a small group of organisms produce few blips, so that a compact, well-defined trace may result. Or, blips may be numerous and continuing when a more-or-less diffuse, layer-like record is obtained. The nature of this record seems to depend on several factors such as the numbers of the organism present[3] and the relation between the sonic frequency (length of the wave) and the cross-sectional area and acoustic properties of the organisms[4]. The latter factors in turn are related to the structure and composition of the exoskeleton and body, and particularly to the inclusion of gas bubbles or other compressible, highly reflective substances[5]. A wide range of organisms has been inferred as causes of scattering; but only a few direct observations have been made, using underwater cameras or television[6].

The problem of relating cause and effect appears to be due to difficulties encountered when working in the open sea. A layer may occur only spasmodically or may not be available readily to the observers, so that a continuing examination is not possible. Sampling presents numerous problems, not the least of which is knowing whether the organisms collected were the effective scatterers on a particular occasion[7,8]. Furthermore, the relationship between pelagic organisms and their environment is a dynamic one which can lead to short- and long-term fluctuations in the species composition of the layer and in its structure, depth of location or expanse[8].

These and related problems could well be examined more effectively in a scattering layer in which comparatively few species are effective scatterers and the species composition is not subject to excessive change. Additional advantages would accrue if the areas concerned were accessible, the layer a permanent feature, and the oceanographic conditions reasonably stable. Accumulating evidence has suggested that such conditions apply in Saanich Inlet, Vancouver Island, British Columbia. Accordingly, an investigation of the scattering layer there has been undertaken.

Saanich Inlet is a fjord, approximately 24 km. long and from 0·5 km. wide in the upper to 6 or 7 km. wide in the lower reaches; the mouth is about 3 km. wide. A sill across the mouth is about 75 m. deep; inside the sill and extending along the length of the inlet for 16 km. are depths near, or in excess of, 200 m.

Two years of observations of temperature, salinity and oxygen[9] indicate that exchange of water between the inlet and outside (Georgia Strait) is slow; periodic flushing of deeper waters has not been observed. Hydrographic conditions therefore appear to be relatively simple. However, an unusual feature (in British Columbian inlets) is the presence of water deficient in oxygen, typical values being 0·1–0·2 ml./l. The deficiency becomes apparent at depths between 80 and 100 m. and extends to the bottom[9]. This condition may be contributed to by substantial quantities of organic material, mostly crustacean exoskeletons, which are found below the scattering layer and are apparently settling on to the bottom. This material is probably in continuous supply and it may well be in excess of the oxygen available for its aerobic decomposition in a water body in which renewal is slow.

Preliminary investigations have included surveys of the scattering layer throughout the inlet. An *EDO* sounder (12 kc./s.), associated with a precision depth recorder, was used for elucidating changes in the density of the scattering and the distribution of the layer in time and place. Sampling of the planktonic organisms was carried out below, in and above the layer, using the 'Catcher', a high-speed closing sampler[10], towed horizontally at speeds between 4 and 8 knots. As well, Clarke–Bumpus samplers, a 1-metre ring net, the Currie–Foxton vertical closing net[11] and a 3-ft. Isaacs–Kidd midwater trawl were used on one or more occasions. A large mid-water trawl with a mouth-opening 11 m. wide was hauled for fish. An echo-sounder transducer was attached to the head rope of the trawl so that it could be fished at any desired depth. It was hauled, usually for 1 hr., at about 2 knots, in conjunction with the plankton tows, but from another vessel.

The presence of a scattering layer in Saanich Inlet has been known for the past two years. It has been investigated briefly on numerous occasions over the past 12 months. The layer is now believed to be a consistent feature of the inlet within which it is confined and over the whole of which it appears to extend. However, the density of the scattering varies from place to place and apparently may do so from day to day at selected positions. The layer lies above the oxygen-deficient water; during daylight it is well defined and about 38 m. deep with its upper surface at about 72 m. After sunset (as detected by a 30-kc./s. Kelvin–Hughes, model 29 *F*, sounder) it has been shown to rise to the surface as a layer at a rate of about 2 m./min. With the 12-kc. *EDO* sounder, on an occasion several weeks later, the scattering appeared to diffuse upwards until its density was more or less uniform between the day-level of the layer and the surface. These differences may be due to changes in the behaviour of the organisms in the layer with time or they may be more apparent than real and result from the use of frequencies of 30 and 12 kc./s. Clearly, problems are posed concerning the biology of the organisms causing the scattering and the nature of the scattering and its relationship to the frequency of the pulse.

Results from the plankton hauls with the catcher (when a net of 10 meshes/in. was used) indicate that euphausiids and amphipods are the predominant 'large' organisms' of the layer along with rare occurrences of shrimp, medusæ, ctenophores and small squid. Of these, one species of euphausiid (*Euphausia pacifica* Hansen, 1·5–2·5 cm. long) and one species of gammarid amphipod (*Orchomenella* sp., 1·5–2 cm. long) far outnumbered any others collected. *Euphausia pacifica* occurred in numbers up to 175/m.^{3} of water filtered, and the gammarid up to 27/m.^{3}. These two species constituted as much as 95 per cent of the total organisms of samples from within the layer (excluding Copepoda, which were not collected in representative numbers by the net of 10 meshes/in.). Biomass was determined on the basis of the wet-weight (filtered and drained) of the total fauna collected with the net of 10 meshes/in. The biomass was nil in oxygen-deficient water; 0·03 gm./m.^{3} just below the layer, 0·01 gm./m.^{3} above and clear of the layer, and 5·2 gm./m.^{3} within the layer. The latter value is very high when compared with 0·1–0·15 gm./m.^{3} in the Black Sea[12]. Copepods, in samples collected with a net of 40 meshes/in. in the catcher, ranged from the larger *Calanus* spp., and *Euchaeta japonica* Marukawa, down to small species such as *Oithona* spp., and *Microsetella* spp. They occurred in high numbers in the layer, but were almost absent below, and sparse above it. The biomass of the copepods has not been determined; but it is less than that of the euphausiid and amphipod. The other sampling gear collected much the same range of organisms as the catcher, but the samples have not been enumerated in detail.

Stratification of the common species of euphausiid and ampliphod is indicated from hauls made at different depths in the layer; the amphipod appears to have predominated at the lower and the euphausiid at the higher levels. In two night hauls near the surface, only *Euphausia pacifica* was captured.

Three myctophid fish (*Lampanyctus leucopsarus* (Eigenmann and Eigenmann)), up to 6 cm. long, as well as two young hake (*Merluccius productus*), of about 13 cm. length, were captured by the catcher. These two species of fish and others were collected by the large trawl as well.

The 11-m. trawl was fished with the mouth opening either in, or encompassing the lower edge of, the layer. For most of these hauls, the 30-kc./s. sounder had shown few or no traces of fish in association with the layer and totals of only 20–40 fish were collected. These consisted of herring (*Clupea pallasii*), young hake (*Merluccius productus*) about 15 cm. long, whiting (*Theragra chalcogramma*) up to 30 cm., a few dog-fish up to 75 cm., *Sebastodes flavidus* and *S. diploproa* 35–40 cm. long, and a few myctophids. On one occasion, scattering attributed to fish was detected immediately below the layer. In a haul of 10 min. through this the catch was in the vicinity of 1,000 lb., consisting of hake, dogfish and a few rockfish (*Sebastodes*). It would seem from these results that fish were not the main cause of the scattering during the period these collections were made. Equally, they contributed to it at times and must be considered as potential scatterers in the layer.

The guts of a number of the fish were examined. A few were empty and the fish may have disgorged, or had not been feeding recently; most contained either the common amphipod or euphausiid or both. The same applied to the stomach contents of a 1·5-kgm. salmon (*Oncorrhynchus kisutch* (Walbaum)) and 0·8-kgm. smelt ((*Hypomesus pretiosus* (Girard)) caught in daylight on a rod and line.

Because the scattering layer in Saanich Inlet is accessible continuously, several features may be investigated. The ecology and biology of the plankton and the fish can be studied to establish relationships between species throughout the year and their distribution in relation to the layer, as well as their distribution horizontally and vertically within the inlet. Relationships of the organisms in the layer to hydrographic conditions must be established, especially to the oxygen-deficient water; it may be possible to demonstrate as well, from field observations, the tolerances of species to low oxygen content.

The determination of the manner whereby scattering is effected can be attempted, and an investigation made of the effects obtained when using a range of frequencies in conditions which are known.

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A SIMPLE SURFACE POTENTIAL METHOD FOR THE DETERMINATION OF SMALL METAL FILM AREAS

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EVAPORATED metal films deposited at low temperatures and partially stabilized by gentle annealing have been widely used to investigate chemisorption. A typical film may weigh 5–10 mgm. with a geometric area of 50–100 cm.^{2} and a true area of the order of 10^3 cm.^{2}, determined by the Brunauer–Emmett–Teller (B.E.T.) method with krypton Improved stability and uniformity of the surface may be obtained by further sintering or by deposition at higher substrate temperatures. The true area may then be only twice the geometric area and the B.E.T. krypton method will be inaccurate unless the volume of the system is very small. This limitation is inconvenient, preventing, for example, the use of an ionization gauge. An alternative is to use another adsorbate with a lower vapour pressure. Thus, ethane[1] has been used at −183° C. on copper films. The method is limited to those cases where the chosen gas does not react chemically with the films. Only an inert gas could have general applicability. Xenon has a low enough vapour pressure at either −196° C. or −183° C., but is difficult to obtain without krypton as an impurity. Preliminary experiments with xenon at −183° C. on a nickel film with an area of about 10^3 cm.^{2} showed that the krypton impurity accumulated in the gas phase. This complication and the need for precise temperature and pressure measurement are avoided by the following method.

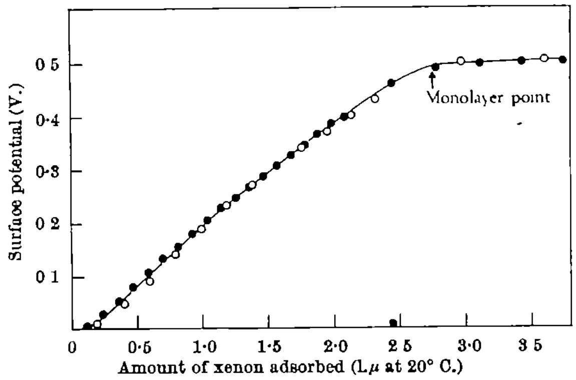

Fig. 1. Adsorption of xenon at −183° C. (full circles) and −196° C. (open circles) on a gold film deposited at −183° C. and sintered at 60° C.

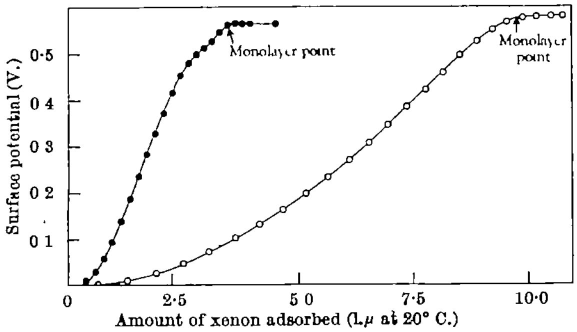

Fig. 2. Adsorption of xenon at −183° C. on a copper film deposited at −183° C. after sintering at −78° C. (open circles) and 60° C. (full circles)

The surface potential of adsorbed xenon[2] may be measured very simply by the diode method, the only necessary addition to the film vessel being a small tungsten cathode at the bulb centre. For metals such as copper and gold the cathode may even serve as the evaporation source. The method of measurement has been described in detail elsewhere[3,4]. Initial experiments with gold films showed a nearly linear dependence of the surface potential on coverage up to a point beyond which the surface potential changed very little and simultaneously a small, but growing, pressure was detectable with a Pirani gauge. It seems reasonable to associate this point with completion of the monolayer, and the succeeding nearly non-polar adsorption with second layer formation.

Early data of Mignolet[5] indicated that pre-sorbed gases reduce the xenon surface potential. Ehrlich and Hudda[6] conclude from field emission studies that the binding energy of an inert gas atom depends critically on how closely it may approach the metal atom array. Because of the relatively large size of the xenon atoms in the first layer it is probable that second-layer atoms are much less polarized and less strongly bound than the first-layer atoms, the strong polarization on clean surfaces of which is indicated by the surface potential. Thus we might expect the monolayer to be practically complete before second layer adsorption starts. This condition is required by both the B.E.T. and the present method if reliable monolayer volumes are to be obtained. In agreement with the ideas of Ehrlich and Hudda it is found that the maximum xenon potential is considerably reduced after sintering.

For the clean surfaces so far examined (copper, gold, nickel, platinum ?) the monolayer point at −183° C. has been reached before a significant pressure (~ 3×10^{-5} mm. mercury) developed. At this pressure the amount of xenon in the gas phase is about 10^{-3} l.μ compared with an adsorbed quantity of one l.μ or more. Neither volume calibration nor accurate pressure measurement is required. A further useful feature of the method is that the result is independent of temperature between −196° C. and −183° C., as shown for a gold film in Fig. 1. Liquid air of any composition may therefore be used. The monolayer point is not always as obvious as in the case of gold or copper (Fig. 2); but in practice the point at which doses of xenon fail to affect the surface potential is quite clear in such cases as nickel (Fig. 3).

The result for platinum (Fig. 3) is probably unreliable. The film was extremely thin and gave only a small positive surface potential with hydrogen instead of the negative effect observed by Mignolet[7] for clean platinum films. Thus the small positive gradient following the monolayer point may be due to second layer xenon adsorption or to xenon adsorbed

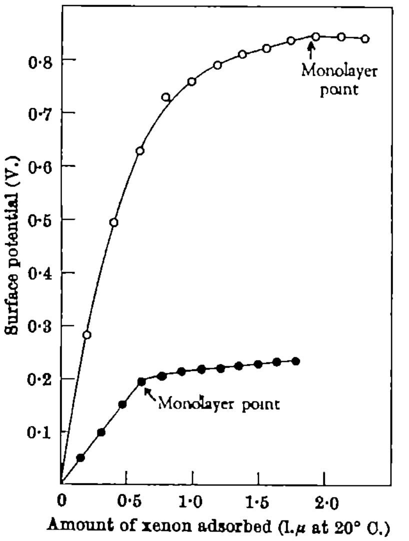

Fig. 3. Adsorption of xenon at −183° C. on a nickel film (open circles) and a platinum film (full circles)

above impurities, the 'monolayer' then being xenon adsorbed between impurity atoms. The very small apparent monolayer volume is noteworthy. In general it is found that pre-sorbed gases markedly affect the apparent monolayer volume and the maximum xenon surface potential. The effect appears to be one of exclusion and gives useful information on the arrangement of adsorbed molecules on the surface. When xenon is totally excluded from direct contact with the metal, for example, in the case of copper saturated with carbon monoxide, it is impossible to determine a monolayer volume as adsorption occurs only at higher pressures and with scarcely any surface potential. Pre-sorbed hydrogen does not appear to affect the xenon monolayer volume.

Having obtained a xenon monolayer volume, a value must be assumed for the effective area of a xenon atom in order to obtain a surface area. A comparison of the lattice constants of crystalline krypton and xenon suggests a ratio of 1 : 1·22. A variety of values has been used for the effective area of a krypton atom. A value of 19–20 Å.[2] appears to be widely accepted. On this basis the area of a xenon atom may be taken as 24 Å.[2]. To check this point a copper film of about 10 mgm. weight was deposited at −196° C. and stabilized by keeping at −78° C. for a day. The film area was then determined by the B.E.T. method with krypton at −196° C., and by the xenon method at −183° C., the respective monolayer volumes being 26·5 l.μ and 21·3 l.μ respectively. These values are consistent with the value 24 Å.[2] for the area of the xenon atom. The absorbed volumes are quoted in units of litre-micron (l.μ) at 20° C. Each unit is thus equivalent to an area of 79 cm.[2].

I thank Mr. W. A. Crossland for assistance with the comparison of the krypton and xenon areas.

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VARIATIONS OF SCALE HEIGHT IN THE UPPER ATMOSPHERE

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THE 'density scale height' H in the upper atmosphere is a measure of the rate at which air density ρ varies with height y, being given by $H = -\rho/(d\rho/dy)$. The value of H, which is important because (together with the molecular weight of the air) it determines the air temperature, is not yet well established at heights above 200 km. The most popular method for evaluating H is from the slope $d\rho/dy$ of the curve of air density (or electron density) versus height; but measurements of this slope are inevitably somewhat inaccurate, because of the errors in ρ.

There are two other viable methods for finding H: (1) from the decrease in the perigee distance r_p of an artificial satellite; (2) from the decrease in the orbital period T of a satellite in a small-eccentricity orbit. Both methods are potentially accurate, since the decreases in r_p and T are directly proportional to H—the decrease in r_p, for example, being given to a first approximation by[1]:

$$r_{p0} - r_p \simeq \tfrac{1}{2}H\ln\frac{e_0}{e}$$

for eccentricities e between 0·02 and 0·2, where suffix 0 denotes initial values. Previous attempts to use these methods[1–3] were not entirely successful, partly because of poor data and partly because the theory needed further refinement. Recently the methods have been further developed[4], taking into account the effects of: (1) the third, fourth, fifth and seventh harmonics in the Earth's gravitational potential; (2) atmospheric oblateness[5]; (3) lunisolar perturbations; (4) the variation of H with height. The methods were then applied to all suitable satellites for which adequate orbital information was available, and the 44 values of H obtained, corrected to 'average day/night' conditions, are shown in Fig. 1. Most of these values are independent of each other, in the sense that they stem from independent values of r_p and T. The points in Fig. 1 fall into a consistent pattern when grouped according to year, as shown by the encircling broken lines, and indicate that the value of H (at a given height) decreased considerably as solar activity declined between 1958 and 1961.

In Fig. 2 curves drawn through the three groups of points in Fig. 1 are shown (unbroken lines), together with selected values from other sources. The 1958 unbroken curve refers to early 1958, since all the relevant points in Fig. 1 are for dates between January and May; similarly, the relevant 1959 points

Table 1. SCALE HEIGHT h AND TEMPERATURE τ, FROM THE VALUES OF H IN FIG. 2

Year	Early 1958			Early 1959			1960–61			Error per cent
Height (km.)	200	250	300	250	350	450	270	350	450	(*S.D.*)
h km.	51	61	66	50	61	64	45	58	57	5–10
M (ref. 7)	27	26	24	26	22	19	25	22	19	10 ?
τ °K.	1,530	1,720	1,680	1,400	1,400	1,230	1,220	1,230	1,090	10–15 ?

are for January–June; and the 1960–61 points cover dates between June 1960 and August 1961. It is estimated that the unbroken curves represent H with errors (*S.D.*) of 5–10 per cent.

Of the other curves in Fig. 2, the broken line shows the values of H in the COSPAR International Reference Atmosphere[7], which refer to 1959 and agree quite well with the early-1959 unbroken curve, though of opposite curvature. The values for late 1960 from ref. 8 (line of short dashes) also agree to within 7 km. with the 1960–61 unbroken line, though obtained by a completely different method. It was noticed[9] in 1959, and has since been confirmed by various authors[8,10–12], that the variation of H with height exhibited a pronounced minimum at some height between 200 and 300 km., as is apparent from the values from refs. 8 and 10 plotted in Fig. 2. The new results for 1959–61 are consistent with these previous findings, since they indicate low values of H (about 35 km.) at heights near 250 km., so that some pause in the increase of H with height is needed for a satisfactory link-up with the values shown at heights below 170 km., which are generally assumed[12] to be little affected by solar activity.

Fig. 2 also shows that, although H continues to increase with height up to 450 km., the rate of increase is much slower above 350 km. This tendency is also evident in refs. 6 and 8, though most other authors (for example, refs. 7, 12–15) have obtained or assumed near-linear increases of H with height in this height-band.

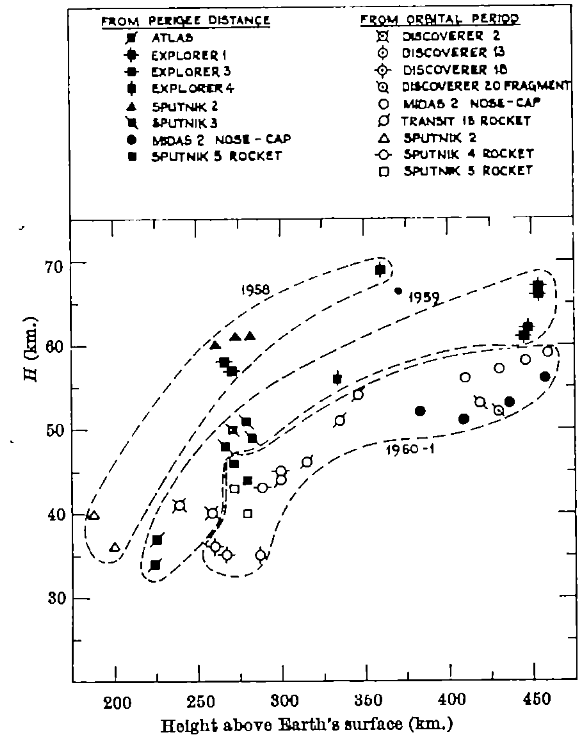

Fig. 1. Values of density scale height H obtained from the changes in perigee distance and orbital period of various satellites

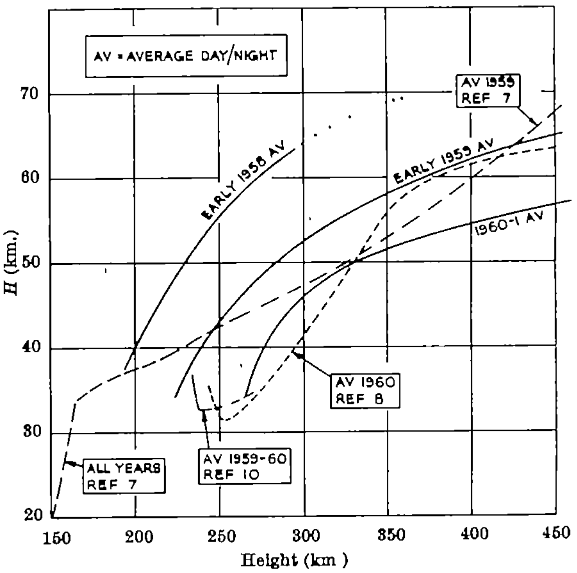

Fig 2. Values of H obtained from Fig. 1 (unbroken lines) compared with selected values from other sources

The scale height h of the upper atmosphere, as usually defined, differs slightly from H, being given by $h = -p/(\mathrm{d}p/\mathrm{d}y)$, where p is the air pressure. From the equation[16] between h and H, it is possible to derive values of h from the curves of H in Fig. 2. If the mean molecular weight M of the air is also known, the air temperature τ can be found. The currently accepted values of M may, however, be in error by perhaps 10 per cent (*S.D.*). Table 1 lists values of h, and also values of τ obtained on taking M from ref. 7.

Thus, between early 1958 and 1960–61, the average day/night temperature at heights of 250–400 km. appears to have decreased by about 500° K. The temperatures obtained here show a maximum at a height between 250 and 300 km., that is, near the maximum of the F_2 layer of the ionosphere; but this is probably not significant, in view of the possible errors.

A full account of this work[4] will be published elsewhere. The results could be greatly improved, both in scope and in accuracy, if more accurate orbits were available for short-lifetime satellites.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Terrestrial Accretion from the Solar Wind

A SERIES of communications has appeared in *Nature* under the above title. We would like to discuss in particular those by de Turville[1,2], who has proposed that the oceans have resulted from the accretion of solar protons, and that by Frith[2], who summarized recent data on stratospheric water-vapour content and showed that this substantiated the hypothesis of de Turville. We will discuss three main points and cite several others, the combination of which seems to indicate that no more than a minor fraction of the water present in the world's oceans has resulted from the accretion of protons from the solar wind. Our main points are these: (1) The solar wind has been measured, and the flux is a factor 10 lower than that used by de Turville in his first communication, and a factor 100 lower than the value used in his second communication. (2) de Turville fails to consider the possibility of escape of hydrogen from the exosphere, which, even if his other assumptions were correct, means that his accretion value is only an upper limit. (3) The observed increase in the water-vapour mixing ratio in the stratosphere cited by Frith does not necessarily mean that the water is of extra-terrestrial origin. There is evidence of large-scale vertical exchange processes in the polar winter stratosphere which can transport the moisture aloft.

The flux of positive particles outside the Earth's magnetic field has been measured by American and Russian space probes. The most extensive Russian data were taken by *Lunik II*. This work was reported by Gringauz *et al.*[3], who quote positive particle fluxes of about 2×10^8 cm.$^{-2}$ sec.$^{-1}$. The American measurements were recorded by *Explorer X*, and were recently reported by Bridge *et al.*[4]. They gave fluxes which varied between 5×10^6 and 10^9 cm.$^{-2}$ sec.$^{-1}$, with an average value of about 4×10^8 cm.$^{-2}$ sec.$^{-1}$. The flux $(n_1 v_1)$ assumed by de Turville in his first communication[1] was $5{\cdot}05 \times 10^9$ cm.$^{-2}$ sec.$^{-1}$ and the value used in the second communication[2] was $4{\cdot}75 \times 10^{10}$ cm.$^{-2}$ sec.$^{-1}$. His values are thus respectively one and two orders of magnitude too large, and the Earth could only accumulate the mass of the oceans during geological time by capturing all protons out to 30 Earth radii, or if fluxes of about 10^{12} cm.$^{-2}$ sec.$^{-1}$ are present in cosmic-ray events associated with solar flares. The latter seems unreasonable in the light of Van Allen's[5] measurements of high-energy ($E > 30$ MeV.) particle fluxes of 2×10^4 cm.$^{-2}$ sec.$^{-1}$ during the strong November 12, 1960, events. Furthermore, as Harrison[2] has pointed out, most authorities expect that the Earth's magnetic field will exert a shielding effect, rather than tend to capture solar particles.

The rate of escape of hydrogen from the Earth's atmosphere is very difficult to calculate, as this rate is dependent on two factors which can only be estimated. These are the concentration of hydrogen and the temperature at the base of the exosphere. However, it is known that both helium-3 and helium-4 do escape. The production-rate of these gases can be estimated within certain limits and they, together with the known concentrations, give atmospheric residence times of the order of 10^7 years[6]. Residence times of this magnitude imply that the atmospheric concentrations represent a steady state determined by escape and production. Thus it is certain that a fraction of hydrogen present in the high atmosphere will be lost, whether it comes from dissociation of water or from the solar wind.

The rate of escape of hydrogen at the base of the exosphere must be balanced by the sum of the hydrogen flux from lower layers, where an equilibrium with oxygen and water is present, and the accretion from the solar wind. Depending on the conditions the hydrogen flux from the lower layers can be positive or negative, but at present we are not even able to attempt to estimate the sign of this flux. Certainly, any amount of hydrogen which could be established as being captured by the Earth (and its magnetic field) can only be an upper limit of the actual amount accreted.

Frith[2] cited data which indicate an increase of the water-vapour mixing ratio in the stratosphere above 15–20 km. in low and middle latitudes and newer data[7] tend to confirm this. However, the data available at present do not necessarily show that this water-vapour in the mesosphere is of extraterrestrial origin. The data of water-vapour mixing ratio do not cover the polar regions, and there is increasing evidence that large-scale vertical mixing processes do occur in the polar winter stratosphere which could provide for the required upward transport of terrestrial water-vapour.

Large-scale vertical motions in the polar stratosphere are indicated by the spring rise of ozone and the explosive warmings in late winter. Recent results of the rhodium-102 high-altitude tracer experiment[8] show that, during winter, air from about 60 km. moves down to the lower stratosphere, resulting in an exchange rate of 0·05–0·2 year^{-1}. These downward movements must be counter-balanced by corresponding upward movements, which most likely occur also over the winter pole and which can carry water-vapour from the tropopause to the mesosphere. Average stratospheric temperatures over the winter pole are such that mixing ratios of about 0·03 gm./kgm. air can penetrate to higher layers and this is about the mixing ratio found in 25–30 km. by various methods. If this is the source of stratospheric water-vapour one would not expect the mixing ratio to exceed 0·1 gm./kgm. air, which means that it must remain fairly constant at altitudes above 30 km.

It is interesting nevertheless to assume that the dry layer present at 15–20 km. extends over the entirety of the globe, and to see how much water would have accumulated on the Earth during geological time. We shall assume a constant mixing ratio above 30 km. in the range between 0·03 and 0·1 gm./kgm. air, an exchange-rate above 30 km. between 0·05 and 0·2 year^{-1} (the same limits found by Kalkstein[8] for the atmosphere above 60 km.), and that the age of the Earth is between $3{\cdot}3 \times 10^9$ and $4{\cdot}7 \times 10^9$ years. We thus obtain a lower limit of 23 per cent and an upper limit of 400 per cent of the mass of Earth's oceans accreted throughout geological time, in confirmation of Frith's results. In the light of our previous discussion of measured solar-proton fluxes, expected shielding due to the Earth's magnetic field, escape of hydrogen from the exosphere, and mixing processes which occur in the winter polar stratosphere, it appears that the coincidence of the integrated influx with the mass of the oceans is entirely fortuitous. However, the importance which is attached to an extra-terrestrial origin for even part of the stratospheric water-vapour means that detailed high-altitude water-vapour measurements, especially in the polar stratosphere, could be of great scientific value.

Finally, one should mention that the oceans account for only 0·025 per cent of the mass of the Earth. Urey[9] indicated that an even greater amount of water was probably held in hydrated silicates in the material which formed the primitive Earth. There is geochemical evidence that the Precambrian atmosphere had much less oxygen than is present on the Earth to-day[10]. It seems more likely that the present oxidized character of the atmosphere has resulted from the dissociation of water and subsequent escape of hydrogen, rather than the ultra-violet dissociation of rocks proposed by de Turville[1]. During the early phases of the Earth's evolution, at which time the speed of rotation was faster and free oxygen in low abundance, the former process probably proceeded quite rapidly.

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THE new observations reported by Gringauz and Bridge appear to confirm each other and, if they are correct, the resulting hydrogen accretion-rate will certainly be too small to account for the bulk of terrestrial hydrogen. Moreover, it is now certain that terrestrial deuterium is definitely not due to a slow accretion of solar deuterium. The previously proposed rate of deuterium production at the solar surface (3·3 × 10^{11} atoms cm.$^{-2}$ sec.$^{-1}$) through neutron capture by hydrogen would imply a flux of 2·2-MeV. gamma radiation of 3·5 × 10^{6} photons cm.$^{-2}$ sec.$^{-1}$ at the orbit of the Earth, whereas Northrop and Hostetler[1] have set an upper limit of only 0·5 photon cm.$^{-2}$ sec.$^{-1}$ for this flux. It seems inescapable that terrestrial deuterium was produced *in situ* by neutron irradiation of terrestrial hydrogen at an early stage in the evolution of the solar system, as proposed by Fowler *et al.*[2]. In view of these considerations, and the difficulties raised by oxygen supplies, the suggestion I made earlier concerning the origin of the oceans has lost all plausibility and I wish to withdraw it.

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GEOPHYSICS

Orbital Acceleration of Satellites during Geomagnetic Disturbance

JOULE heating of the upper atmosphere can explain the fluctuations of orbital acceleration of artificial Earth satellites with geomagnetic disturbance[1,2].

Consider a model thermosphere in which the height (z) rate of change of scale height, H ($= kT/mg$), is constant, that is, $\mathrm{d}H/\mathrm{d}z = \beta =$ constant ($k =$ Boltzmann's constant, $T =$ temperature, $m =$ molecular weight of gas, $g =$ acceleration due to gravity). Then[3]:

$$p/p_0 = (H/H_0)^{-1/\beta} \qquad (1)$$

where p, p_0 are the pressures at height z and at a base level z_0. Assuming $m =$ constant, it follows that:

$$n = n_0(T/T_0)^{-(1+\beta)/\beta}.(g/g_0)^{1/\beta} \qquad (2)$$

A crude match of the model to the actual thermosphere is suggested by taking $\beta \approx 0 \cdot 6$ (corresponding to $\frac{\mathrm{d}T}{\mathrm{d}z} \approx 10°$ K. per km. or a downward flux of about 1 erg cm.$^{-2}$ sec.$^{-1}$). Neglecting the factor $(g/g_0)^{0 \cdot 6}$ over the height-range of present interest (150–650 km.) we have:

$$n \approx n_0(T_0/T)^{\gamma} \qquad (3)$$

where $\gamma \approx 2 \cdot 7$. This shows the sensitivity of thermospheric densities to density and temperature at the base-level.

At high magnetic latitudes joule heating peaks at an altitude of about 150 km.[4,5] and falls off with a scale length of about 25–50 km. above and below this height. Those electric currents flowing in the ionosphere to cause geomagnetic variations (*Sq*) and disturbance (*Ds* and *Dst*) (see following communication) modulate the density and temperature at this height and hence at all heights in the thermosphere.

The rate of heating, $Q(Sq)$, attributable to *Sq* is about 10^{-8} erg cm.$^{-3}$ sec.$^{-1}$. Elsewhere[6] I have shown that such a Q value can modulate the temperature at 150 km. height (0 level) by tens of degrees K., say 50° K. for sake of calculation. Such a change of T_0 will not greatly affect β when $T_0 \approx 500°$ K. originally. There will be a 10 per cent increase in n_0 due to change of scale height at 0 level. It can be seen from equation (3) that the percentage change in n will increase with height until it is about 30 per cent at 650 km. The rate of heating $Q(Ds)$ in low latitudes appears on the average to be about equal to $Q(Sq)$ (see following communication). Thus the average fluctuations of density attributable to joule heating of the ionosphere can amount to about 20 per cent at 150 km. to 60 per cent at 650 km. This compares favourably with variations actually observed[2], namely, ± 20 per cent at 250 km. and ± 50 per cent at 650 km.

At times of severe magnetic disturbance, conditions common to the auroral zones spread to lower latitudes. At these times Q can rise to order 10^{-6} erg cm.^{3} sec.$^{-1}$

(*Continued on page 75*)

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BEYOND THE 'TWO CULTURES'

Towards a Third Culture

By Charles Davy. Pp. 178. (London: Faber and Faber, Ltd., 1961.) 18*s*. net.

THE conception of two cultures, the humanist and the scientific, has given rise to animated discussion, to which Mr. Davy's book is a welcome contribution because it attempts a constructive understanding of the situation. Two important ideas form the core of his thesis—that of the evolution of consciousness and that of the possible development of a "third culture" together with a new mode of consciousness, which would be a synthesis, and more than a synthesis, of the two with which we are acquainted.

Naturally, the argument is to a large extent speculative; but it is conducted with adequate knowledge, both of science and history, and with logical awareness. Mr. Davy believes that the gap between the old and the new culture is rapidly widening and that an entirely scientific culture may become so dominant that the values of the older culture would "survive only as eccentricities". The evolution of consciousness so far has been manifested in the rise of the scientific culture which has been associated with a new mode, or focus, of consciousness. The names chosen by the author for the two consciousnesses are sufficiently indicative of their nature—the 'participating' and the 'onlooker' consciousness. It is an important part of Mr. Davy's thesis that the scientific culture presupposes a consciousness which is aloof and distinct from the object, from 'nature'. The discussion of the gain and the loss which result from the transition to the onlooker consciousness is well worth attention.

There is no attempt to minimize the value or the inevitability of the scientific outlook; it is maintained, however, that there are other ways of knowing and experiencing which can display aspects of reality with which the scientific method cannot deal. The character of the third culture and the more comprehensive consciousness must remain largely conjectural, since they exist so far only in rare and eccentric individuals. Mr. Davy brings in here references to 'seers', which will disturb some readers; Rudolf Steiner is prominent among them. I hope that this part of the book will at least be read with respect, for it would be wrong to reject the 'seers' out of hand as victims of illusion. It must be owned, however, that their visions are incapable of verification and do not altogether cohere with one another. How far they indicate 'something beyond' the Nature studied by science is an open question. *Towards a Third Culture* is a stimulating book, of interest to all who are concerned about the nature of human intelligence and its probable future. Does consciousness evolve and, if so, how and whither? These are fascinating questions.

W. R. MATTHEWS

ORGANIZED THINKING AND EVOLUTION

The Nature of Life

By Prof. C. H. Waddington. Pp. 131. (London: George Allen and Unwin, Ltd., 1961.) 18*s*. net.

PROF. WADDINGTON is distinguished not only as a professional biologist but also as a leader of thought. In earlier centuries he would have been called a philosopher; but to-day's restriction of philosophy to logic, metaphysics or semantics forces him into the slightly pejorative category of 'thinker'.

In this short book he continues to illuminate our thinking about life, human as well as organic, in respect of its nature as a process, its methods of operation, and its results.

He begins by pointing out that science must always be concerned both with understanding and control, and that scientific theories which suggest new advances are often preferable to logically tidier, but less suggestive, theoretical models. He emphasizes the barrenness of 'nothing-but' views which vainly seek to explain complex results in terms of simpler origins, and proceeds to a useful discussion of the two main types of approach now prevalent in biology, which he calls "atomistic" and "continuum" theories, the one aiming at analysis of biological processes and structures into their ultimate components, the other at understanding them as wholes, operating through organized systems in which the parts are interrelated. Both are necessary; but over-emphasis on either leads nowhere. Elsewhere he uses the terms "substance" and "architecture" to characterize the two approaches: one might also suggest 'particulate' and 'organizational'.

He later points out that biological systems, from the genetic outfit to the cell, the individual, the population, and the ecological community, are all organizations with some capacity for self-regulation, and stresses the importance of unitary self-regulating fields and gradients in development.

He very usefully summarizes his own special contributions in this area. First, the realization that the process of development is genetically 'canalized' into a limited number of paths, each of them resistant to interference. For such genetically initiated and largely self-regulating systems of developmental change, he proposes the useful term *creode*, or "necessary pathway". Secondly, his discovery of *genetic assimilation*, whereby, through the operation of orthodox natural selection on genetic capacity to react to environmental modification, the Lamarckian inheritance of acquired character is simulated.

Both this pseudo-Lamarckian mechanism and that of creodic canalization involve cybernetic feed-back, and therefore cannot be adequately described in purely deterministic causal terms. In fact, 'arguing in a circle', or rather in a feed-back cycle, must be a feature of our new cybernetically based biological thinking. This applies also to evolution. At all levels there is a constant feed-back system operating between organism and environment, and between the past and the future direction of the course of evolution. As Waddington says, the implications of this fact are of enormous importance, but have scarcely begun to be studied or worked out.

Waddington proceeds to point out that the frequent assertion that biological evolution is based on chance is entirely untrue. 'Chance' events furnish its raw material, but the process itself is directional—self-steering, but automatically steering itself in a definite direction. This is because, in Ernst Mayr's phrase, natural selection is not a random but an 'ordering' mechanism. It is quasi-finalistic, or, to use Pittendrigh's useful term, 'teleonomic'—it orders chance events into directional channels.

As Waddington continues, "the most important of all the lessons to be learnt by man from the consideration of evolution arises, not from the methods by which evolution has been brought about, but from the nature of the results which it has achieved". The most important of these results is the achievement of progress. Are the later products of evolution on the average 'higher' than the earlier ? Are we, Waddington writes, "in any way better than the worms ? A short answer to this is similar to Dr. Johnson's answer to those who queried the reality of the external world. He kicked his foot against a stone. We might say we will take seriously the worm's claim to be our equals when the worms come and present it, but not before".

On the basis of an interesting analysis, Waddington concludes that we must assuredly recognize the existence of progress in evolution. But his analysis would have been more complete and his conclusion more firmly grounded if he had not equated biological progress with biological improvement. For one thing, his "we may expect natural selection always to effect an improvement" is too extreme—Darwin was both more comprehensive and accurate in the *Origin of Species* a century ago, when he wrote that natural selection inevitably leads to the improvement of the majority of organisms in relation to their conditions of life. In restricting the improvement to the majority of organisms he was presumably thinking of the inevitable limitations of all improvement. An improvement in a detailed adaptation such as cryptic resemblance cannot go beyond a certain degree of accuracy: an improvement in the efficiency of a way of life, such as that seen in the evolution of the horse, cannot proceed beyond a certain degree of specialization. All evolutionary trends tend to reach a limiting value and to become stabilized there.

Darwin's other caveat was inherent in his phrase "in relation to the conditions of their life". Biological improvement, in other words, is always relative. It may be relative to the efficacy of a single adaptation, such as Batesian mimicry; to life in an extremely narrow ecological niche, as with leaf-miners or clothes-moths; to specialization for a particular mode of life, such as grazing on open plains or internal parasitism or predation in the open sea; or finally to efficiency of general organization, as in mammals as against reptiles or teleosts as against other fish.

Progress may thus be broadly defined as improvement in general organization in relation to the entire evolutionary situation at that time, with the further proviso that it is itself progressive and does not bar the way to further improvement. We must therefore distinguish between 'improvement', which is general and indeed universal, and the particular and very special occurrence of 'progress'.

The book ends with a short chapter on "Biology and Man", which rightly stresses the fact that the nature, mechanism and course of evolution change radically on its passage from its biological to its human or psychological phase. In this field again—as set forth more fully in his earlier book, *The Ethical Animal* [see *Nature*, **193**, 409; 1962], Waddington has made an important contribution, by pointing out that human behaviour and human ethics are based on what he calls authority-bearing or authority-acceptance systems in the human baby. In the present chapter he follows up this point by a discussion of free-will, self-awareness, 'unconscious' mind and other psychological matters in a somewhat tentative way.

In an earlier book, Waddington very properly took the physicists to task for neglecting to take mind and consciousness into account while yet claiming that physiochemical "explanations" of Nature were the best and most scientific. When he returns to this topic, as I am sure he will, I hope that he will focus his treatment on the central biological fact of psycho-physical unity, and on the most important of all progressive trends, the trend towards 'more mind', with the brain as the organ of improved awareness.

Meanwhile we are grateful to him for his clarification of many important factors in the all-important process of evolution. JULIAN HUXLEY

POETIC IMPRESSIONS OF A SCIENTIFIC ERA

The Poet and the Machine

By Dr. Paul Ginestier. Translated from the French by Martin B. Friedman. Pp. viii + 183. (Chapel Hill, N.C. : University of North Carolina Press; London : Oxford University Press, 1961.) 30*s*. net.

DR. PAUL GINESTIER is lecturer in French in the University of Hull. The theme which he is considering is an old one, and we may refer to the formulation of the problem by T. E. Hulme[1] nearly forty years ago: "The association of art and machinery suggests all kinds of problems. What will be the relation to the artist and the engineer ? At present the artist is merely receptive in regard to machinery. He passively admires, for example, the superb steel structures which form the skeleton of modern buildings, and whose gradual development in a parasitic covering of stone is one of the daily tragedies to be witnessed in London streets. Will the artist always remain passive, or will he take a more active part ? The working out of the relation between art and machinery can be observed in many curious ways. Besides the interest in machinery itself, you get the attempt to create in art, structures whose organization, such as it is, is very like that of machinery. . . ."

Dr. Ginestier makes several claims. He holds that imagery from the mechanical world is now being widely and effectively used in English, American and French poetry; from which he quotes freely and pertinently. The imagery he perceives as part of the archetypal patterns which emerge from the subconscious of Freudian man. There is thus a possibility of the re-vitalization of poetry, and an extension of its profound significance: "After the inhuman harshness of the industrial revolution which is coming to a close, the poets of the machine age are re-introducing the most profound and poetic humanism in a civilization in which we are beginning to lose hold because the speed of its progress made it lose touch with the realities of life" (p. 178). (At least two serious assumptions are latent in the quotation.)

So, he says, we must study the poetry of the machine: of fire, mine, forge, factory, building, bridges, traffic, harbours, motor-cars. The list of poets is formidable; it ranges from Villon, Milton, Musset (there is, curiously, no reference to Blake), Eliot, MacNeice, Carl Sandburg, E. E. Cummings, Hart Crane, Wallace Stevens; less familiar are the quotations from Cendrars, Emmanuel Aergeter, Ivan Goll, Loÿs Masson—and a host of others. The quotations, which are usually brief, are analysed in terms of standard Freudian imagery, with anthropological reinforcements. The outcome seems to be a kind of catharsis. "The poetry of the machine effects the transference of dreaming into the current of life, giving it a new value." Flight, for example, allows the poet to achieve "man's liberation from the Icarus complex, his feeling of being unable to effect 'the unification of the force which moves and the object which is moved'. Through the poetry of industrial effort, man will be able to recover from that psychopathology of failure which characterizes our age" (p. 175).

The author writes with great energy and conviction; the bulk of his reading and learning is enormous. His analysis is applied with great ingenuity, and sometimes with a certain *naïveté*; I might cite as examples the explication of the extract from Verhaeren, *Les Villes tentaculaires* (p. 57), or of Hugo's *La Conscience* (p. 84). Is it really valuable or valid to write (after quoting Ashley and Allard), "Thus the railway station has an obvious value for mental catharsis. One can dream about travelling or drop off into space without fear of being carried away. The freight train is one of the machine age's answers to man's fundamental need for a scapegoat [here there is a reference to *The Golden Bough*] but it has acquired an element of nightmare horror" ? (pp. 85–6).

For all Dr. Ginestier's enthusiasm and ingenuity, the central dichotomy seems to me still unresolved. What the poets have indeed done is extend the range of their imagery; they are probably using more contemporary idioms from the world of science than at any time since the seventeenth century. But the poet does not seem to have accepted the machine, integrated it into his poetic vision, any more than did the Romantics. Blake, Shelley, Carlyle, Morris revolted against the Age of the Machines for reasons that were predominantly social. The modern poet protests against the mechanical society as the destroyer of man's meaning as an individual. His fear is that of man as automaton—see Auden's *Epitaph to an Unknown Citizen*, or MacNeice's *Prayer before Birth*.

The quotations set out in the book suggest this protest, in varying degrees of bitterness, loneliness, frustration: and it is not enough to select examples of imagery, to point to their archetypal nature, and to conclude, merely because the imagery can be shown to have Freudian significance, that any poetic symbiosis has taken place. Before we can reach such a deduction each poem would have to be investigated as a whole, in the totality of its statement; and this the method of the book does not provide for. A mere study of the imagery, in isolated passages, is insufficient to establish the striking and optimistic claims which the author makes for the high destinies of poetry.

T. R. Henn

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[1] *Speculations*, 105 (1924).

ORIGINAL THINKING IN SCIENCE

The Inspiration of Science

By Sir George Thomson. Pp. x + 150. (London: Oxford University Press, 1961.) 18*s*. net.

THIS small volume from the pen of a very outstanding British physicist is somewhat unique in many ways. The title corresponds reasonably well with the recurring theme of the author, who concerns himself, as a physicist, with the mode of action of scientists of great achievement. His thoughts on the manner in which they proceeded to establish their fame through their various works are sure to ring a responsive chord in the minds of those who have themselves given thought to the question of how the notable advances have come about. The author illustrates his views as to the reasons for success with many illuminating examples.

It is no easy task that is assumed in this work. The subject is not straightforward history of science (though there is a good deal of that inserted in the form of stories of the outstanding men of science) nor yet is it explored in text-book fashion, though here again the author pays enough attention to physics in particular to make his theme clear to those of limited knowledge. In my own case I had good cause to believe that Sir George succeeded very well in this second matter because I kept losing the volume until I found that one of my sons, fourteen years old, was removing it for his own perusal. Judging by his obvious enjoyment of the work there is every reason to conclude that the book should appeal to a wide audience, that many reasonably informed laymen, or students of the arts, should find it of considerable interest and value to them. Yet, even if it is judged from the point of view of the physicist, there is much of great value and, in my own case, the method of presentation of familiar matters was found to be most stimulating. To take one example, the discussion on Chapter 5 of mass, energy and matter is confined to less than eleven pages; but it concerns itself with a very fundamental subject indeed and the thinking of the author comes through admirably. This is surely something that should concern more and more scientists—the need to let others see how they think about the really important concepts of science, and no one could say that the author shirks this duty. One of the most educative processes in life is to be found in the opportunity to 'listen in', so to speak, to the thinking of acknowledged experts, an idea which lay behind the habit in the past of young scientists to attach themselves for a period to a school under some great man such as Rutherford. This method of learning is not so obviously followed to-day when the tendency is to go to a centre housing some expensive machine, not necessarily the home of a great thinker or thinkers. This may well be a fault in our present system. For this reason and many others, we must be grateful to the author for giving us so much of his own philosophy in his volume.

The book is a most welcome effort to explore an aspect of science as yet inadequately covered, and it can be highly recommended to professional scientists and to laymen who definitely wish to understand more of science and those who practise it. It could prove very attractive to senior school pupils in science who wish to acquire a clear picture of the breadth and depth of the fields ahead of them.

S. C. CURRAN

THE MIND OF THE CRIMINAL

The Psychology of Crime

By David Abrahamsen. Pp. xii + 358. (New York: Columbia University Press; London: Oxford University Press, 1960.) 48*s*. net.

AFTER his first comprehensive study, *Crime and the Human Mind* (New York, Columbia University Press; 1944), Dr. Abrahamsen offers in his recent book a reappraisal and critical assessment of the dynamics of criminal behaviour and, based on this, on the treatment of offenders and the prevention of crime. The book is an American counterpart of Edward Glover's *Roots of Crime* (Imago Publishing Co., London, 1959). It is one more contribution to criminology from the point of view of psychoanalysis, discussed in the light of the author's practical experience in the clinical examination of criminals and his research project on sexual offenders in the New York prison of Sing Sing.

Dr. Abrahamsen reaffirms certain fundamental results of dynamic psychology, the importance of experience in early childhood, the part played by emotional disturbance in the origins of a social and anti-social behaviour, and the significance of the unconscious for the explanation of apparently motiveless crimes. The psychoanalytical doctrine of the development of the child's mental life through different distinct stages offers an explanation of the manifest immaturity of many criminals as a regression, a fixation of an earlier stage, especially in the sexual sphere.

The multiple and relative character of crime causation is clearly seen with the infinite combinations of inclinations, environmental conditions and the degree of personal resistance. In the author's experience, emotional deprivation with its damaging effect on future personal relationships is a frequent cause of deviant behaviour. The latter may be either neurotic or criminal, an important distinction with regard to the origin and forms of manifestation and to the choice of treatment. As a forensic expert on insanity, Dr. Abrahamsen is, at the present state of our knowledge, reluctant to say in accordance with the Durham-doctrine whether a particular crime is the outcome of a specific mental disturbance. He rather prefers to interpret the McNaghten rules liberally; 'knowing' the wrongful character of one's act means not only intellectual perceiving, but also emotional understanding (p. 254). Prisons, though indispensable for secure segregation of dangerous criminals, are failures so far as treatment is concerned (p. 273). Only a detailed knowledge of the offender's character development from early childhood onwards and of the personal influences around him provides a reliable prognosis of persistent dangerousness, or susceptibility to treatment which should bring to the surface any positive traits on which something constructive could be built.

It is the merit of this book that it is not a mere collection of facts and figures but an interpretation of the empirical material. A psychoanalytical exploration of the unconscious relies on the interpretation by the examining psychiatrist. In an admirable chapter on the examination of offenders (p. 208 *seqq.*) the author rightly asserts that it requires intuition, if not art, to diagnose a human personality. At the same time, theoretical doctrines have to be adapted to the realities of present-day life. While traditional standards become unstable and relative, the treatment of deviant behaviour is less concerned with neurotic effects of suppression than with character disorders "reflecting a distorted ego and super-ego structure acting out" into crime (p. 64).

The lasting contribution of modern medical psychology to the study of crime is the insistence on intensive examinations of individual offenders. This is the keynote of the present book. Only when it comes to conclusions, certain generalizations and typified analytical conceptions are not always avoided. Guilt feeling is certainly often the basis of anxiety, but one likes to see more evidence of a craving for punishment as a frequent motivation of crime; the accident proneness of offenders may often be due to indifference, recklessness or, among certain personalities, to excessive day-dreaming. There are probably more and different motivations of shoplifting by women than the sexual interpretation offered by psychoanalysts. Perhaps the time will come when psychologists and sociologists join hands in a common effort to master the complex phenomena of crime.

M. GRÜNHUT

PLANNING FOR EXCELLENCE IN EDUCATION

The Year Book of Education, 1961

Concepts of Excellence in Education edited by Prof. George Z. F. Bereday and Prof. Joseph A. Lauwerys. Pp. x + 503. (London: Evans Brothers, Ltd., 1961. Published in association with the University of London Institute of Education, and Teachers College, Columbia University, New York.) 63*s*. net.

THE editors have chosen this year to concentrate on a field of study which is the scene of acute contemporary controversy. In doing so, they have performed a valuable public service, and if the *Year Book* suffers, as it always must, from the very varied degrees of excellence in its own contributions, it cannot be faulted in its main conception. America, Europe (including Britain) and the Soviet Block are all concerned to-day with concepts of excellence in education and with the practical question of how the education of those who excel should be planned. Debate on the subject has become heated, particularly in Britain, and this massive addition to our information should do something to weaken the force of arguments which are based on nothing but a combination of 'hunch' and ignorance. "It is really most astonishing", the general introduction points out, "that successes achieved in the U.S.S.R. with a common non-selective secondary school system should be used in Western European countries like Britain to justify the existence of a selective secondary system."

The first section of the book, which consists of a series of essays on different concepts of excellence, is the weakest. One gets, for example, an interesting account of the Yugoslav school system, a historical sketch of the development of American education, and a socio-economic essay on Asian society: but little about 'excellence'. It is unfortunate that the English contribution should repeat the legend of Arnold's responsibility for the cult of games as part of the education of the gentleman.

The second section is much more valuable and contains some excellent discussion of the different systems adopted to educate that aspirant after excellence, the 'gifted child'. Three main patterns emerge. The American and the Russian are firmly committed to the common secondary school, while the European and British show a tradition of segregated schools being modified in the direction of the 'comprehensive principle'. It is particularly interesting, for example, to read a classic exposition of the English 'tripartite' division by Lord James, immediately followed by a defence from Roger Gal of the French preference for postponing 'positive differentiation' until about the age of fourteen or fifteen. There are excellent descriptions of the Swiss and Italian segregated systems, but on the whole the major weight is given to the comprehensive schools of North America and the Soviet Block. This is probably justified if the editors are right in their feeling that the trend of world opinion is towards a common school with varying devices for differentiation within it, until the age of fifteen.

The device used to provide for the gifted child in the Soviet Block is the provision of a wide range of study circles outside school hours. These circles study mathematics, physics or languages in a more adult way and to a more advanced level than is provided for in the school curriculum. In North America the same objective is sought by the twin methods of 'enrichment' and 'acceleration'. The case studies in the third section of the book are mainly American and show that 'acceleration' is no more than the promotion by ability rather than age with which English public schools with a wide ability-range have long been familiar. Enrichment classes seem not unlike the Soviet study circles except that they find their place inside the curriculum instead of out.

A short review cannot do detailed justice to a *Year Book* containing forty-three separate articles, some, like Prof. Bereday's, of surely lasting value. But clearly no one ought to discuss the 'Grammar/Comprehensive' controversy further without referring to it. The proof reading has been at times curiously lax. It is startling, for example, to read that "the Muslims of Dacca were abolished to promote the cloth from Bradford and Manchester". A. D. C. PETERSON

ACTION AT A DISTANCE AND METAPHYSICS

Forces and Fields

The Concept of Action at a Distance in the History of Physics. By Dr. Mary B. Hesse. Pp. x+318. (Edinburgh and London: Thomas Nelson and Sons, Ltd., 1961.) 35*s*. net.

THIS book deals historically and philosophically with the concept of action at a distance up to its present almost complete eclipse by field theories. The scope is indicated by the headings of the eleven chapters, of roughly equal length: the logical status of theories, the primitive analogies, mechanism in Greek science, the Greek inheritance, the corpuscular philosophy, the theory of gravitation, action at a distance, the field theories, the theory of relativity, modern physics, the metaphysical framework of physics. There are two appendixes, a bibliography and indexes. It is a scholarly work, obviously the result of long study. The style is pleasant, but occasionally long sentences make the going heavy, for example, there are sentences of eight lines each on pp. 260 and 298. A reader unversed in the techniques of mathematical physics will find some of the book difficult. This is not due to proliferation of mathematical formulæ (there are few), but because, from the time of Newton, physics can scarcely be understood without its mathematical formulation. To those with the requisite background I recommend the book, particularly to young physicists who, by studying the queer ideas of their forebears, may come to see that they too may think queerly without knowing it.

However, in spite of the high merit of the book, the respect I have for the author's erudition, and the shrewd and penetrating remarks scattered through the pages, I ended it with some feeling of isolation. The party was on, I had been invited; but somehow I could not join in because I was wearing the wrong clothes. The thing was brought to a head in the closing paragraph of the book, which I now quote with the addition of my own italics:

"Our discussion has not enabled us to answer the question 'Do bodies act at a distance'—this in any case is not *the business of the philosophy of science*. It has not enabled us to show that physics itself gives a definite answer, because although we have maintained that physical theories are asserted as facts, the problem of action at a distance is not so much a problem of particular theories as of *metaphysical framework*. The discussion has, however, enabled us to compose some variations on a theme: namely, the historical, heuristic, and logical importance for physics of ideas and assumptions commonly called *metaphysical*. A society which is uninterested in *metaphysics* will have no theoretical science."

Reading this over, particularly the italicized parts, I oscillate between extremes of humility and arrogance. Dr. Hesse handles with assurance certain technical expressions which I do not understand, and modesty demands that I creep into a corner, leaving her to pontificate in her field of expertise. But, on the other hand, I am a theoretical scientist, and I begin to wonder whether my inability to understand what she says about theoretical science may not be due to the absence of meaning in her words. In this mood, I feel inclined to throw them back at her, telling her that the philosophy of science and metaphysics are nothing but two empty battered tin cans drawn behind the triumphal car of science on strings two or three centuries long. It would help me to find an equilibrium between these two extremes if Dr. Hesse would look over my shoulder when I am trying to understand or solve some scientific problem, and say, as I write down one formula: "That is philosophical!" and, as I write down another: "That is metaphysical!" provided that in the circumstances self-consciousness did not prevent me from writing down any formulæ at all.

I searched the *Oxford English Dictionary* for a meaningful meaning of 'metaphysics', but did not find it; I found instead a quotation to end this review with: Shakespeare, *Taming of the Shrew*, I, i, 37 "The Mathematickes, and the Metaphysickes, Fall to them as you finde your stomacke serves you".

J. L. SYNGE

THE COSMOLOGICAL PROBLEM

Fact and Theory in Cosmology

By Prof. G. C. McVittie. (A Survey of Astronomy, Vol. 3.) Pp. 190. (London: Eyre and Spottiswoode (Publishers), Ltd., 1961.) 15*s*. net.

PROF. McVITTIE, formerly of the University of London and since 1952 professor of astronomy in the University of Illinois at Urbana, is one of the leading authorities on the interpretation of the empirical data bearing on the cosmological problem. In his latest book, to quote his own words, he has attempted "to weld together the astronomical observations relevant to cosmology with cosmological theory without entering into detailed mathematical proofs". He is an unashamed empiricist in his outlook, and there is a refreshing air of 'realism' about his approach to a subject in which the inadequacy of the data tends to encourage dogmatic theorizing from intellectually seductive 'principles' and postulates. In his opening chapter on the nature of cosmology, he exposes the variant of the 'argument from ignorance' fallacy whereby it is asserted that a statement is to be taken seriously if no observational evidence 'against' it can be adduced. Nevertheless, although McVittie lays his emphasis on the empirical data, he recognizes the necessity of theory for their interpretation.

Chapter 2 is devoted to an introductory account of the fundamental problem of distance determination, including a careful description of the use of Cepheid variables to determine distance moduli of local galaxies. Prof. McVittie brings out very clearly the uncertainty in our knowledge of the distance of so familiar an object as the great nebula in Andromeda. Chapter 3 is on the galaxies in general, and is useful for bringing together both visual and radio astronomy.

The next chapter, on theories of the universe and links with observation, is followed by one in which the effects of the red-shift are described, in particular its relation to distance. McVittie emphasizes that this relation is, in general, non-linear and that the linear form holds only for small shifts.

In Chapter 6 we encounter the central problem of using the observational data to select a particular world-model. McVittie shows that the red-shift data are at present sufficient only to narrow the class of admissible models by restricting the range of possibilities for the expansion factor. To determine the space-curvature index, appeal must be made to estimates of the mean density of matter in the universe. The principal snag is that, unlike their red-shifts, the distances of remote galaxies can be determined only by prior choice of a model universe and cannot be assigned uniquely on the basis of the relevant empirical data (apparent magnitude determinations).

Hopes have been expressed in recent years that these obstacles might be by-passed by studying the distribution of class II radio sources. McVittie indicates the difficulties of this approach, and the restrictive assumptions underlying the belief of most radioastronomers that, if these sources are uniformly distributed in space, the law expressing their number as a function of limiting flux-density must involve the exponent $-3/2$.

The final chapters are devoted to the steady-state theory and to a brief summary in conclusion. There is an appendix of useful data relating to clusters of galaxies and references to literature.

The book can be warmly recommended as an authoritative introduction to the subject at a level intermediate between the popular essay and the advanced mathematical treatise. The print is very clear and the price is extremely reasonable.

G. J. WHITROW

A CONSERVATIVE VIEW OF THE PLANETS

Planets and Satellites

Edited by Gerard P. Kuiper and Barbara M. Middlehurst. (The Solar System, Vol. 3.) Pp. xx + 601 + 106 plates. (Chicago and London: University of Chicago Press, 1961.) 12.50 dollars; 100*s*.

THIS is the third of what was to have been a series of four volumes, now increased to five, dealing with members of the solar system. The first two, on the Sun and the Earth, respectively, are already well known. It must be at least ten years since Prof. Kuiper first planned the series, years during which the successful developments of rockets, artificial satellites and 'space probes', as scientific tools, have brought a revolution in the possibilities for research within the solar system. These developments have led naturally to a great revival of interest in important problems, comparatively neglected until recently, concerning the physical nature of our Moon and the planets.

The book, therefore, appears at a very opportune time. "Designed to be a reference book for the specialist and also a source of information for non-astronomical scientists working in allied fields of study" it gives a reliable picture of some of what we have learnt so far, starting from ground-based observations in both optical and radio frequencies. Some subjects of particular present-day interest have been reserved for Volume 4, including mapping of the lunar surface, planetary spectroscopy and comets. There is no discussion of what at present must necessarily be speculative theories, for example, of the origin of the planetary system. As mentioned in the preface, there has been some delay in publication, and evidence elsewhere shows that at least one or two articles were originally prepared more than five years ago, although with some later revision. However, this should not deter the reader; most of the subject-matter is of the less-ephemeral kind, and there is much which is not only of contemporary interest but also should remain valuable for many years. A good deal of the material appears here for the first time, while some has been available until now only in scattered publications.

S. Fritz and H. Wexler give a short, abundantly illustrated and very instructive article on photographs of the Earth from *Tiros I*. Clyde W. Tombaugh describes the search for trans-Neptunian planets. One of the best chapters in the book is a critical review of the orbits and masses of planets and satellites, by Dirk Brouwer and G. M. Clemence. It is followed by a much more specialized article, by Y. Hagihara, on the difficult problem of the stability of the solar system. Rupert Wildt gives a clear, authoritative account of the theory of planetary interiors, a subject of much interest to physicists as well as astronomers. M. Minnaert, whose clarity of exposition is well known, deals with results from photometry of the Moon, and one of his sentences may perhaps be quoted: "Provisionally we are inclined to picture the lunar surface as very porous (photometric properties), all the details being covered by a thin layer of dust (polarimetry)". D. Barbier discusses the photometry of lunar eclipses, with special reference to properties of the Earth's upper atmosphere. Daniel L. Harris's chapter on the photometry and colorimetry of planets and satellites contains many hitherto unpublished measurements, made at the McDonald Observatory. There are two particularly good contributions by Audouin Dollfus, the first on polarization studies, the second on visual and photographic studies of planets at the Pic du Midi, which should lead to a wider appreciation of our debt to the author and his mentor, Bernard Lyot, who have greatly influenced present ideas about planetary surfaces and atmospheres. Edison Pettit describes early thermocouple measurements of planetary temperatures, and is followed by William H. Sinton on more recent radiometric work.

There are three chapters on radioastronomy. In the first, Cornell H. Mayer describes radio measurements in general, especially as they relate to thermal emission. Perhaps the most interesting result, now well known, is the high temperature, about 600° K., shown by Venus at 10-cm. wave-length. The next two chapters, which might have been combined, are by B. F. Burke and Roger M. Gallet, respectively, both dealing with the intense bursts of radio emission from Jupiter, not yet fully understood. Milton L. Humason contributes a selection of planetary photographs taken with the 200-in. telescope and W. S. Finsen some interesting colour photographs of Mars. Although the illustrations are very good, I have the impression that there has been a perceptible loss of quality, probably unavoidable, in reproductions of planet photographs here and elsewhere in the book. The final chapter is by the senior editor, who winds up with an account of searches for intra-Mercurial planets and for new satellites.

The book is to be welcomed as a valuable addition to the rather sparse literature of this corner of astronomy. As in most collective works of this kind the articles do not appear to be all equally valuable, but their general standard is very satisfactory; half a dozen of them impressed me as unusually good. Editorial policy appears to have been to avoid subjects or theories which are pretty certain to change rapidly in the next few years. This may not suit the taste of some readers, but it ensures a longer useful life for the book.

R. O. Redman

THE EARTH TODAY

The Earth Today

Edited by A. H. Cook and T. F. Gaskell. (A collection of papers dedicated to Sir Harold Jeffreys by some of his students and colleagues on the occasion of his 70th birthday.) Pp. xi+404. (London: Royal Astronomical Society, 1961. Distributed by Oliver and Boyd, Ltd.). 80*s*. net.

It was a happy idea of the Royal Astronomical Society to arrange a special issue of the *Geophysical Journal*, also available as a bound volume, containing papers by pupils and colleagues of Sir Harold Jeffreys in celebration of his seventieth birthday. The title appropriately recalls the four editions of Sir Harold's great work *The Earth*, which is a landmark in the application of mathematical methods to the solid Earth. The papers are restricted to those on subjects which Sir Harold discusses in *The Earth* and therefore reflect the influence of his ideas. The first group of papers discusses the motion of satellites around the Earth and the consequent determination of the Earth's external gravitational field from observations of them. The next two groups deal with seismic body and surface waves. The fourth with the free oscillations of the Earth, the fifth with problems of heat conduction in the Earth. The two articles on the theory of the excitation of the Eulerian nutation and the Cromwell equatorial current and two on instruments complete the volume.

The volume, perhaps appropriately, respects Sir Harold's views on 'the Earth to-day'. Our planet is presented as being a perfectly elastic body, entirely non-magnetic, no drift of continents is allowable, and of course no convection in the mantle—silence hanging over the recent apostasy of oceanographers on the latter topic. Even Sir Edward Bullard, discussing heat-flow through the Atlantic Ocean, only hints at convection in the mantle. To maintain this static view of the Earth it is necessary to ignore some very important experimental results, and following Sir Harold's example in the fourth edition of *The Earth*, where the subject of palæomagnetism is refuted in two self-contradictory paragraphs, F. F. Evison explains away the rock magnetism data by a hypothesis which any field geologist could as simply refute as Dr. Samuel Johnson's famous rebuttal of Berkeley's philosophy. Or perhaps these critics of continental drift are believers who are following the precepts laid down by Cornfor in *Micro-Cosmographica Academica*, that ancient guide to academic politics, which advised those who wish to get new ideas accepted to argue weakly against their own side.

One may conclude, therefore, that while all geophysicists will wish to possess this valuable book, it would be a pity if anyone thought that it was in any real sense a survey of the growing points in geophysics to-day. Yet the subject has now reached a stage where it seems that the exciting hypotheses that the Earth has formed by accretion, the continents have drifted, the ocean floor has recently been formed and thermal convection is occurring in the mantle, can all be finally checked by new experimental methods.

S. K. Runcorn

EARLY MODELS OF THE UNIVERSE

The Astronomical Works of Thabit B. Qurra

By Francis J. Carmody. Pp. 262. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1960.) 48*s*. net.

Dr. Carmody's book is the first comprehensive collection that has ever been available to Western scholars of the works of the Arab astronomer Thabit ben Qurra. The work is not a translation; the author has reproduced principally, but not exclusively, the texts of Gerard of Cremona (1114–87), to whom we must always be indebted for the translation into mediæval Latin of much of the Arabic corpus of astronomy.

Thabit ben Qurra (Bencorath) was born about A.D. 836 at Harrân in Upper Mesopotamia. He became the leading member of a group of four translators who, following the enlightened practice established by al-Ma'mûn, Caliph of Baghdad A.D. 813–33, continued the translation of the Greek astronomical manuscripts into Arabic. His predecessor, al-Hajjâj, had translated the Almagest. Thabit had used the work of al-Hajjâj and realized that there were errors in some of Ptolemy's basic data. He tried to lessen the error by using a tropical reference for the Sun which did not neglect the apparent precession of the stars. To this end he wrote his two most important works, *De Anno Solis* and *De Motu octaue spere*. In the former he quotes Ptolemy's statement that the difference between the two types of solar year—referred to fixed equinox position and referred to star-positions—was negligible. Thabit endeavours to establish a method of reckoning the solar (anomalistic) year by making his reference point the solar apogee. Dr. Carmody has carefully checked all Thabit's calculations and has re-computed both forward and backward. By doing so he has revealed the reason for the rather indifferent results obtained by the Arab; for despite using carefully chosen parameters, Thabit has used a number of unjustified approximations.

In *De motu octaue spere* Thabit goes further into the problem of precession. He adopts the theory of trepidation—that the zodiac moves advancingly by about 8° and then retrogrades by the same amount, at the rate of about 1° in 80 years. Dr. Carmody gives two Latin versions of the work, and a very full analysis of the mathematical methods used, and also examples of the Toledan tables derived from Thabit's work. There follow three shorter papers, *De Hiis indigent expositione antequam legatur Almagesti*—"For those in need of an exposition before the Almagest is read"; in fact, for beginners or students. In the paper *De quantitatibus stellarum et planetarum et proportio terre* are some interesting contemporary ideas of planetary distances and dimensions.

The final work reproduced, in two parallel Latin versions, is *De Imaginibus*; a book of magic giving instructions for the making and burying of small images to increase one's chances of succeeding in various undertakings. Dr. Carmody finds Thabit's insistence on using astrology in connexion with the images strange; but as he states, Thabit was a Sabean. He was, in fact, a pseudo-Sabean, a Harrânian. The inhabitants of Harrân had been forced by the Caliph al-Ma'mûn, during the life-time of Thabit's parents, to adopt some recognized form of religion. They chose that of the Sabeans, a semi-Christian sect of Babylonia, tolerated by the Muslims, and mentioned in The Koran. Harrân was the provenance of that small group which had preserved since the time of Alexander the Great the Hellenistic and Babylonian cultures. The true Sabeans were star-worshippers versed in astrology and magic, and Sabeanism, at least as a cloak for their practices and beliefs, suited the Harrânians. Thabit was but one of many brilliant scholars and scientists from the same town who communicated a great deal of Greek and Babylonian knowledge to the Muslims.

In his valuable appendixes, Dr. Carmody gives a full account of his own critical method in preparing the book, and lists with meticulous care all his sources. He includes many clear diagrams, some extracts from the Arabic texts, and an analysis of many of the Arabic–Latin equivalents used by the early translators.

Dr. Carmody has not written for a popular market. This book is a further example, and an excellent one, of his devoted work in assisting present and future scholars in their research into the history of science.

Leonard W. Clarke

A CLASSIFICATION OF LIVING ANIMALS

A Classification of Living Animals

By Lord Rothschild. Pp. vii + 106. (London: Longmans Green and Co. Ltd., 1961.) 25*s*. net.

During his studies of spermatozoa, Lord Rothschild found that he was often frustrated by having to consult a number of books, instead of one, to find an up-to-date classification of the animal kingdom. It occurred to him that other scientists might have the same difficulty, and he has now produced a single-volume classification of living animals intended for "physiologists, biochemists, biophysicists and those biologists who are not familiar with the classification of animals". No extinct groups are mentioned, although occasionally members of such groups turn out not to be extinct at all, like the coelacanth and the mollusc *Neopilina*.

The author explains briefly the purpose of his book and how to use it. A summarized classification of the animal kingdom follows, together with the approximate number of described species in each group. A more detailed classification of living animals is given in Chapter 3, down to orders and sub-orders for all but the smallest phyla. Under the small phylum Entoprocta only families are given and under Sipunculoidea and one or two others only some genera are mentioned. These classifications have been discussed with specialists on each group or groups and should, therefore, be authoritative and up to date. The author admits, however, that he has not always taken the advice of the specialists, that any errors are his own responsibility, and requests that errors or misprints be pointed out to him when detected. Alternative classifications are given for three major groups, sponges, platyhelminths and nematodes, the preferred classification being given first in each case. (In the preferred classification of Parazoa (sponges), the order Calcarea has inadvertently been placed in the class Nuda instead of in the class Gelatinosa.) The Latin names of some "better known genera" are given throughout the text (Chapter 3) and additional examples are included in the index, together with the order or sub-order to which the genus belongs. A certain number of English vernacular names is also mentioned and some of these may be questioned by purists. Where an animal causes a disease which has an English name, it has been inserted after the generic name of the animal thus: *Plasmodium* (malaria).

The book and its index can be used to find out how the animal kingdom, or parts of it, are classified, which are the decapod crustaceans or the decapod molluscs, what are Rotifera and Squamata, etc. But they cannot necessarily provide answers to questions concerning the systematic positions of individual genera. A genus may be well known to one scientist and quite unknown to another, the choice of "better known" examples is bound therefore to be arbitrary. There seems to be a discrepancy concerning the number of generic names included, that given in the introduction being considerably higher than that implied in the publisher's note on the dust cover. But whichever it is, it represents a very small percentage of the estimated total number of known genera. If the limitations of the book are kept in mind, it should prove extremely useful to many others besides physiologists and biochemists. Parasitologists, for example, should find in it valuable information on the classification and systematic position of the hosts which harbour the parasites. As a work of reference it ought to find a place in all biological libraries, and many research workers will want to own a copy. May the author find that in this instance diligence is not without its reward.

ISABELLA GORDON

BIG GAME OF THE PLANT WORLD

Anatomy of the Monocotyledons

Vol. 2: Palmae. By Dr. P. B. Tomlinson. Edited by C. R. Metcalfe. Pp. xiii + 453 + 9 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1961.) 63*s*. net.

PALMS have for centuries stirred the imagination of dwellers in chilly temperate regions. They are among the most romantic of plants, conjuring up visions of moonlit Pacific islands and of ill-lit Victorian conservatories in the intervals between dances. For the botanist they have the added attraction of being among the least studied groups of flowering plants, largely because of their intractability in the herbarium. To make adequate specimens of the large palms requires a collector with the determination of Beccari, who pressed a whole inflorescence of *Amorphophallus titanum*. The identification of palms is thus no easy matter and any new evidence which helps to improve the existing imperfect classification must be more than usually welcome to taxonomists.

Dr. Tomlinson places both anatomists and taxonomists in his debt for the thoroughness and skill with which the large amount of original work contained in this volume has been carried out, as well as for the comprehensive survey of previous investigations which he gives.

The book is divided into two main sections, the first dealing with classification and the morphology and anatomy of the vegetative parts of palms, and the second describing in more detail the anatomical features of the genera which have been investigated.

The methods used in cutting sections of stems give some idea of the difficulties which faced Dr. Tomlinson and his predecessors; these include treatment with steam, soaking in hydrofluoric acid for weeks and even grinding thin sections, as with fossils. The leaves, however, are easier material for the anatomist, and, unlike the stems, show features of value in delimiting the groups which are here referred to sometimes as sub-families and sometimes as tribes. On the basis of these characters the elevation of "two groups formerly of minor taxonomic rank to the same status as the other main units" is recommended. A key, based mainly on leaf anatomy, is provided for the recognition of these 'groups'; it would have been easier to use if the trichotomies had been avoided, but it is an interesting example of the taxonomic uses of anatomy.

The other topics dealt with in Part 1 include germination and seedling morphology and the general morphology and anatomy of the stem, the leaf and the root. We are reminded of the sometimes enormous elongation of the cotyledon (up to 12 ft. in *Lodoicea*), of the great diversity of the juvenile leaves, and of the frequent occurrence of diffuse secondary growth in palm stems, among many other interesting and little-known features.

Part 2 follows in the main the plan of the previous volume in this series and provides a systematic account of the anatomical features of all genera so far investigated. There are numerous excellent illustrations, an extensive bibliography and indexes to authors and subjects.

Part 1 can be read with pleasure and profit by any botanist, Part 2 is more for the specialist in anatomy; the book should certainly be in every botanical library.

Dr. Tomlinson and Dr. Metcalfe are to be congratulated on an excellent volume which will still further whet the appetite for what is to come.

T. G. TUTIN

KEEPING SEEDS ALIVE

Seed Preservation and Longevity

By Dr. Lela V. Barton. (Plant Science Monographs.) Pp. xvii + 216 + 13 plates. (London: Leonard Hill (Books), Ltd.; New York: Interscience Publishers, Inc., 1961.) 50*s*. net.

IN 1948 Dr. Barton, in collaboration with W. Crocker, published *Twenty Years of Seed Research* (see *Nature*, 162, 594; 1948). The present book is to some extent an extension and amplification of the earlier one and reprints from it some graphs and plates, including the single plate in colour.

Some records of the survival or reputed survival of old seed are noted, and the interesting point is made that longevity of buried seeds is often longevity of moist or even fully imbibed seeds, and must depend on a dormancy differing from that which maintains viability under the low-moisture and low-temperature conditions which are experimentally favourable for survival.

A chapter is devoted to considering the effects of moisture on the life of seeds. Other external influences and internal factors affecting seed life are rather briefly reviewed. Subsequent chapters summarize work on the storage life of seeds of different types; vegetables, flowers, cereals, other crops, aquatic plants. Anyone engaged in the horticultural seed trade will be particularly interested in Chapter 11, concerned with the effect of different containers or packets on live seed storage.

Dr. Barton has come to acknowledge, a little grudgingly perhaps, the proved usefulness of chemical methods, in particular the now very widely used tetrazolium test, for determining rapidly the viability of some kinds of seed.

As data can obviously be classified according to either the species of seed concerned or the different factors influencing viability, a somewhat disconnected and patchwork arrangement results. Fortunately, a subject index and a separate author index do much to remedy this, although the indexing is not perfect. For example, no reference could be found to the quite important paragraph on the International Seed Testing Association and its rules. (Incidentally, the last published revision of these rules is based on the Oslo meeting of July 1959). Work on the duration of life in seeds and related storage conditions has been long continued, voluminous but scattered, and not only strictly experimental and scientific, but also commercial and merely observational. It is therefore not easy to write about it as a coherent whole. Moreover, Dr. Barton is careful to record objectively and generally refrain from criticism of the work of other investigators. Throughout the book the author's own critical appraisal of results reported, and suggestions for the integration of established facts, would have been very welcome.

The extensive, although of course still incomplete, bibliography is a most valuable part of the book. There is also a glossary for the non-botanical reader. *Seed Preservation and Longevity*, despite some limitations, brings together a tremendous amount of information and may remain for a long time the standard work of reference on its subject.

S. CLAY

TROPICAL FRESHWATER FISHERIES

Tropical Inland Fisheries

By Dr. C. F. Hickling. (Tropical Agriculture Series.) Pp. xvi + 287 + 68 plates. (London: Longmans Green and Co. Ltd., 1961.) 42*s*. 6*d*. net.

ALTHOUGH man has fished in tropical freshwaters for several thousand years it is only recently that he has taken a scientific interest in the subject. Indeed, twenty years ago this book would have been little more than a review article. As is clearly shown in the bibliography, the researches on which it is based had then only just begun. Of the 148 references cited, 18 were published before 1940, and most of the remainder appeared between 1950 and 1960.

A substantial part of the book's value lies in its intricate synthesis of diverse subjects and the clear statement of general principles which have emerged from this fast-evolving field. Its value is not, however, confined to biologists. If the pace of tropical fisheries research is to continue, the subject must have the sympathy and understanding of non-scientific administrators and politicians. For these purposes there could scarcely be a better advocate than Dr. Hickling. His style is enthusiastic and lucid, and the book is a well-balanced rendering of the numerous biological, technological, sociological and political factors that go to make up tropical inland fisheries.

The subject is treated in three broad divisions. First, there is a five-chapter account of the hydrological principles against which the biological factors and ultimately the fisheries themselves are reviewed. This section should be of particular value to students in tropical universities and to those with only a background of temperate climates. In a mere fifty-four pages we are given a clear account of hydrological conditions in rivers, lakes, dams and swamps. There is also a discussion of problems connected with sewage disposal, river pollution and swamp clearance. The diversity of conditions within tropical waters cannot fail to be impressive.

The biological factors of food supply, the condition of the water and of migrations are dealt with in the next three chapters. Here, too, Dr. Hickling gives a comprehensive introduction to a very complex subject, ranging from the anatomy of feeding mechanisms to an analysis of food chains and the interaction of the biological with the physico-chemical environment. Although this account reads smoothly and carries the reader across the boundaries of many subjects, no attempt is made to hide the unsolved problems which beset the fisheries biologist. Like the previous section, these chapters should serve as a good introduction for biology students within and without the tropics.

A chapter devoted to fishing methods, fish-preservation and marketing is a self-contained and fascinating essay. The fishing gear described includes both indigenous and imported kinds, and ranges from complex fishing machines like the 'day' of the Mekong River to what is surely the simplest known device, stirring up bottom mud and dissolved gases so as to asphyxiate the fishes.

The last seven chapters deal with the fisheries themselves and cover lake, swamp, river and flood-plain fisheries. One chapter is confined to the stocking of lakes and dams. These chapters could easily have been a dreary recital of comparative statistics, but they are far from being so. Indeed, it is in this part of the book that the first two sections are fully integrated with the main theme (a point which should show the importance of pure research to those politicians who sometimes consider it a dispensable luxury). Dr. Hickling takes a very broad view of his subject, and he is fully aware of the profound influence which social and political factors can have on the fisheries. To quote one example of these interactions, "... the construction of an all-weather road, to open up to a a reliable and lucrative market new areas of water, will do more to relieve fishing pressure on a non-migrant stock of fish than all the regulations at present applied, even assuming that these regulations were in fact enforceable at economic cost". No one can accuse Dr. Hickling of sheltering in an ivory tower. He has visited workers in the field and has directed a tropical fisheries research station for two years. This practical knowledge has clearly influenced much of what is written in these chapters.

Over-fishing is a hotly argued subject in the tropics, particularly among those concerned with fisheries in the major African lakes. Dr. Hickling takes a rather optimistic view of future developments, his optimism being based, as he says, on "the astonishing powers of recuperation of a tropical inland fishery". Certainly there are data which seem to support this point of view as applied to certain fisheries, principally those of the flood-plains and the impermanent Lake Rukwa But I believe that this optimism should be tempered so far as the fisheries of some lakes are concerned. In these waters, the conditions and the principal species are different, and above all, the fishery is continued throughout the year. Furthermore, although "recuperation" may describe the return of the fishery to economic levels in Lake Rukwa after partial desiccation, it does not describe the conditions obtaining in a relatively static lake. Perhaps 'resistance' would be a better word to cover the actions and reactions involved in the fishery of a permanent lake ? If the resistance is lowered, some form of strict control (as is imposed naturally in Lake Rukwa) may be necessary if the fishery is to recuperate; by then we have overfished and the economic consequences will be manifest.

Flood-plain fisheries obviously fascinate the author, and his detailed descriptions of their development and exploitation are of great interest since it seems inevitable that water conservation measures will eventually bring about their extinction. Here again is a subject which concerns both the biologist and the administrator.

There are a few minor taxonomic errors in the text and the outline drawings of fishes are weak (and sometimes incomplete). These are very minor blemishes in a book which must be considered as a major contribution to tropical hydrobiology and a most readable and important text-book in the field of fisheries research. P. H. GREENWOOD

THE MAMMALIAN EGG

The Mammalian Egg

By Dr. C. R. Austin. Pp. viii + 183. (Oxford: Blackwell Scientific Publications, Ltd., 1961.) 42*s*. net.

SINCE Dr. Pincus's monograph, "The Eggs of Mammals", appeared in 1936, considerable advances have been made in the study of the mammalian egg, which to-day is attracting the attention of an increasing number of workers. Consequently, a real need has developed for an up-to-date, authoritative treatise on this subject. This new book is, therefore, especially welcome. The author, Dr. Austin, is well known for his research work into the process of fertilization in mammals. His numerous papers published during the past decade provide the fabric on which the book is based.

To quote from the preface, "this book is an attempt to review in detail available information on mammalian eggs and to discuss briefly the trends of research from the point of view of the cytologist". It is divided into five main sections under the following headings: "General Biology of Eggs", "Structure and Function in Mammalian Eggs", "Cytoplasm", "Membranes and Investments", and "Manipulation of Eggs".

The processes of maturation, fertilization and cleavage are described in considerable detail, with special attention being directed to abnormal forms. This part of the book will prove especially interesting to those concerned with the study of early embryonic development. The reader becomes aware of many deficiencies in present knowledge; for example, the important phenomena of capacitation and fertilization *in vitro* are but poorly understood. Techniques described in the final section will assume considerable importance if egg transfer can be translated successfully from a laboratory research technique into a practical method of improving livestock.

The book is well illustrated with 76 text-figures, 17 of which are in full colour. However, it is debatable whether quite so many colour photographs should have been included, particularly in view of the cost of reproducing colour plates. Unfortunately, a number of the text figures appear out of sequence; for example, Fig. 46 follows directly after Fig. 39, while Fig. 40 is located some 23 pages later.

There is a most useful and commendably up-to-date bibliography, containing nearly 700 references, more than half of which date from 1950 onwards. The very high proportion of recent references gives a good indication of the present degree of activity in this field. In addition to a subject index, there is a complete index of organisms referred to in the text. Two appendixes, one on egg transfer and the other on culture of eggs *in vitro*, contain in summarized form the main details of relevant papers with references. Attention is directed to two errors in Appendix 1: on p. 125, "Belgian Hare" is classified as a species, whereas it is a breed of rabbit, and on p. 141, it is stated that Hunter, Adams and Rowson (1954) transferred "unfertilized eggs to ewes which were then mated". They did, in fact, transfer fertilized eggs to ewes which had been mated previously with vasectomized rams.

Although relatively expensive, few reproductive physiologists can afford not to have such a useful reference close at hand, while the general biologist will also find in it much of interest. The whole book is very well produced. C. E. ADAMS

WHAT PROGRESS IN RADIOBIOLOGY?

Mechanisms in Radiobiology

Edited by Maurice Errera and Arne Forrsberg. Vol. 1: General Principles. Pp. xv + 534. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 16 dollars.

Studies on Quantitative Radiation Biology

By Prof. K. G. Zimmer. Translated by H. D. Griffith. Pp. 124. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 15*s*. net.

Radiobiology

Proceedings of the Third Australian Conference on Radiobiology, held at the University, Sydney, 15–18 August, 1960, by the Australian Radiation Society. Edited by P. L. T. Ilbery. Pp. xi + 314. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 63*s*.

Progress in Photobiology

Proceedings of the Third International Congress on Photobiology, Copenhagen, 1960. Edited by B. Chr. Christensen and B. Buchmann. Pp. xv + 628. (Amsterdam: Elsevier Publishing Co., Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 126*s*.

THE first of the works listed above attempts (in its two volumes) a general review of radiobiology and may be taken as an occasion for some general comments on the state of this subject. As the second volume on multicellular organisms appeared first (and has already been reviewed—*Nature*, 190, 72; 1961) it is possible in assessing the subject-matter to take this into account. The earlier chapters of the first volume deal with: (1) the physical principles of ionizing radiations; (2) the radiation chemistry of water and simple organic and inorganic solutions; (3) the action of radiations on certain biological macromolecules, isolated and *in situ*. This is followed by chapters on various biochemical processes, on various cytological effects including replication, on radiation genetics and the use of radiations in plant breeding. The last two chapters would have been more happily placed in Volume 2, while Eldjarn's and Pihl's article in Volume 2 on the mechanisms of protective and sensitizing action really belongs to Volume 1.

The authors of the articles in this volume have produced a valuable guide to much of the literature of their subjects. From a study of these articles it will be evident that the subjects considered are at very different stages of development. The radiation chemistry of simple solutions is relatively advanced, and a good deal has been learnt about the effects of radiation on biological macromolecules, such as proteins and nucleic acids. Hutchinson and Pollard's chapter on the latter concentrates on the 'target theory' aspects, largely ignoring the fairly extensive knowledge of chemical changes produced, which are, however, considered to some extent by T. Alper under "Sub-cellular Units".

However, a study of the later chapters shows that there is a large gap to be filled between the knowledge of the actions of the radiations on biological substances and the consequent effects on the life of the cell. Notwithstanding an enormous amount of experimentation, largely supported by the various atomic energy authorities, very little has been certainly established about the mechanisms of these effects.

There is, of course, a great deal of information about the overall effects of radiations, for example, lethal action, genetic effects, effects of oxygen, specific effects in certain organs such as bone marrow, but knowledge of the essential origin of these effects seems to have progressed very little in the past ten years. It is still impossible to say which is the sensitive site (or sites), why some cells are much more sensitive than others; which of the innumerable biochemical effects are important and primary, and which secondary. There is a very good reason for this *impasse*—until the main biochemical processes involved in living cells and how they are co-ordinated with each other are known at least in outline, it is unlikely that we can pin-point the effect of the perturbations caused by ionizations. This stage has not been reached. Even in the case of chromosomal replication and synthesis of deoxyribonucleic acid which have long been thought to be sensitive processes, little is known of the critical step or steps at which radiations act; but this is not surprising as the sequence of reactions involved is only now being unravelled. A study of the relevant chapters will, I think, show that many researches have been undertaken on the basis of a purely temporary conception of the importance of one process or another. It could not be asserted that no useful knowledge is obtained in this way, but it has to a great extent been unfruitful. It seems a pity that these very considerable efforts could not have been applied to the examination of normal functioning of cells as a necessary basis for a non-empirical radiobiology which must eventually find its expression in biochemical terms.

The other volumes listed above are more limited in their scope. Prof. Zimmer's is of a more physical character and for the most part is an up-to-date and critical assessment of 'target theory'. The final chapter describes micro-wave techniques for identifying transient radicals, which have demonstrated that in dry or semi-dry systems, such as seeds, free radicals formed under the action of radiations persist for appreciable times; but although the radicals giving signals can be identified in the case of some pure substances, identification in biological material is very uncertain and may remain so in view of the vast variety of substances present.

The third volume consists of papers contributed to a conference on radiobiology in Australia. Although these cover a wide range, it is difficult to trace any connected theme running through them —in fact, the volume is a collection of papers on a variety of topics, some being in the nature of reviews and others apparently original papers. Compilations of this kind have a rather limited usefulness since the reviews often repeat those given elsewhere and the original papers would be easier to find in some recognized journal. Indeed, one cannot help but think that in this subject far too many records of conferences are being published. There have been occasions when the papers given at a conference provide a useful summary of recent work, but this is doubtfully so in the present case.

The last volume listed is in a rather different category. It does cover comprehensively most of the lines of photobiological research which are being pursued at the present time. Although much of the work recorded is to be found in the literature elsewhere, it is undoubtedly valuable to have all these fairly brief statements of results, which together give an excellent bird's-eye view of the subject, collected in one volume. J. A. V. BUTLER

MODERN AEROBIOLOGY

The Microbiology of the Atmosphere

By Dr. P. H. Gregory. (Plant Science Monographs.) Pp. xv+251+7 plates. (London: Leonard Hill (Books), Ltd.; New York: Interscience Publishers, Inc., 1961.) 60*s*. net.

THE microbiology of the air is a subject of interest to workers in such diverse fields as phytopathology, palynology and the study of allergy, and this intellectually satisfying and exhaustive treatment of the subject will, therefore, be greatly welcome. Aerobiology is no new subject. It throve in the latter half of the past century. In evidence of this it is only necessary to recall Pasteur's *Mémoire sur les corpuscles organisés qui existent dans l'atmosphère* (1861) and Miquel's long book *Les organismes vivantes de l'atmosphère* (1883). However, the subject then languished, to be revivified in recent decades. Through Dr. Gregory's own researches, and through those he has inspired, the quantitative study of the composition and movement of the air-spora (composed of such varied elements as bacteria, algae, yeasts, spores of fungi mosses and ferns, pollens of plants and cysts of protozoa) has made spectacular progress in the post-war years. In this progress the development of a satisfactory mathematical theory of the dispersal of spores in turbulent air, and the invention of suitable techniques (notably the Hirst spore-trap) for accurate quantitative sampling of the spore load of the atmosphere, have played decisive parts.

The subject is presented in a most logical manner. After a full historical introduction, the atmosphere is considered as the medium in which the aerial plankton or spora is suspended. Then problems of 'take-off' of fungal spores and of other elements of the spora are briefly reviewed, followed by a consideration of the horizontal eddy-diffusion of the spore cloud. Some of the older biologists, including myself, may find the derivation of the formulæ somewhat difficult; but the author does his best to make the mathematical argument as painless as possible. Deposition of spores is fully considered both under the controlled conditions of a wind-tunnel and in Nature. Then techniques of sampling the spora are critically evaluated. A detailed account is given of the composition of the air spora near the Earth's surface, in the upper air, and indoors. Deposition of spores is fully considered and the difficult mathematical treatment of deposition gradients is explored in detail. The controversial subject of long-distance dispersal of pathogenic fungi is presented clearly and without bias. The book ends with the author looking outwards from our planet: "After exploring the stratosphere, we shall then be better able to tackle outer Space. Time is short; before we know where we are, we shall have the Moon at our doorstep and Mars and Venus on our hands".

There can be little doubt that this book is a landmark in biology, and it required a man with a mathematical outlook, with the gift of experimental ingenuity and with imaginative insight to achieve this fine synthesis.

The book is well and clearly written and beautifully produced—perhaps too beautifully. There are four pleasing coloured plates of air-spora painted by Miss Bunce. They add to the general attractiveness of the book without, perhaps, contributing much to its scientific value. Line drawings would have been nearly as effective and surely a black-and-white diagram might, with positive advantage, have replaced the half-tone plate of the capillary impinger. All libraries with an interest in botany, medicine or plant pathology will want to have this book, but all too few individuals will be able to afford it.

C. T. INGOLD

THE MONOGENETIC TREMATODES: A RUSSIAN VIEW

Monogenetic Trematodes

Their Systematics and Phylogeny. By Boris E. Bychowsky. Edited by William J. Hargis, Jr. Translated from the Russian by Pierre C. Oustinoff. Pp. xx+627. (Washington, D.C.: American Institute of Biological Sciences, 1961.) 10 dollars.

THIS formidable monograph on the monogenetic trematodes was completed in 1955 and first published in Russian in 1957. It represents a comprehensive study with 115 Russian and 235 other citations from the original literature on the subject, but some important papers were not consulted by the author and others were known to him only by abstracts, so that the book is neither up to date nor fully documented. The author's attitude is dogmatic, his treatment of the subject is pedantic and the style of his writing is seldom lucid. The reader is irritated by imperious statements and wearied by verbosity, and he senses most acutely the harrowing enormity of the task which occupied editor, translator and several other persons for 30 months.

The book is divided into three parts, each of which has several chapters. Part 1 is concerned with morphology, biology, embryology and life-cycles, and there is a supplement on matters of a developmental nature. There is much information here which did not emanate from the U.S.S.R., and also 260 out of a total of 315 illustrations—line diagrams without annotations. The treatment of structural detail adds very little to what was known already, but the Russian feeling for ecological matters is evident in the chapters dealing with biology and life-cycles. The monogenetic life-cycle is often regarded as simple, but when the succession of forms which arise during the different seasons of the year is considered in relation to cyclical changes in the life-cycle of the host and the interplay of variable environmental factors there is added complexity which is informative. This is especially true when the host is a migratory fish dividing its activities between some river or the littoral zone and the open sea.

Part 2 of the book is concerned with the occurrence of monogenetic trematodes on their hosts and with the analysis of what is generally called specificity. Bychowsky is commendably cautious about the use of this term 'specificity', which he defines as "a potential aptitude for determined relations with the host, or, in other words, the ability to exist on this host" (p. 326). He prefers to consider the 'occurrence' of the parasite, that is, the 'realization' of its possibilities of existence on a given host under conditions which are determined by ecological factors. Much information from the original literature is collated here in various ways. Of the 958 known species of the Monogenea, 806 (84·1 per cent) occur on species of fishes belonging to a single genus and only 152 (15·9 per cent) on fishes belonging to more than one genus. Of the grand total, 903 species occur on host genera belonging to a single family and only 55 species on host genera belonging to more than one family, more than two-thirds of these occurring, however, on fishes belonging to a single order. There are 154 genera of Monogenea which infest fishes and 95 of them are found each on a single family of hosts only, and of these 67 are monotypic genera and 28 are genera usually having 2–6 species (three exceptions with 10, 13 and 23 genera). These and other analyses lose some of their value, however, because the author does not utilize "a certain amount of data" (p. 240) and "faulty or doubtful data" (p. 280). Another drawback is that specific and other diagnoses are taken at their face value. Any future evaluations of degrees of specificity will call for careful consideration of possible errors of diagnosis.

Part 3 of the book is concerned with systematics and phylogeny. Bychowsky here exposes himself to adverse criticism. He would promote the order Monogenea to the status of a class (which also involves raising the status of the Digenea) and would change the name to Monogenoidea because "the contemporary data on morphology, development and life-cycle of these groups do not provide any basis to suppose the commonness of their origin", and because "similarity in structure . . . is a purely converging phenomenon which does not in any way indicate their actual phylogenetic consanguinity" (p. 563). The Monogenea, we are told, had a more recent origin than the Digenea by an ancestral line of Turbellaria from which the Cestoda also arose. The Udonellidae is removed from the Monogenea because "this group descended from Turbellaria quite independently" and because the larva is not "equipped with a cercomere and its chitinous armature" (p. 570). The Microbothriidae is threatened with removal from the Monogenea for a similar reason (p. 463). Other readers may also consider that Bychowsky attaches too much importance to the hooklets of larvæ. At first sight, his taxonomic scheme has an alien look, but when it is compared with the scheme it is intended to replace there are many common features, although the unit of super-family is discarded. There are 29 families: 16 of them already existed, 11 are promotions from the status of sub-family, and only 2 are new, and these have only one genus each. The "Monogenoidea" is divided into 2 sub-classes, the Polyonchoinea (with 3 orders and 17 families) and the Oligonchoinea (with 3 orders and 12 families). There is some ambiguity in the delineation of these groups. The Polyonchoinea are defined as forms having 12–16 marginal hooklets (p. 405), the Oligonchoinea as forms having only 10 (p. 481), so that the prefixes to these names are misleading. The sub-classes are derived from two types of larvæ which are said to have 14–16 marginal hooklets and 10–12 marginal hooklets respectively (pp. 100–101), the line of demarcation between the two groups now becoming arbitrary. Hooklets are not mentioned in the diagnoses of families of Oligonchoinea, but the statement about 10 marginal hooklets is repeated for the largest order of this sub-family, the Mazocraeidea (p. 498). However, for some families of this order the full complement of marginal hooklets cannot be determined, because the larvæ develop precociously and emerge from the egg with an imperfect complement of hooklets and with some of the haptorial accessories of the adult already formed. This is true of Microcotylidae and Discocotylidae. Other families of the order contain forms with 6 pairs of larval hooklets which must be considered marginal and it is not legitimate to discount any of them on account of size. In *Octostoma scombir* (Mazocraeidae) the lengths of hooklets of the first 5 pairs "fluctuate around 0·02 mm." and the length of the hooklets of the sixth pair is "about 0·022 mm." (p. 215), and in *Diclidophora luscae* (Diclidophoridae) the comparable lengths are 0·025 mm. and 0·028 mm. respectively (p. 218).

To say the least, Bychowsky's taxonomic system is built on weak foundations. We shall need to know much more about larval hooks and hooklets and especially about the relationships between them—which can be revealed only by a study of the formative processes during development—before we can erect a reliable system of classification which is based on their numbers and their various arrangements. The Russian system has produced some oddities. The names of Odhner's sub-orders Monopisthocotylea and Polyopisthocotylea, which have a wealth of meaning for students of the monogenetic trematodes, have been restricted to sub-orders within the Polyonchoinea. The well-known genera *Gyrodactylus* and *Polystoma*, which are parasites of cyprinid fishes and Amphibia respectively, are both placed in the order Gyrodactylidea. These are specialized types, *Gyrodactylus* in respect of viviparity and *Polystoma* in respect of structural complexity. Each has 16 marginal hooklets arranged as 8 pairs, but this is an adult character of the former genus and a larval character of the latter genus.

BEN DAWES

NEMATODES OF PLANTS

Principles of Nematology

By Prof. Gerald Thorne. (McGraw-Hill Publications in the Agricultural Sciences.) Pp. xiv + 553. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1961.) 116*s*. 6*d*.

THE first thing to be said about this book, which is in some ways admirable, is that its title is extremely misleading. Far from dealing with nematodes as a whole, it is devoted almost exclusively to those which are parasitic in plants with a few representative free-living, soil-inhabiting species. In fact (p. 91), representatives of only seven out of thirty-one superfamilies of Nematoda recognized by B. G. Chitwood (1959, no reference in bibliography) are mentioned. Free-living freshwater and marine nematodes and those parasitic in animals are only referred to very briefly in passing. A comprehensive treatment of the phylum Nematoda as a whole would have expanded the book beyond the limited aims defined in the introduction. Nevertheless, the appropriation of the term 'nematology' is unfortunate. If this term means anything, it implies the study and knowledge of the whole group and not just an arbitrarily selected part. Entomology includes both butterflies and fleas and no one would wish it otherwise. It is also disturbing to read on p. 82 the words "Nematology, like entomology, zoology and other branches of biological science. . . .". Nematology is *part* of zoology, not equivalent to it. Prof. Thorne is not the only specialist to commit the sin of equating his own speciality to the whole of the subject of which it is a part, but it is none the less unfortunate.

Apart from these comments the book is a valuable contribution. The first chapter begins with an interesting historical introduction illustrated by photographs of distinguished workers in the particular field of nematology with which the book deals. This is followed by short discussions on the importance of nematodes to agriculture, methods of control, including a short section on soil fumigation and useful suggestions for the training of nematologists. Chapter 2 is concerned with collection of material and estimates of population density, which, as the author points out, are "subject to great error". In Chapter 3 great emphasis is laid on the thorough training of nematologists in microscopic techniques and the use of first-class optical equipment essential for the study of the small and often elusive details necessary for the identification of these difficult animals. There are useful sections on fixation, dehydration and the preparation of "whole mounts" as well as on identification and the description of new species. In connexion with the last-mentioned aspect the author makes the refreshing comment "Select short, easily pronounced names. A name does not have to be descriptive of the organism although it is desirable that it be so when feasible. It is far better to draw from four to six letters at random and arrange them into a pronounceable barbaric name than to create a jaw-breaking monstrosity". It may be anathema to the purists but how much one recalls Leach, who, in the last century, created quite euphonious names for genera of isopod Crustacea such as *Conilera*, *Lonicera*, *Cirolana*, *Anilocra* and so on, all anagrams or near-anagrams of the name Caroline.

The rest, and the major part of the book, 400-odd pages, is devoted to a systematic account of soil nematodes most of which are, or are suspected of being, harmful to crops but also including some saprozoic, bacteria-feeding and predacious species. This is, for the systematist and 'practical' man, the most important part of the book. The descriptions are clear and succinct and the illustrations numerous and good. In the case of species of proved economic importance good accounts are given of the damage caused, host plants, distribution and methods of control.

This book is the production of a systematist, with, as would be expected, a strong bias towards the *ad hoc* methods of control. He displays little awareness of modern work and methods of approach to the nematode problem or to the physiology of these important animals. The word 'principle' has been much abused in many books, but there are here only methods, no principles as I understand the term. Nevertheless, this is, within its limits, a useful and much-needed book, and is therefore sincerely welcomed. It is very well produced and I have noticed no printing errors. A. D. HOBSON

TOXICOLOGY OF AROMATIC HYDROCARBONS

Toxicology and Biochemistry of Aromatic Hydrocarbons

By Dr. Horace W. Gerarde. (Elsevier Monographs on Toxic Agents.) Pp. xiii + 329. (Amsterdam: Elsevier Publishing Co., Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 30*s*.

THIS useful monograph consists of two parts. Part 1 is devoted to a general treatment and includes nomenclature, sources, analysis, toxicology and biochemistry of aromatic hydrocarbons as well as a chapter on the treatment of exposure to these compounds. Part 2 deals, in handbook style, with twenty aromatic hydrocarbons of industrial importance. These include benzene, the mono-, di- and poly-alkylbenzenes, and di-, tri- and poly-cyclic aromatic hydrocarbons. Each compound is classified in a similar manner to Part 1 and given brief treatment. A chapter on solvents, fuels and lubricants, and a glossary of terminology are also included.

To the analytical toxicologist, the outstanding feature of this book is the omission of any mention of vapour-phase chromatography. On page 87, Dr. Gerarde states that "If sufficiently sensitive methods of analysis were available, breath analysis could serve as a test for exposure to these hydrocarbons . . .".

One is of the opinion that, by a combination of vapour-phase chromatography and infra-red spectrophotometry, sufficiently sensitive methods are in fact available. Perhaps, too, application of such techniques might eliminate the need to wait for the appearance of leucopenia or thrombocytopenia as evidence of exposure to hydrocarbons; the 'biochemical lesion' may thus be prevented from occurring. One might pertinently enlarge the discussion here and say that, in Great Britain at least, there is a great need for the establishment of a centre for research into toxicological analysis and for the provision of a routine service for the practical application of the fruits of such research. This would be one way, at least, of solving one of the problems posed in Dr. Gerarde's statement on page 87, that "The accidental ingestion of chemicals is an infrequent occurrence in industry but regrettably too common in the home".

Chapter 4 contains one or two blemishes. For example, on page 38 reference is made to ". . . benzene and its more volatile homologues . . .". This statement is ambiguous; it suggests that the homologues of benzene are more volatile than benzene itself. This, of course, is inconsistent with the statement on page 26 that ". . . progressive decrease in volatility with increasing molecular weight accompanies the lengthening of the alkyl chain".

The sentence beginning "The aromatic hydrocarbons have . . .", on page 47, should begin, "The alkyl derivatives of benzene have . . .", to make the paragraph intelligible.

It is interesting to note in Chapter 6 that the threshold limits for benzene and toluene in U.S.S.R. are lower than the American and British values; one wonders whether the Trades Unions are aware of this difference.

The binding of this soft-covered book is poor; several pages came away from my own copy after the third opening. The quality of the paper is good.

Notwithstanding these criticisms, the monograph provides a useful compilation of hitherto somewhat scattered information and it should find a place on the bookshelves of all those whose work, whether routinely or infrequently, brings them into contact with toxicology. HAROLD V. STREET

ORGANOPHOSPHORUS ANTICHOLINESTERASES

Organophosphorus Poisons :

Anticholinesterases and Related Compounds. By D. F. Heath. (International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 13.) Pp. vii + 403. (London and New York: Pergamon Press, 1961.) 80*s*. net.

THIS book deals with the chemistry, biochemistry and pharmacology of the organophosphorus anticholinesterases and is intended primarily for the research worker in this field. The idea is sound, for probably nowhere else is so much related information collected conveniently together into one volume. Although the book demonstrates the author's considerable experience in all three disciplines, no one person can be expected to possess all the detailed knowledge necessary to write such a book. This is borne out in the section on pharmacology in mammals where numerous errors or distortions of the accepted facts occur. Two examples suffice. Thus on p. 260 the general statement is made that complete tetanic fusion in skeletal muscles occurs at a stimulation frequency of about 25/sec., when 100/sec. would probably be nearer the correct average for mammalian muscles. On p. 267, the author attributes to Zaimis the conclusion that decamethonium blocks neuromuscular transmission by potentiating the action of acetylcholine. From her vast experience in this field, Prof. Zaimis has always been convinced that decamethonium acts by virtue of its own powerful depolarizing action.

For a single author to complete an up-to-date review of this length on such a complex subject he must of necessity prepare his manuscript in a hurry, and some parts of the book, particularly the references, strongly convey this impression to the reader. Fortunately, most of the errors in the references appear to consist only of misspelling of the author's names. However, one reference to work by Blaber and Creasey (p. 186) contains four errors: in the initials of one of the authors, in the year of publication, in the page number of the article and in the species of animal used.

The author is a well-known research worker in the fields of chemistry and biochemistry and the sections of the book concerning these aspects of the subject are written with authority; they contain a wealth of information and should be extremely useful to those starting research in this field. However, the section on pharmacology in mammals is not to be recommended, especially for those inexperienced in pharmacology. W. C. BOWMAN

OPTICAL DESIGN IN INSTRUMENTS

Instrumental Optics

By G. A. Boutry. Translated by R. Auerbach. Pp. xvi+544. (London: Hilger and Watts, Ltd., 1961.) 168*s*.

THE first thing that should be said about this rather expensive book is that the quality of production is outstandingly good. A great deal of attention has clearly been paid to the choice of type, to the setting out of the tables and the mathematics, and to the clarity of the diagrams, in which the essential information is boldly portrayed while the non-essentials are ruthlessly omitted. All this, combined with the quality of the paper and the striking red and black cover, makes it a book which from the æsthetic point of view alone is a delight to possess.

The next thing that needs to be said is that the title gives a rather misleading impression of the contents. There is considerably more straight lens theory in its 22 chapters than might be expected in a book on instrumental optics. The scope of the book can best be judged from the titles of the five sections into which it is divided, dealing with: principles; optical systems with symmetry of revolution; systems with symmetry about a plane; vision and image formation in optical instruments; and main types of optical instruments. Only in the final section can the author be said really to come to grips with optical instruments, more particularly in the case of microscopes. The elegance of the diagrams is also best exemplified in this section, with a very simple but effective illustration (Fig. 360) showing how the exposure spreads through a photographic emulsion, as especially noteworthy.

It might have been expected that a book on instrumental optics would include chapters on interference and polarization and their use in optical instrumentation. Then, too, the special problems associated with, say, optical surveying instruments or spectrophotometers might have been discussed. But perhaps to ask for more is itself a tribute to what the author and translator have already provided.

Special mention should be made of the section entitled "Systems with Symmetry about a Plane". The chapter here on trains of refracting prisms should be particularly helpful; a second rather unusual chapter in this section discusses the symmetry and displacement of the image produced by various sequences of reflecting surfaces; while the third chapter covers the relatively unfamiliar subject of cylindrical lenses.

The original French text was written during the War years 1940–44, so that optical developments which have taken place during the past twenty years are not discussed. At certain points, therefore, there is a slightly dated outlook, as for example, in the reference on p. 320 to the techniques being developed for producing anti-reflexion coatings. This situation has been met by footnote references given by the translator to more up-to-date publications. Other examples are the Stiles–Crawford effect and eye movements which would be given greater prominence in a modern discussion of perception through visual instruments.

The task of translation must have been a mammoth one and it has been carried through with devotion and dedication by Mr. Auerbach. The opportunity has also been taken to correct certain errors in the original French edition. It is perhaps a little disappointing that the opportunity was not also taken to add a bibliography, an omission from the French text which could no doubt be attributed, in part at least, to the conditions prevailing when the book was first written.

W. D. WRIGHT

ROGER JOSEPH BOSCOVICH

Roger Joseph Boscovich, S.J., F.R.S., 1711–1787

Studies of his Life and Work on the 250th Anniversary of his Birth. Edited by Lancelot Law Whyte. Pp. 230+4 plates. (London: George Allen and Unwin, Ltd., 1961.) 32*s*. net.

IT is doubtful whether the reputation of any other natural philosopher of the first rank has suffered such an undeserved eclipse as that of Roger Joseph Boscovich, S.J. Nor is the word 'eclipse' an idle metaphor; for less than fifty years ago this luminary shone with a light undiminished by the passage of a century. As late as 1905 J. J. Thomson drew inspiration from the 'Boscovichian curve'; two years earlier Kelvin had described his own position as 'Boscovichian'; Maxwell gave a critical exposition of his theory in the article "Atom" in the *Encyclopaedia Britannica*, and in his *Dynamical Theory of Gases* based his argument on a mutual 'repulsion' of mass-points (his attribution of actual mass to Boscovich's 'puncta' was in fact an error); Faraday regarded Boscovich's point-centres of force as the only plausible form of 'atomicity', from which he turned away only as his own conception of the field came to dominate his thought; John Robison gave a long and careful exposition of Boscovich's theory from the chair of natural philosophy in Edinburgh in the closing years of the eighteenth century; and, finally, so struck was Priestley by the importance of Boscovich's ideas that he wrote to him with the view of having the *Philosophiae naturalis theoria redacta ad unicam legem virium in natura existentium* (Vienna 1758; revised, Venice 1763) translated.

Despite this cloud of witnesses, and the existence since 1922 of J. M. Child's fine translation of the *Theoria*, it is not uncommon to meet scientists who have not even heard of Boscovich. Fellows of the Royal Society were reminded of the exploits of their former colleague by Mr. L. L. Whyte's article in *Notes and Records* (13, 38); the book of studies now extends this opportunity to the general public: and there is scarcely any member of that public, now increasingly avid for reconstructions of the past, who would not derive pleasure and profit from its perusal.

Most of the articles make no very great claims on the technical equipment of the reader: the admirable "Biographical Essay" by Prof. Elizabeth Hill may be read with enjoyment and profit at almost any level of historical sophistication; for it deals with a fascinating and critical period of European history into the diplomatic, dynastic and ecclesiastical *coulisses* of which the young Jugoslav Jesuit was drawn by his urbane manner (at least in his early years), sympathetic understanding of human relations, elegant Latinity, and capacity for sustained hard work; while his technical realism brought him into consultation about the Pontine Marshes, the dome of St. Peter's, canals, cartography, geodesy, and the equipment of the Brera Observatory near Milan. He became *persona grata* in many quarters—not least in his own native country, the Republic of Dubrovnik, which he cleared of an alleged breach of neutrality towards the British Crown. His intuition of the contingent dominance of Euclidean geometry is only one example of his astonishing insights.

Since the book includes eight further articles on different aspects of Boscovich's scientific work, space does not permit of even listing them; I am able to give them no higher praise than to record that many evenings spent in reading them were followed by days of commerce with many of the original authors referred to. There are an admirable bibliography of Boscovich's works and of numerous relevant secondary sources; also a helpful index of proper names, and of topics dealt with by Boscovich.

Publishers, printers, editor, and Sir Harold Hartley (for his engaging foreword and the part he has played in re-awakening interest in Boscovich) all deserve thanks for a very attractive volume. A few errors—typographical and orthographical—were noted: in the transcription (p. 115) of extracts from Child's translation of the *Theoria* the reference to Newton should be in square brackets, 'found' should read 'bound', and in the next paragraph 'terms' should be 'powers' (*potentias* in the original).

William P. D. Wightman

OEUVRES COMPLÈTES—AND NOTES FOR POSTERITY

Oeuvres Scientifiques Complètes

Par Frédéric Joliot-Curie et Irène Joliot-Curie. Pp. viii+916+10 planches. (Paris: Presses Universitaires de France, 1961.) 100 N.F.

TWO years after the death of Pierre Curie in 1906, his collected works were published by la Société de Physique. In 1924, Marie Curie added a biographical sketch of her husband as preface when this collection was reprinted. Thirty years later, and twenty after her own death, Marie Curie's scientific publications, collected by her daughter, were published as an act of homage by Polska Akademia Nauk in Warsaw. Irène Joliot Curie, in her preface to that volume, wrote: "For the most part these publications no longer have more than an historical interest, such has been the rapidity with which our knowledge of radioactivity has [recently] advanced".

Irène Curie died in 1956, and Frédéric Joliot in 1958; now, in the volume under review, we have the *Oeuvres Scientifiques Complètes* of this second generation of Nobel prize-winners. The preface is signed jointly by their children. Again, in a sense, what is collected is of no more than historical interest—for the rapidity of advance of knowledge has not slackened in the past decade—but the collection is different from its forerunners in one respect which must be mentioned, and its interest is greater in consequence. The novelty is that the present volume contains material that has not previously been published. Let us look first, however, at its main content, at the papers which appeared in the scientific journals over a period of some thirty-five years, and greatly advanced our knowledge, and then return to the rest.

The whole impact of the published work, as all physicists must surely know, is impressive in the extreme. If we look for the highlights they are there: the joint work which resulted in the discovery of 'artificial' radioactivity, and the work of Frédéric Joliot and his collaborators, both before the fall of France and after the liberation, on fission. Equally substantial is the work of Irène Joliot Curie and Savitch, on the radioelement of 3·5 hr. lifetime, which for eighteen months before the discovery of fission held the secret of that process at so short a distance from their grasp. Earlier, there is the note of January 18, 1932, entitled "The emission of protons of high velocity from hydrogenous materials irradiated with very penetrating γ-rays". That, too, was epoch-making. Indeed, the whole corpus is monumental: here is recorded the work of two experimenters of genius.

The unpublished material consists in part of extracts from the *Notices de Titres et travaux* which Irène and Frédéric Joliot Curie wrote in retrospect concerning their published work. Precise dates are not given, but from internal evidence the interval between experiment and gloss must sometimes have been as great as ten years. The result is not uniformly satisfactory, though respect must naturally be accorded to an investigator's personal views of origins and significance when his own work is in question. In the preface to the Warsaw volume, Irène Curie wrote of her mother's early papers, composed in her native language, "That is why it has seemed preferable to publish them without translation—so as to preserve their character as [original] documents". If a similar restraint had motivated the editors of the present volume, the testimony of the original documents here collected would not, in places, have been obscured by the gloss. In his account of the discovery of the neutron (pp. 351–3), Frédéric Joliot comments: "We started to repeat the experiments [of Bothe and Becker]. . . . We learned later that Webster . . . at Cambridge was carrying out experiments by the Wilson method (however without success). . . . This effect escaped Webster's observation . . . which explains why Chadwick did not take up his crucial experiments until we had published our account of the projection of nuclei [by the penetrating radiation]". It is only honest to record the fact that the original documents in the present collection provide convincing evidence in themselves of the inadequacy of the first statement (concerning Webster's experiments) and of the ingenuousness of the plural usage 'nuclei' in the last sentence of this quotation. It is unfortunate that any such rebuttal of witness should be necessary—as it is necessary—in the present context.

The unpublished material is of further interest to the historian in that it includes the full text of the five applications for patent in relation to nuclear reactors and nuclear explosives which Joliot and his collaborators made in May 1939 and April and May 1940. Over the space of a generation the attitude of scientists towards the exploitation of their discoveries had sharpened. In the preface to the Warsaw volume Irène Curie had written, "Pierre and Marie Curie had had no desire to take any patent on the method of extraction of radium. Industry had taken over the process which they had developed almost without modification. . . ." So we have the draft patents of 1939 and 1940—but not the judgment of the law on their validity.

N. Feather

PROGRESS IN SOLID MECHANICS

Progress in Solid Mechanics

Vol. 1. Edited by Prof. I. N. Sneddon and Prof. R. Hill. (Series in Physics.) Pp. xii+448. (Amsterdam: North-Holland Publishing Company; New York: Interscience Publishers, Inc., 1960.) 100*s*.
Vol 2. Pp. xi+331. (Amsterdam: North-Holland Publishing Company, 1961.) 70*s*.

THE problem of acquainting engineers with the essential features of recent research by mathematicians, as well as that by workers in pure science generally, in fields relevant to engineering science and practice, is ever present. This in itself is sufficient justification for these volumes edited by Profs. Sneddon and Hill, which contain review articles of high standard addressed to engineers as well as to mathematicians and physicists.

Volume 1 contains eight chapters. The first, by S. C. Hunter, reviews progress in studies of waves in viscoelastic materials, such as synthetic plastics, and includes accounts of theoretical and experimental investigations. Then K. Marguerre provides an introduction to recent papers on the use of matrices of transmission in problems of vibrating beam systems. The method is described fully for the example of a vibrating beam, and references to the treatment of more complex problems such as vibrating frameworks are given. The present state of knowledge of the mechanics of dynamic expansion of cavities in solids, with particular reference to ductile metals, is surveyed by H. G. Hopkins in Chapter 3. This field involves elastic and plastic behaviour and embraces phenomena due to explosions within solids such as soils, as well as metals. In the fourth chapter, W. T. Koiter reviews general theorems for elastic-plastic solids including uniqueness theorems, minimum principles, plastic collapse theorems and limit analysis and shakedown theorems. Part of this chapter is relevant to the study of the ultimate load-carrying capacity of structures made of ductile metal, but the method of presentation is not well suited to the needs of engineers. Work on dispersion relations for elastic waves in bars is reviewed by W. A. Green in Chapter 5, then in Chapter 6 P. Chadwick surveys thermoelasticity with particular reference to the dynamical theory. This recognizes the interaction of the thermal state and deformation of an elastic solid in time-dependent problems. The seventh chapter, by B. A. Bilby, provides a review of analytical studies of continuous distributions of dislocations relating to the explanation of plastic behaviour of crystalline material. Finally, the treatment of asymmetric problems of the theory of elasticity for a semi-infinite solid and a thick plate in Chapter 8 by R. Muki deals mainly with his own recent work on such problems.

Volume 2 begins with a review by J. E. Adkins of the development of the theory of large elastic deformations with particular reference to the rapid progress made in recent years due to the stimulus provided by the use made of vulcanized rubber. This is followed in Chapter 2 by a survey by M. J. P. Musgrave of recent progress in the theory of propagation of elastic waves in anisotropic media which has applications in seismology and ultrasonic techniques of studying materials and their defects. Then there is a review by J. D. Eshelby of work on effects of elastic inclusions and inhomogeneities in materials, with particular reference to the special problem in which the inclusion or inhomogeneity has the form of a general ellipsoid. The effects are concerned with the elastic behaviour and stress distribution. Mathematical theory of plastic waves, being concerned with the propagation of stresses which are sufficiently large to cause inelastic behaviour of ductile metals, is reviewed by J. W. Craggs in Chapter 4. It is noted that the theory is far from complete at present. Chapter 5, by K. W. Hillier, is unique among the contents of these volumes, since it is concerned entirely with experimental work. Resonance, wave-propagation and direct stress-strain methods of studying the dynamic elastic properties of materials are reviewed. Under the heading "Discontinuity Relations in Mechanics of Solids", R. Hill provides a detailed treatment, with historical background, of the basic analysis for dealing with discontinuity in the dependent variables and their derivatives, in boundary-value problems of continuum mechanics. The final chapter, by M. R. Horne, surveys the field of stability studies of elastic–plastic structures. This chapter is valuable to structural engineers, for the problem of the stability of frameworks in metal which are capable of elastic followed by plastic behaviour is fundamental to the study of the ultimate load-carrying capacity of multi-storey building frames made of steel. It is, moreover, the subject of much present-day research.

The suggestions for future research contained in these volumes are valuable, though more than a year has elapsed since any of the contributions was written. However, the numerous references to original work provide a useful guide to probable sources of present-day research. The editors say in the preface (Volume 1) that the main emphasis in these volumes will be on mathematical techniques of continuum mechanics but that they hope to include surveys of recent experimental investigations in future volumes. This aim has been followed in Volume 2, from which it seems that some aspects of research in theory of structures are also within the scope of the series, to judge by Horne's contribution. Chapters reviewing research into behaviour of frameworks due to dynamic loading and behaviour of shell structures might, then, be appropriate in future volumes.

T. M. CHARLTON

ADSORPTION AND CATALYSIS

Advances in Catalysis and Related Subjects

Vol. 11. Pp. x+384. 12.50 dollars. Vol. 12. Pp. x+324. 11 dollars. Edited by D. D. Eley, P. W. Selwood and Paul B. Weisz. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959 and 1960.)

VOLUME 11 opens with an article by G. Natta and I. Pasquon in which the authors show in detail how kinetic analysis of propylene polymerization by the triethylaluminium/$TiCl_3$ catalyst system helped to establish the now familiar process of stepwise insertion at a metal–carbon bond. R. V. Culver and F. C. Tompkins give a timely review of surface potentials and the adsorption process on metals. The experimental side of the subject is very properly emphasized and there are some useful tables of the more reliable data. Gas reactions of carbon are described by P. L. Walker, jun., F. Rusinko, jun., and L. G. Austin (87 pages). This is a good, critical account of the subject, and there is an important discussion of the part played by mass transport.

C. Kemball has written a clear and concise chapter on catalytic exchange of hydrocarbons with deuterium. The principles of exchange reactions are followed by examples studied from the point of view of hydrocarbon structure. J. J. Chessick and A. C. Zettlemoyer treat immersional heats and the nature of solid surfaces in a chapter which is a welcome *apologia* for a method that could certainly be more widely used. The last article, on catalytic activation of hydrogen in homogeneous, heterogeneous and biological systems, is by J. Halpern. By collating advances over a broad field and emphasizing the success of electronic interpretations in homogeneous catalysis, the author has written a stimulating chapter.

In Volume 12, T. B. Grimley begins with a short account of the wave mechanical approach to chemisorption. The move away from metals towards semi-conductors as being the most amenable solid type for theoretical study is evident here, and this is strongly accentuated in a later chapter by Th. Wolkenstein (F. F. Volkenstein) on his elegant electronic theory of catalysis on semi-conductors. This is particularly welcome as being the first account of Wolkenstein's work to appear in English. The longest chapter (80 pages) is by D. E. O'Reilly, entitled "Magnetic Resonance Techniques in Catalytic Research". The theoretical background to nuclear magnetic resonance and electron paramagnetic resonance is presented in rather excessive detail, but about a dozen examples of the applications of these methods in various fields of catalytic interest are discussed. In sharp contrast is an article by R. A. van Nordstrand, who presents his extensive experimental data on the X-ray K-absorption edges of the 3*d* metals and their dependence on chemical environment. The correlations here, although purely empirical, are very clearly set out. The link between homogeneous and heterogeneous systems is evident in an authoritative chapter on base-catalysed reactions of hydrocarbons by H. Pines and L. A. Schapp. The authors develop a consistent mechanistic interpretation of these reactions on the basis of carbanion intermediates. The final chapter is a comprehensive review by D. J. C. Yates of dimensional changes in porous solids (notably porous glass) during physical adsorption, together with the related infra-red work on changes in the OH absorption bands. It is always salutary to be reminded that the solid is not an inert partner in adsorption.

These two volumes deserve to be widely read by all those interested in the fundamental aspects of adsorption and catalysis, and the editors are to be congratulated on maintaining such a high level of authorship.

F. S. STONE

DETERGENCY

Surface Activity and Detergency

Edited by K. Durham. Pp. xiii+250+14 plates. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1961.) 35*s*. net.

THIS book is based on a series of lectures given at the Liverpool College of Technology during the winter of 1957–58 by K. Durham, H. E. Garrett, T. G. Jones, A. S. C. Lawrence and D. G. Stevenson.

The first two chapters deal with the properties of detergent solutions with particular reference to surface activity and micelle formation. Some attention is given to the increasingly important non-ionics. The three chapters that follow are concerned with applying these ideas to the basic processes of detergent action—wetting, dirt removal and the prevention of redeposition of the dirt. The separate consideration of these processes is logical since detergency is so complex that no complete theory seems possible. A clear account is given of wetting phenomena and their relation to the washing process, with emphasis on the difficulty of applying simple theoretical models to the wetting of a fabric. Removal of dirt is considered in terms of the modern theories of colloid stability (Derjaguin, Vervey and Overbeek) assuming that 'deflocculation' occurs in the disruption of the dirt-fabric complex. A useful theory of builder action is presented. The importance for efficient washing of maintaining the dirt in suspension is emphasized and the fundamentals of stability are discussed. Then follows an account of ancillary effects in detergent action, including some particularly good photographs of dirt removal from fibres and the cleaning action of foam. Lawrence has written an interesting chapter describing mainly his own work at Sheffield and has presented a plausible picture of the mechanism of detergency of most greasy and oily dirt. The remainder of the book deals with kinetic effects, a subject not discussed in other books in this field, and a brief summary of the evaluation of detergent efficiency.

It is clear from reading this book that there is much more work to be done before a general theory of detergent action can be constructed.

The book successfully combines a reasonable amount of theory with as much practical information as possible—a comprehensive account of the whole field of detergency is not intended. Unfortunately, work published since the lectures has not been included.

In general the book is well written but contains a number of misprints, and more care could have been taken over the lettering on the diagrams. However, these are minor faults and the book is a useful addition to the literature on colloid and surface chemistry. It is highly recommended to technologists working in this field.

G. D. PARFITT

UP TO DATE ON LIGNIN

The Chemistry of Lignin

Supplement Volume, Covering the Literature for the Years 1949–1958. By Friedrich Emil Brauns and Dorothy Alexandra Brauns. Pp. x+804. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 18 dollars.

INCLUDED in this critical literature review are a few papers, mostly from the U.S.S.R. and Japan, which were not available for inclusion in the original standard work by Brauns published in 1952. *The Chemistry of Lignin*, which is produced in a similar type and style, is of the same length as its predecessor, testifying to the widespread interest, except apparently in Britain, in lignin chemistry and the large amount of work in this field over the past ten years. There is little repetition of the information in the original book and frequent references are made to its contents, although unnecessary disruptions due to this are avoided.

This is an essential reference work for all interested in the chemistry of cellulosic raw materials, for those with little knowledge of the subject it presents in a most digestible form the results of a specialist lignin chemist's struggle to keep abreast with the literature and reconcile the many contradictory claims.

The original literature has been consulted in the majority of cases. As this includes a great number of Japanese papers the author was particularly favoured by the opportunities provided by a Fulbright lectureship at the University of Osaka. This enabled him to obtain assistance from the staff with the translation and with criticism of many of the chapters.

The principal authorities throughout the world have co-operated in providing reprints and overall résumés of their work. Merewether is the only author of a complete chapter, which is that on "The Linkage of Lignin in the Plant", of which he has made a special study.

Excellent author and subject indexes are provided and the twenty-seven chapters are selected and sub-divided in a most convenient logical form, similar to that in the original book, to permit a rapid selection of relevant information. In the preface the author expresses his realization that such a work can neither be complete nor infallible; certainly on first reading no omissions could be found and every effort has undoubtedly been made to make it as comprehensive and accurate as possible.

Brauns and his wife have produced a most pleasing work, and it was a joy to read in such comprehensive detail about one of the remaining major mysteries, as little progress has been made towards the elucidation of the lignin structure in the past decade. It is a pity that so little interest is taken in the United Kingdom in what, after all, is the second most abundant naturally recurring material on this planet. It is to be hoped that this work will provoke more scientists into entering this field, particularly as there is an increasing possibility of workable sources of raw material becoming available in Great Britain, based on the extensive pulping feasibility studies now in progress in Government and industrial laboratories.

J. LUCKINS

PHYSICS FOR THE NON-PHYSICIST

Physics for the Inquiring Mind

The Methods, Nature and Philosophy of Physical Science. By Prof. Eric M. Rogers. Pp. x + 778. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 48*s*. net.

AT first sight it might seem paradoxical to suggest a course of physics for the non-physicist, but the use of this book will indicate what a wealth of common sense and intelligent understanding lies at the back of the conception of such a course of study. The book is definitely written for the non-scientist, in the hope that he will be brought to appreciate the value of physics as part of a liberal education, and to understand the subject so that he can enjoy the contacts with science and scientists which are part of our modern civilization.

This is surely a very commendable objective, since scientific thought and method are so much a part of our daily lives. I myself think that the book could also be used by bona fide students of physics, since if they pursue the course here prescribed they will certainly get a greater fundamental knowledge of the subject than many of them get by following the more usual courses.

The book is so different from the usual text-book, and it is the author's intention that it should be so. He deals intensively with a series of topics—not so many that learning becomes superficial and hurried, and not so few that he misses the connected nature of scientific work and thinking—to form a co-ordinated structure of knowledge. He does not merely describe experiments and give facts; he aims rather to encourage and stress the value of critical reading, good and sound reasoning and clear thinking, qualities, one would think, sorely needed in our world to-day by men and women whether they are scientists or not.

The mathematics used in the book involves only the simpler parts of algebra and geometry, but a special feature is the unusual type of problem found in copious numbers at the end of each chapter and which are designed to get the student to discuss and reason.

In this particular book the subjects covered are matter, motion and force, astronomy, molecules and energy, relativity, electricity and magnetism and atomic and nuclear physics, but, as the author points out, any other selection of subjects could be made and treated in a similar way.

If one is expected to make one word of criticism it is perhaps that certain parts suffer from over-simplification, but this remark must not be taken too seriously, for the course has been brilliantly conceived and worked out by the author, who deserves congratulations and every encouragement.

I am pleased to have had the opportunity of inspecting this book and of mentioning a few of its many virtues.

T. M. YARWOOD

A CONCISE COURSE OF PHYSICS

A Textbook of Physics

By Dr. R. C. Brown. Pp. xxi + 1424 + xxxvi. (London: Longmans Green and Co., Ltd., 1961.) 60*s*.

THIS voluminous work of more than 1,400 pages incorporates all the material published in separate volumes in the author's *Mechanics and the Properties of Matter*, *Heat*, *Light*, *Sound and Electricity and Magnetism* and is, of course, eminently suitable for all students preparing for the Advanced Level of the General Certificate of Education.

Throughout the book Dr. Brown has made use of his experience as an examiner to take special care in presenting certain topics which he has found to be only partially understood by students. This is a good thing, for there is no doubt that quite a number of candidates enter for the examination with an inadequate knowledge of fundamentals, and these are strongly emphasized in this book.

An appreciable amount of the book deals with work suitable for the Ordinary Level of the General Certificate of Education, and one wonders whether it might not have been better to have had two smaller volumes, one for each level. This is in no sense any criticism of the contents of the book, for Dr. Brown develops his advanced work from the elementary principles quite successfully and thoroughly.

While recognizing the value of M.K.S. units in applied electrical theory and engineering where they are used extensively, the author does not appear to advocate their use in the rather different intellectual climate of advanced physics, though he has an open mind about the use of any particular system of units. He suggests that students who have passed their General Certificate of Education examinations and have to use M.K.S. units afterwards will understand their work better after the firm foundation given by the present syllabuses. This would certainly be true of the good physics student who would have no difficulty with the transition, but the weaker brethren might not find it quite so easy. In connexion with this it is perhaps relevant to point out that at least one of the British universities sets alternative questions based on M.K.S. units in the magnetism and electricity paper, while, of course, the system is widely used in the United States.

Reference is also made in the book to the discussion going on concerning the introduction of more modern topics into the advanced syllabus, and Dr. Brown, very wisely in my own opinion, warns against the inclusion of any such topics if they are allowed to crowd out the more basic parts of the subject. He agrees that all students should know something about modern developments in pure and applied physics, and this belief would be shared by most people; any change, however, would have to be thought out carefully. To stimulate interest among students in the newer work and to serve as an introduction to further reading, two chapters at the end of the book deal with electronics, radioactivity and atomic structures.

This is an excellent and well-written work, and, as one would expect from such an experienced author, a book from which a student should derive great help. T. M. YARWOOD

METALLURGY OF 55 UNCOMMON METALS

Rare Metals Handbook

Edited by Clifford A. Hampel. Second edition. Pp. xvi+715. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1961.) 160*s*.

THE first edition of this hand-book set out existing reference data on thirty-four elements which at that time (1954) were not available in large quantities or were too expensive to warrant wide usage. In the intervening years, many of those originally referred to as 'rare' have become more plentiful in supply and more widely used in industry and laboratory. In this second edition (1961) additions have been made, (chiefly from the rare earth group) and chapters formerly devoted to two metals have warranted separate treatment.

Inevitably, the only method of compilation in a volume of this type is to use specialists in each field and an editor of broad outlook. The result more than justifies the new edition, and this book will be invaluable to all metallurgical libraries, whether concerned with manufacturing processes, development, or research.

In addition to thirty-three separate chapters on individual metals or groups from the periodic system, there is a general review in Chapter 1 on occurrence and processing problems, and a final chapter summarizing physical properties in a form easy to consult, with metals not covered in the hand-book included for reference. Many tables in this chapter show an order of merit in addition to the usual alphabetical list, a fashion which might be more widely adopted in similar reference books.

Each chapter on separate metals gives a full treatment on occurrence, preparation, refining where applicable, properties and applications, with many references for further exploration. Information on such metals as beryllium, germanium and silicon is still obviously affected by commercial security, but extensive publications in recent years on uranium, plutonium and titanium are more fully covered. I was somewhat amused to find niobium described under columbium, and a quarter page of text devoted to an explanation of why the American name has been adopted. The inclusion of metals of the rare earth group is most welcome. During the past few years work in this field has been mainly concentrated in the United States and U.S.S.R. and there is no doubt that interest in these metals is bound to increase with improved methods of separation, refining and fabrication.

This book utilizes the two-column format, which suits a reference book, and the text, diagrams and photographs are excellently reproduced. In spite of the price, this is a work of reference which inevitably will find its way into every laboratory dealing with materials science. C. R. TOTTLE

ANALYTIC FUNCTIONS

Analytic Functions

By R. Nevanlinna, H. Behnke and H. Grauert, L. V. Ahlfors, D. C. Spencer, L. Bers, K. Kodaira, M. Heins, J. A. Jenkins. (Princeton Mathematical Series, No. 24.) Pp. vii+197. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 40*s*. net.

THIS book contains the principal addresses delivered at the conference on "Analytic Functions" held during September 1957, at Princeton, New Jersey. The authors are all very distinguished, and their articles, which are concerned with the most recent advances in the fields covered, are of a very high standard indeed. In a review of this kind it is impossible to give more than the following brief outlines of the contents.

R. Nevanlinna gives a short account of an absolute calculus. He then proceeds to prove the theorem of Liouville concerned with conformal transformations in Euclidean space of dimension greater than 2.

H. Behnke and H. Grauert contribute a survey article which will be of immense value to those interested in complex analysis of several variables, especially as it contains an extensive bibliography. It deals with attempts to arrive at a suitable analogue of Riemann surfaces. There is considerable dependence on topology of a high degree of sophistication.

Teichmüller showed that the space of Riemann surfaces with generators of genus g greater than unity has a natural metric by which it is topologized as a $6g - 6$ dimensional manifold. L. V. Ahlfors in his article shows that over this topology is a unique complex analytic structure with respect to which the elements of the Riemann matrix are analytic functions.

D. C. Spencer applies a technique that he and Kodaira used earlier to describe the deformation of complex analytic structures to other structures. Particular attention is directed to real foliate structures and to structures defined by pseudogroups in the sense of E. Cartan.

After an introduction to quasi-conformal mapping and Fuchsian groups L. Bers proves a theorem due to Teichmüller. This result, which leads to the natural metric considered in Ahlfors's address, is that between any two Riemann surfaces of genus g greater than unity there is an essentially unique quasi-conformal mapping of constant dilatation.

K. Kodaira deals with structures of complex analytic surfaces, that is, complex analytic manifolds of dimension 2.

M. Heins surveys results concerned with a certain aspect of conformal mapping of Riemann surfaces. The author has made many contributions to the subject, which is still in an early stage of development.

J. A. Jenkins introduces an idea due to Teichmüller that certain kinds of extremal problems in geometric function theory are associated with quadratic differentials. The author then states his 'general coefficient theorem'. Best possible inequalities are found for the first few coefficients of univalent functions, including the known bounds for the third coefficients in the regular and meromorphic cases.

J. CLUNIE

MODERN ECONOMICS

Economics

The Science of Prices and Incomes. By H. Speight. Pp. 671. (London: Methuen and Co., Ltd., 1960.) 42*s*. net.

IT is difficult to do justice to Mr. Speight's book in a short review: it is a very long book (670 closely packed pages), it contains much that is excellent, it gives ample proof of the author's wide knowledge of, and enthusiasm for, his subject, and yet I am uncertain to what extent the book meets the requirements of university students and students reading for qualifying examinations of professional associations for whom the book is intended.

Mr. Speight writes (p. 290) ". . . the function of a text-book is to present its subject as it exists to-day . . ." and this he has done. The result is a most modern text-book. But this modernity has exacted a price; a long book and, in spite of the length, the omission of some basic material (for example, formal analysis of selling outlays, indifference curves and the demand for complementary goods), and a brevity of exposition of basic theory which, though in the main sound, is sometimes incomplete (for example, references are made to the convexity of a consumer's indifference curves but no formal analysis is made of the convexity) and occasionally ambiguous or wrong (such as the discussion using Figs. 52 and 53, p. 207, which is wrong about the response of the intramarginal firms to an increase in market price). Little that has concerned economists in the post-war period has escaped Mr. Speight's net, from the theory of games to the Radcliffe Report on the working of the monetary system. Mr. Speight tells us that "one of the most difficult tasks in the actual writing of the book was deciding where to stop". There is plenty of scope for controversy on what should be included in a basic text-book, but my preference is for a number of short but thorough books which go out of their way to meet the difficulties of students. Perhaps it is time we decided that the "subject as it exists to-day" is too big to be dealt within a single volume.

The book covers almost the whole range of economics. Part 1 deals with the nature of economics, wealth and income. Part 2 deals with the theories of demand and production. Parts 3 and 4 deal with welfare economics and the economics of money and banking. Part 5 deals with the theory of income and employment, the trade cycle, public finance and fiscal policy, international trade and finance, and concludes with a chapter on stability and growth. The latest analytical devices are used throughout. It must also be mentioned that the book contains a lot of interesting factual material on economic structure and institutions, and some which is relevant to the assessment of the validity of the theory being discussed (such as the influence of the rate of interest on investment decisions).

The later chapters are not claimed to be more than introductions to the major themes of their subjects, and if they stimulate the reader to further reading they will have done their job. This is equally true of many of the earlier chapters such as those on the theory of the firm and those sections dealing with welfare economics. Supplementary reading is automatic in the universities and is laid on to supplement lectures and as a basis for written work. Much of Mr. Speight's material will be valuable in this connexion as a first approach, for example, the various sections of his book dealing with the rate of interest. The private student, however, will not find a number of parts of this book easy going and is likely to wish that the author had spent more time on their potential difficulties at the expense of some of his marginal material.

The book is well produced, attractive to look at and the contents are very clearly laid out. It is good value for money. A few minor errors, however, need correction. The reference on p. 79 to Fig. 9 should be to Fig. 10*a*. The text on pp. 83 and 84 refers to letters N, Q, D and Y on Fig. 12, but this figure does not contain these letters. The three sections of Fig. 43 on p. 194 should be labelled A, B and C. On p. 498 there is a reference to $K = \frac{1}{2}$; this should read $c = \frac{1}{2}$ or $K = 2$.

H. BURTON

FOSSIL MAMMALS

Traité de Paléontologie

Tome VI, Premier Volume: Mammifères—Origine Reptilienne—Évolution. Publié sous la direction de Jean Piveteau. Pp. viii+1138, 970 figs. + 1 planche. (Paris: Masson et Cie., 1961.) Broché 240 N.F.; Cartonné toile 254 N.F.

WITH the publication of the first volume of Tome VI, only one volume (Tome IV) remains to complete Piveteau's *Traité de Paléontologie*, which began publication in 1952. Of the eight volumes comprising the work, three are devoted to invertebrates, two to the lower vertebrates and three to the mammals, one of the latter being exclusively devoted to Primates—a heavily anthropocentric bias. Nineteen authors contribute to the volume under review, and of these fourteen are French, the major contributions being made by Piveteau, Lehman, Viret and Dechaseaux. The first third of the volume deals with mammal-like reptiles; the second with mammalian anatomy, classification, geographical and stratigraphical distribution and the Mesozoic Mammalia; the final third treats systematically with Carnivora, Cetacea and Artiodactyla—an oddly unnatural grouping of orders.

The *Traité* is one of a series of recent French science treatises, a field which was previously so typically Germanic. Piveteau's treatise is a worthy successor to Zittel's long-outdated *Grundzüge der Paläontologie*. This volume of the *Traité* will be a valuable reference work for zoologists and geologists in general besides mammalian palæontologists in particular. The illustrations are plentiful and clearly reproduced, considering the diverse quality of many of the originals. The table of contents is very inadequate, just over one page in a work of more than 1,100 pages, and lack of systematic tabulations within the text will impair the work's ease of use. A stricter editorial policy would have made for more uniform treatment of subjects and more consistent revisions, for some parts give the impression of having been written a decade ago. The work will be used by many without a detailed knowledge of stratigraphy, yet it lacks a correlative table of stratigraphical terms.

Space does permit comment on each order. The editor is to be congratulated in obtaining the services of Orlov and Tchudinov to describe the Russian therapsid material, all too little known in the West. The therapsids are comprehensively treated, abundantly illustrated and the problems of their relationships, so much *sub judice* at present, are succinctly summarized.

A brief chapter is devoted to geographic and stratigraphic distribution of Tertiary mammals, with maps of each continent and lists of main sites; by a peculiarly Gallic twist of logic, Europe comprises only France, Western Germany and Spain. The palæontologist would have welcomed more maps and fewer illustrations of palæolithic cave art, so well dealt with elsewhere. The inclusion with each section of an account on brain-casts is imaginative and laudable.

In the section on carnivores, practically all genera up to 1945, except those the validity of which is suspect, are mentioned and their characteristics briefly noted. No less than fifty new genera of fossil carnivores have been described since 1945 (excluding Kretzoi's re-naming of known forms) and of these only eleven are mentioned. This omission of several important new faunas described within the past two decades is an unfortunate defect. Other groups are admirably up to date, as, for example, the Artiodactyla, where the treatment of this very large and complex order has been skilfully handled by Viret.

It is unfortunate that so useful a volume should be priced outside the reach of many individuals.

R. J. G. Savage

THE DIVERSITY OF URBAN LIFE

British Towns

A Statistical Study of Their Social and Economic Differences. By C. A. Moser and Wolf Scott. (Centre for Urban Studies: Report No. 2.) Pp. xii + 169. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 30*s*. net.

At the 1951 census there were in England and Wales 157 urban areas with a population of not less than 50,000. Diversity was perhaps their most striking characteristic. In size they ranged from Harrogate with 50,000 to the Administrative County of London with 3·3 millions. The proportion of the population over 65 years varied from a mere 5 per cent in Dagenham to 25 per cent in Worthing. Over the two previous decades West Ham had declined in size by 42 per cent while in contrast Huyton had increased by 973 per cent. Annual retail sales per head of population ranged from £38 in Huyton to £244 in Worcester. Using 57 variables and data relating mainly to 1951, Moser and Scott present an empirical study of the rich variety of urban life in these 157 centres of population. The variables embrace demographic characteristics, housing, economic activities, voting behaviour, indices of health, and some aspects of education. Chapters 2 and 3 describe this diversity in some detail. Chapter 4 considers the matrix of 1,596 zero order correlations between the variables taken in pairs. Chapter 5 uses multivariate analysis to extract four principal, orthogonal, components of the form: $\xi_i = \Sigma a_{ij} x_j$, where the x_j are the original variables in standard form, which together absorb 60 per cent of the variance. Chapter 6 considers each town as represented by a point in four-dimensional space with the ξ_i as co-ordinates. All but two of the points thus derived are grouped into an arbitrary number of clusters so that the mean distance of points in a cluster from their centre is less than the distance between clusters. This clustering is interpreted as a 14-point classification of the towns of England and Wales. The Administrative County of London and Huyton-with-Roby in Lancashire defy classification. Chapter 1 is a short summary of the volume, and the book closes with a series of appendixes on the original data and a brief comparison with Scotland.

The authors are scrupulously fair about the limitations of their method. The factors yielded by component analysis will depend on the selection of the original variables. That selection in turn depended in part on the authors' ideas of what was relevant to a description of the varied pattern of urban life, but perhaps more on the availability of comparable data. Some of the diversity will arise simply from the arbitrary nature of the boundaries of our towns. Carve out a slice of the low-status area of north Merseyside, label it 'Bootle', treat it as a separate urban area, and it must be expected that its characteristics will differ markedly from those of Liverpool with its rich mixture of all classes. Let Parliament re-draw the Local Government map and the method may yield quite different components. There is little even to suggest that similar results will follow from the use of data shortly to become available from the 1961 census. But the experiment must certainly be tried. If the same components again emerge then it is time to carry the investigation much further. As the authors themselves remark, this is a pilot study. It is perhaps best regarded as a meticulous set of laboratory notes, awaiting further evidence and, above all, awaiting a unifying theory.

H. Silcock

TERMS OF REFERENCE

The International Dictionary of Applied Mathematics

Editor-in-Chief: Prof. W. F. Freiberger. Pp. viii + 1173. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London, D. Van Nostrand Company, Ltd., 1960.) 187*s*. 6*d*.

In the preface to this considerable compilation, the editors state that the dictionary "defines the terms and describes the methods in the applications of mathematics to thirty-one fields of physical science and engineering". This is a fair claim, but a prospective reader may pertinently inquire for whose benefit the set of definitions is intended. The dust-cover declares that the "range", "scope" and "number" of "the more than 8,000 entries in this volume bring to those of many disciplines—from physicist to chemist, from aeronautical engineer to metallurgist—an invaluable aid for ready use in the laboratory, plant or office wherever mathematical applications are needed to-day".

To this end, the editors have obtained, from some forty distinguished authors and translators, contributions which are models of succinctness and these have been disposed in alphabetical order and cross-referenced. The terms defined range from "Bethe-Salpeter Equation" and "Blunder" to "Yang-Feldman Formalism and the S-Matrix" and "Year". This bespeaks comprehensiveness perhaps, but alas, too often it happens, in face of the requirement for accurate and concise definition, comprehensibility flies out of the window. Moreover, we may not follow her flight since, in general, we have not the radar of references. Nor do the essential multilingual indexes in German, Russian, French and Spanish help us in our difficulty.

Is this dictionary then a dead loss ? (A term, by the way, it has not chosen to define.) Not entirely. But by its present standard of production it must count as a curate's ostrich egg; for besides the disparity of content, the typography shows many signs of inadequate proof correction (for example, SCARE, PYTHAGOREAN What a title for schoolboys' science fiction !)

In according such a qualified reception to a work which must have taken a large number of man-hours, it remains to offer an opinion about the reasons for disappointment. Principally, it would appear that the contributors did not have a common audience or readership in their several minds. This is an editorial shortcoming basically, as to a large extent are the other faults mentioned above.

To obtain the standard expected of a good work of reference, a clearer vision of the likely consultant and his requirements, a considered policy for references and bibliography and greater care in proof correction seem essential.

The present edition may serve a limited purpose in libraries. M. J. P. Musgrave

A LEXICON FOR THE SCIENTIFIC ERA

Webster's Third New International Dictionary of the English Language

Editor in Chief: Dr. Philip Babcock Gove. Unabridged. Pp. 56+2662. (London: G. Bell and Sons, Ltd.; Springfield, Mass.: G. and C. Merriam Co., 1961.) 420*s*.

To quote the statement inside the jacket of this monumental work: "A new full-scale 'Merriam-Webster' dictionary comes but once in a generation". It is now twenty-eight years since the last edition of this dictionary, or a dictionary planned on the 'International Webster', lines appeared. The present dictionary is, in fact, an entirely new one, being called *Webster's Third New International Dictionary* rather than the third edition of *Webster's New International Dictionary*.

While there has been a large number of additions and retractions from the 1934 edition, *Webster's Third New International Dictionary* maintains the traditional Webster style, and, with more than 450,000 entries, many of which are illustrated, can be considered as almost an encyclopædia in miniature. It represents a great development on the second edition and to some extent as a reduction (3,210 pp. to 2,662 pp.). This can be accounted for by the fact that not only has the Bibliographical Section been removed, but also the Gazetteer Section. The main developments and extensions lie in the way in which the editors have capably handled the flood of scientific, engineering and medical terms which have evolved during the past twenty-eight years. This has been admirably done by the permanent scientific editorial staff, supplemented by some eighty outside scientific consultants.

Biologists are well served in that, besides explanations of general terminologies, family, specific and common names of animals and plants are listed and cross-referenced. For example, the description under the entry "Twayblade" is supplemented by reference to "*Listera*" and "*Liparis*" and vice versa: "Beaver" gives reference to "*Castor fiber*" and vice versa, etc. For the chemist, elements and compounds are treated individually, and information is supplied concerning their properties, occurrence and uses, and in the case of elements this is followed by a detailed list of their more general compounds. The compounds entered under "Nitrogen" extend to some two and a half columns of text. Named effects and laws in chemistry, physics and mathematics, including such entries as Bohr's theory, Krebs' cycle, Euler's formula, Stoke's law, etc., are defined and explained. It is pleasing to see such terms as "Maser" defined, not only technically, but also from the etymological point of view, for it would be a great pity to lose track of the fact that such words are really only abbreviations—in this case, "*m*icrowave *a*mplification by *s*timulated *e*mission of *r*adiation".

Mineralogical terms are defined and formulæ given to minerals with brief outlines of their properties; crystallographic forms are defined and usually illustrated. A full-page illustration is presented of man dealing with the muscular system and skeleton, and in general anatomical and physiological terms are well explained, and also pathological terms with an outline of their ætiology.

The edition which appeared in 1934 was priced at 85*s*. and could be considered to stand in good stead even against such works as the great *Oxford English Dictionary*. First, this was due to the disparity of prices, secondly, a utilitarian difference lay in the disparity of sizes. In a review of the second edition (*Nature*, 134, 603; 1934) it was claimed that "Even the smallest study or office could accommodate "Webster" (which might indeed stand permanently on the corner of a desk), the O.E.D. spreads itself luxuriantly over several feet of shelf-room". This is still the case even with *Webster's Third New International Dictionary* at 21 guineas—the production of which necessitated an outlay of more than 3,500,000 dollars (£1.25 million)—however, it would be necessary to have rather a large desk if one wanted to work and maintain Webster (approximately 10 in. × 13 in. × 4 in.) on it.

Webster's International Dictionary was originally designed for the educated practical citizen of the world. It has now gone much further and is indeed a scholarly and technical reference work, a beneficial acquisition to any office or library.

R. J. Fifield

SHORT REVIEWS

A Scientist Who Believes in God

By Dr. H. N. V. Temperley. Pp. 176. (London: Hodder and Stoughton, Ltd., 1961.) 15*s*. net.

THERE is an increasing number of books by men of science concerning their faith and Dr. Temperley's is a welcome addition to the range. In the author's words: "The central problem is whether the whole body of evidence before us justifies the inference that the universe is directed by a Mind, or whether we must conclude that the existence of the human race is the accidental result of certain forces acting in a particular environment. . . . To sum up, the book is intended to be a survey of, and a guide to, the available evidence rather than an attempt to sell any particular brand of philosophy, theology or churchmanship, or any special scientific theory". Dr. Temperley has succeeded in this objective, and his desire to help young people to make up their minds about fundamental religious questions should receive wide recognition. Every thoughtful reader, whether young or old, can benefit by this adequate and frank discussion of religious problems. No doubt the author's wide experience as teacher and research worker in physics and biophysics, both at Cambridge and in the United States, is reflected in his own confession of faith.

Special attention is directed to the case for the 'materialist evolutionary' theory of the universe, followed naturally by that for a 'theistic' interpretation. Specific lines of evidence such as that from within, from Christianity, and from other sects and faiths are pursued with clarity. Of special interest is a modern modification of Paley's well-known teleological argument. The last chapter of the book is devoted to the author's own position and is all the more convincing as his journey back to belief was made from the realm of complete agnosticism.

H. D. ANTHONY

The World of Learning, 1961–62

Twelfth edition. Pp. xiv+1359. (London: Europa Publications, Ltd., 1962.) 140*s*. net.

THE current edition of *The World of Learning* continues the policy of providing the widest possible guide to the world's cultural institutions, showing the addresses, and names of the leading officials, of the world's learned societies, research institutes, libraries and archives, museums and art galleries, universities, colleges and technical institutes. A list of the professors of most universities is also provided, and this year, in response to numerous requests, the telephone numbers of a large number of universities and certain other institutes have been included. It also includes details of Unesco, giving its origin, aims, functions and activities, followed by, in alphabetical treatment, each of the member countries.

The present edition shows an increase of eighty pages from the eleventh edition, necessitated by the rapid development of the newly emerged nations of Africa and Asia and the intensification of scientific and cultural activities in the rest of the world. The contribution from the U.S.S.R. shows a small increase, but still remains at the regrettably low figure of thirty-three pages, compared with the total of two hundred and twenty from the United States

It is to be regretted that *The World of Learning* still does not include a name index. With present-day 'punch-card' sorting techniques, such a compilation should not represent the impossible task that faced the organizers of *Index Generalis* when completing the twenty-first edition in 1955. The index to this massive work included something like 125,000 entries and extended it by some 400 pages. If necessary, the index to *The World of Learning* could be prepared as a supplement.

Persons and Perception

By Leslie Paul. Pp. 231. (London: Faber and Faber, Ltd., 1961.) 25*s*. net.

THIS volume is interesting more for some of its 'asides' than for its main theme. The author considers the reasonableness of metaphysical inquiry, the impasse over perception, the protest against bifurcation, the analysis of feeling, and the status of percepts. All this is not very much more than a book about books, mostly those of the great philosophers. But at the end of Chapter 1 we find—after a reference to the æsthetic satisfaction of mathematical physics—a noteworthy paragraph on the present obsession with scientific specialization Those, in fact, whose outlook is not scientific would be worse humanists if they were to abandon their own type of wisdom. Metaphysics, unfashionable no doubt, needs no apology or backward glances.

With this in mind, we turn to Chapter 4 and read a charming account of how any graceful bodily action is ruined if the performer becomes self-conscious. He simply does not know how it is done; mastery is completely natural. We all know this, but it is tempting to go a stage further (Dr. Leslie Paul does not quite venture thus far). and apply much the same criterion to accomplishments of the mind. Perhaps the greatest achievements in, for example, pure mathematics are entirely unselfconscious. They become something far less when pressed into service for the satisfaction of examiners, or even for an occasional lecture.

Along these lines, the axiology of thought will have to advance, if the author's metaphysical hopes are to reach fruition.

F. I. G. RAWLINS

The Coil of Life

The Story of the Great Discoveries in the Life Sciences. By Ruth Moore. Pp. xv+418+vii+16 plates. (London: Constable and Co., Ltd., 1961.) 40*s*. net.

RUTH MOORE is a science reporter to an American newspaper, and, in this book, combines flair for journalism and considerable scholarship to produce a dramatic account of the great discoveries in biology and allied sciences over the past 200 years. The story begins with Lavoisier, and, besides more familiar figures, describes the work of Bichat, Beadle, Tatum and other investigators who have made important contributions to the unravelling of the properties of life. Developments over the past ten years are explained with rare skill even if the author's imaginative gifts sometimes exceed her concern for objectivity. With its many fine illustrations *The Coil of Life* could be read profitably by many who wish to know more about the science of life. To help them to a sound start with better perspective, the publishers might remove the dust-covers and their extravagant blurb about great discoveries "which have at last virtually solved the riddle of life". T. H. HAWKINS

The Boy's Country Book

Edited by John Moore. Pp. 320. (London: William Collins, Sons and Co., Ltd., 1961.) 12*s.* 6*d.* net.

ONE of the drawbacks of growing up is a gradual abatement of curiosity. The reaction of many people to the pressures of life is a form of withdrawal, a cutting down of interests, sometimes deliberate, sometimes not, leaving eventually a limited number, often entirely spectator interests or indoor ones. City children of parents who reach this stage may never have their eyes opened to the possibilities of out-door country interests unless schoolmasters or others take a hand. This book should help. It should be in public and school libraries.

Mr. Moore has enlisted the help of some twenty authors of national standing. Each has contributed an outline of his (or her) own particular sport, pastime or recreation which could kindle an interest, and has recommended one or more specialist books where its details can be followed up.

Part 1 has five chapters: route planning, lightweight camping, travel by horse, on not getting lost and on outdoor food. In Part 2, nine chapters range from rock-climbing to gliding, through sailing, angling and other interests. Parts 3 and 4 have nineteen chapters which cover a range from geology and archæology to a wide variety of natural history topics.

No money has been spent on expensive half-tone illustrations. Each chapter is headed by a simple drawing, and in a few instances simple illustrations clarify the text, but in general the book gives value for money in useful print rather than eye-catching pictures. H. DUNNICLIFF

Instructions to Young Naturalists

Part 4: Fossils. By Dr. W. E. Swinton. (The Brompton Library.) Pp. 112+13 plates. (London: Museum Press, Ltd., 1961.) 12*s.* 6*d.* net.

THIS is more than a set of instructions to young fossil hunters, it is the story both of conditions on the Earth's surface and of the animals that have roamed that surface during the past five hundred million years.

Magnificently illustrated with line drawings and photographs, it is a most readable and absorbing account. Dr. Swinton takes care to explain the theoretical problems involved in simple, lucid language; and long, resounding names, which so many young palæontologists will enjoy rolling their tongues around, are provided with their meanings or origins—a truly educational touch.

The practical nature of such a hobby is, nevertheless, always to the fore and constant reference is made in the text to sites at which the fossils described may be sought. The chapter of detailed collecting instructions gives further geographical information, along with a map of fossiliferous localities. This is followed by a useful list of reference books and museums from which further information may be gleaned.

Young fossil-hunting enthusiasts are a demanding race, but all should find stimulation as well as instruction in the pages of this book.

It is not, however, a book for the half-hearted. Dr. Swinton seems to sense their easily induced boredom and his style is too apologetic to rouse them from a prejudiced inertia.

The only serious criticism that can be levelled is the virtual rejection of the plant world. Apart from two photographs and a few passing references to the food of animals, these sustainers of life, on the energy transformations of which the whole of evolution has depended, are completely ignored.

Young naturalists who live in coal-mining areas will find this particularly frustrating, while academic botanists may justifiably feel that the title should have had the word 'Animal' inserted.

J. S. FORDHAM

The Young Scientist's Companion

A Book of Information and Advice to all concerned with Science. By Maurice Goldsmith. Pp. 160+4 plates. (London: Souvenir Press, Ltd., 1961.) 15*s.* net.

THE book claims to be "an information guide for all concerned with science" and its weakness is that it attempts too much. For example, the schoolboy is unlikely to wish to present scientific films or to use wall charts, so that lists of addresses relating to them are not helpful to him, while the science teacher or technologist can get fuller information from professional sources. The information given on careers is necessarily limited, but it could have been extended had the reader been referred to *Whitaker's Almanac* for the addresses of professional bodies, which occupy four pages. There are some useful notes on equipping a 'home laboratory' but few suggestions about what to do with the equipment. The notes on science fiction could well be omitted since the schoolboy is well informed and the adult who is an addict will know his way round the public library.

The best features are perhaps the illustration of scientific method as it was applied in developing the periodic table of elements and the commentary, which contains some interesting extracts from the writings of great scientists and of present-day scientific workers as well as elaborations of points mentioned in the text. The book list is also valuable.

The book is one of a series of *Companions* so that the author was presumably expected to fit a little of everything into 150 pages or so. He is not to be blamed if the resulting volume appears superficial.

K. W. LYON

Vanishing Animals

Preserving Nature's Rarities. By Philip Street. Pp. 232+20 plates. (London: Faber and Faber, Ltd., 1961.) 21*s.* net.

THIS book concerns one of the greatest problems facing naturalists and zoologists throughout the world: the conservation of wild life. Particular emphasis is given to rare species and to those threatened with extinction.

It may, therefore, be thought to be a book for the specialist, but this is not so. The book makes a fascinating study for all animal lovers, and is very easy to read. The author writes lucidly from his wide research on the subject, with the result that few will fail to be entertained, and many will gain a deep interest in the welfare of fauna.

The book covers a wide range. Fresh information on the habits and behaviour of various animals is disclosed: for example, the fact that the sea otter spends much of its time floating on its back with its arms resting across its chest or using its chest as a table for its food. Odd corners of the Earth, such as the Galapagos, Islands, are mentioned. Past, present and future problems are considered.

There are twenty black-and-white plates that are actual photographs. These provide rare glimpses for the layman of some animals: for example, fur seals and the Komodo dragon of Komodo Island. Photographs come from such sources as David Attenborough and Mrs. Peter Scott, as well as from scientists and zoologists of many countries.

B. J. G. Maitland

The Birds of the British Isles

By Dr. David Armitage Bannerman. Illustrated by George E. Lodge. Vol. 10: Scolopacidae (conclusion); Charadriidae; Recurvirostridae; Haematopodidae. Pp. x + 320 + 23 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 63*s*. net.

With the appearance of this tenth of his dozen volumes, Dr. Bannerman approaches completion of his great undertaking. Here he concludes his account of the sandpiper family (mainly those species named in English from the colour of their 'shanks'), deals with the smaller number of the plovers, and ends with chapters on the avocet, the stilt, and the oystercatcher. As in the ninth volume, many of the species are more or less uncommon visitors to the British Isles from distant breeding grounds; it is therefore valuable to have the contributions that Dr. Bannerman has obtained from American, Russian and other ornithologists on aspects of life-history that could not otherwise have been fully covered. There are also British contributions on special topics such as the breeding habits of the dotterel and greenshank in Scotland, the disappearance of the Kentish plover as a British breeding bird (last known to nest in 1956), and the recovery of that status by the avocet under careful protection in East Anglia.

Landsborough Thomson

Living Fishes of the World

By Earl S. Herald. Pp. 304 (300 illustrations). (London: Hamish Hamilton, Ltd., 1961.) 63*s*. net.

Had there been fewer errors in the text, this book could have made a noteworthy addition to the list of popular works on fishes. The colour and monochrome photographs are very good, and some are surely unique. Although much of the text passes muster, there are far too many errors. Some, like the inconsistent spelling of certain specific names and placing Phallostethidae under a heading "Suborder Polynemoidea", may be due to careless proof-reading. Others cannot be so dismissed. There are unsettling contradictions; for example, we are told that the arawara (*Osteoglossum*) and *Scleropages* are both mouth-breeders, and later in the section we are told that the breeding habits of the arawara are unknown, but it is suspected of being a mouth-breeder. The author's views on external gills, their occurrence and homologies are confused. Who would agree that the external gills of *Polypterus* are only comparable with the embryonic gills of larval sharks, and that they are not like the external gills of lung fishes? The section dealing with cichlid breeding habits needs tightening up, and it is a pity that *Tilapia melanopleura* (one of the non-mouth-breeding species) should be used to illustrate "African Mouth-breeders". One final example: in view of the many articles and papers on the cœlacanth it is surprising to read that one of its "anatomical distinctions include . . . a large lung . . ."; it is stretching definitions too far to describe this admittedly large but narrow, fat-filled and solid structure as a lung.

These and other errors could easily be corrected in a revised edition, which I hope will appear, for there is a lot of valuable material in this book which the non-professional zoologist would not easily find without searching widely.

P. H. Greenwood

The Chrysomelidae (Coleopt.) of China and Korea

Part 1. By J. L. Gressitt and S. Kimoto. (Pacific Insects Monograph, No. 1*A*.) Pp. 300. (Honolulu, Hawaii: Entomology Department, Bernice P. Bishop Museum, 1961.) 4 dollars; 29*s*.

As the authors state in their introduction, the Chrysomelidae is one of the largest families of beetles. It probably includes more than 50,000 species in the world. Therefore, any modern work dealing comprehensively with the family as found in a single zoogeographical region of the world is to be welcomed. Unfortunately, it falls between two stools. It is more than a simple catalogue, but much less than a comprehensive hand-book to the fauna of the region. In short, the authors seem to have attempted to do too much in too little time and above all, space. It is a pity that this work was not modelled on that invaluable contribution to our knowledge of the Chrysomelidae, the three volumes by S. Maulik in the *Fauna of India*.

The illustrations in the present work are superb and the descriptions of new species good. The keys to the sub-families, genera and species are clear and a very valuable tool to all workers on the group. The authors are to be congratulated on the monumental effort needed for their compilation. However, it would be useful to have short descriptions and notes on each genus and species. One would then be able to check identifications and to determine new species. It is pleasing to find that the host plants are stated where known; but fuller biological notes on both larvæ and adults would be useful. It is to be hoped that the final volumes of the Chrysomelidae will include a comprehensive and easily used index at the back. The format of the *Insects of Hawaii* is to be recommended for all future volumes.

B. J. Selman

The Yorkshire Jurassic Flora

By Prof. Thomas Maxwell Harris. 1: Thallophyta-Pteridophyta. Pp. ix + 212. 71 text-figs. (London: British Museum (Natural History), 1961.) 80*s*. net.

This is the first of two volumes on the Jurassic flora of Yorkshire. The discoveries of fossil plants made since the early part of the nineteenth century in Yorkshire have probably contributed more to our knowledge of Jurassic plants than those made in any other part of the world. Nathorst, Sir Albert Seward, Dr. Hamshaw Thomas and Prof. Harris have carried out most of the more detailed scientific investigations of this flora. These two volumes by Prof. Harris will replace Seward's *Catalogue* of 1901. He assembles all the important information and has examined, using modern methods, the evidence that the fossils afford. He has also clarified the nomenclature, which was in a chaotic state. He has been able to define specific differences in some critical genera, for example, *Coniopteris*, and gives clear descriptions of species, including many that are new. This first volume is illustrated with numerous line drawings and silhouettes which are for the most part excellent, but there are a few (for example, Fig. 55*B*) which are so simplified that they do not resemble an actual specimen. I should have preferred that use had been made of some photographic illustrations to give an idea of the nature and beauty of some of the fossil material and of the cuticle and transfer preparations that may be prepared from it. Such illustrations would assist in substantiating the statements made and would permit overseas workers who have not handled the material to acquire a greater appreciation of Prof. Harris's methods of investigation.

These volumes will be of great value to palæobotanists and to the curators of the many museums in which there are collections of Jurassic fossil plants.

JOHN WALTON

Advances in Ophthalmology

Vol. 11. Edited by E. B. Streiff. Contributors: G. Ten Doesschate, W. A. Manschot, L. Matiaska, and J. Stepanik. (Bibliotheca Ophthalmologica, Fasc. 58.) Pp. vi+274. (Basel and New York: S. Karger, 1961.) 60 Swiss francs.

THE concept of collagen diseases was introduced about twenty years ago and, in the first paper in this volume, its impact on ophthalmology is examined. Descriptions are given of the essential features of generally accepted collagen diseases and, against this background, the pathological and clinical manifestations of certain disorders of the various tissues of the eye are carefully analysed. The conclusions are presented with an excellent combination of conciseness and clarity.

The second paper deals with the causes of blindness in the neighbourhood of Basle. To those familiar with Sorsby's surveys in England and Wales it will be interesting to find that, in Basle, uveitis is the main disease causing blindness, while primary glaucoma occupies the sixth position, being a less frequent cause of blindness than retinitis pigmentosa.

About half this volume is occupied by a treatise on tonography, dealing thoroughly with the theoretical basis and sources of error of the technique, but far less extensively with its diagnostic value. Adequate accounts are given of ocular rigidity, tonometric calibration, episcleral venous pressure and other factors of fundamental importance in the determination of the facility of aqueous outflow. Nevertheless, one is left with the impression that a somewhat empirical method of evaluating the results achieves the best differentiation of glaucomatous from normal eyes by tonography.

Finally, there is a brief review of the history of ophthalmology which attempts little more than giving a list of the more important references.

J. GLOSTER

Advances in Applied Microbiology

Vol. 3. Edited by Wayne W. Umbreit. Pp. xi+421. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 93*s*.

TEN widely diverse topics are discussed in the third volume in this series. Three are primarily concerned with technique: preservation of cultures by freeze-drying, by R. J. Heckly; the hazards of accidental infection in the microbiological laboratory by M. A. Chatigny; and the application of chromatography to the determination of organic acids likely to be of interest in studies of microbial metabolism, by A. C. Hulme. Microbial metabolism is considered in articles on the cardiac lactones by E. Titus, on the oxidation of aromatic compounds by M. H. Rogoff, and by J. D. Bu'lock, who contributes a stimulating discussion of the relationships between general and secondary metabolites with particular reference to antibiotic synthesis. Taxonomy is represented by a scheme put forward by E. Baldacci for the classification of the actinomycetes. In the remaining articles F. M. Schabel, jun., and R. F. Pittillo discuss the use of micro-organisms in screening tests in the search for anti-tumour drugs, D. J. Merchant and C. R. Eidam consider the potentialities of the large-scale use of animal cell cultures for biochemical syntheses and interconversions, and N. C. Dondero is concerned with the nature and economic significance of the sheathed, slime-forming bacteria of the genus *Sphaerotilus*.

This volume is less parochial in outlook than its predecessor, and it has benefited from the effort that has been made to restore some international flavour to the series.

J. W. G. PORTER

Microbial Cell Walls

By M. R. J. Salton. (CIBA Lectures in Microbial Biochemistry, Vol. 4.) Pp. ix+94. (New York and London: John Wiley and Sons, Inc., 1960.) 28*s*.

THE CIBA Lectures in Microbial Biochemistry were established in 1955 and four of the series have now been published, the latest having been given by Dr. Salton in the spring of 1960. It deals with the isolation, electron microscopy, chemistry, synthesis and degradation of the cell walls of micro-organisms. Although the bulk of the material concerns the walls of Gram-positive bacteria, mention is also made of blue, red and brown, and blue-green algæ, fungi and yeasts, as well as Gram-negative bacteria. A wide range of information is collected in an extremely concise form and there are ample references (more than 200) to the relevant literature.

Much space is devoted to the constituents unique to bacterial cell walls—mucopeptides containing D-amino-acids, diaminopimelic acid and muramic acid; and teichoic acids consisting of polymers of ribitol or glycerol phosphates with attached carbohydrate residues and ester-linked D-alanine. The mucopeptides apparently occur in all bacterial cell walls so far examined, and it is the synthesis of this class of compound which is inhibited by such antibiotics as penicillin, bacitracin, oxamycin (D-cycloserine) and novobiocin.

The author was a pioneer in the development of methods for studying bacterial cell walls and has contributed much from a variety of aspects. The CIBA Lectures provide a welcome opportunity for an expert to give a reasoned account of his special field of interest and this volume is fully up to the standard of its predecessors.

Chromatographic Reviews

Progress in Chromatography, Electrophoresis and Related Methods, Vol. 3. Edited by Michael Lederer. Pp. viii + 187. (Amsterdam: Elsevier Publishing Co., Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 50*s*.

TWO of the articles in this volume have hitherto not been published. H. Bloemendal reviews starch-column and starch-gel electrophoresis in two articles, which form a series with the first article in a previous volume on starch slab electrophoresis. Techniques and applications are adequately covered, though one wonders why the author took the trouble to write so comprehensively about the first subject when his conclusions show that he does not think much of the method. L. Reio continues a painstaking survey of the paper chromatography of phenolic and related compounds, begun in a previous volume, and gives R_F values and colour reactions of hundreds of compounds. Some of his tables contain such a heterogeneous collection of substances that the value of his article would have been enhanced by a more systematic presentation enabling one to locate a given compound. Substances which can undergo slow changes may form more than one zone on a chromatogram. This phenomenon is examined by R. A. Keller and J. C. Giddings. Z. Pučar deals fully with methods utilizing continuous electrophoresis and electrophoresis combined with chromatography. The articles by J. J. Wren are up to date and useful reviews of chromatography of lipids on silicic acid. Finally, the behaviour of inorganic ions on paper chromatograms (M. Lederer) and by ionophoresis (R. A. Bailey and L. Yaffe) are the subjects of comprehensive reviews covering progress in the past few years and complete another useful volume in this series.

R. CONSDEN

Process Chemistry

Vol. 3. Edited by F. R. Bruce, J. M. Fletcher and H. H. Hyman. (Progress in Nuclear Energy, Series 3.) Pp. vi + 474 + xii. (London and New York: Pergamon Press, 1961.) 105*s*. net.

THE first volume on *Process Chemistry* was published in the year following the 1955 Geneva Conference and contained not only a selection of conference papers revised by their authors but also a substantial number of papers specially written for it. By contrast, this third volume contains twenty-seven papers from the 1958 Conference, only one of which is marked as modified, together with a review of its two hundred process chemistry papers. It is disappointing, therefore, to find such a long delay in publication.

The book contains a reasonable selection of papers from four volumes of the official proceedings, covering the treatment of ores, the refining and production of feed materials, aqueous and non-aqueous processes for irradiated fuels and waste treatment. Some deal with operational processes, some review special aspects of the chemistry of the processes and others describe research and development work on new processes.

Much of the information represents the first open publication; the Russian papers in particular were new to the majority of delegates at the Conference. The two Conferences did in fact cover some fifteen years of work originally reported to a privileged few.

This volume does bring together under one cover a fair cross-section of the art in 1958, and is therefore of value to the library which cannot justify the expenditure on a set of the official proceedings. But it must be admitted that interested people are more likely to go to the original sources and can be kept up to date by the excellent and cheap quarterly reviews now available.

A. S. WHITE

Physical Chemistry

By Prof. Farrington Daniels and Prof. Robert A. Alberty. Second edition. Pp. x + 744. (New York and London: John Wiley and Sons, Inc., 1961.) 70*s*.

THE ancestry of this book may be traced back to Getman (1913), *Outlines of Theoretical Chemistry*, which passed through four editions by 1927, when Dr. Daniels took over. In 1955, Getman and Daniels was replaced by the first edition of the present book, which quickly became established as a leading physical chemistry text for university work in the United States. The second edition has been largely re-written and substantially expanded. The treatment is more mathematical, with emphasis on the rigorous treatment of chemical thermodynamics, the laws of which are established in the opening chapters, and quantum mechanics. This has necessitated the pruning of older and more elementary topics. New chapters on spectroscopy and statistical mechanics emphasize the thermodynamical outlook. Simple molecular orbital theory is included and there are new chapters on one-component systems and surface phenomena. Problems (836) are grouped according to difficulty at the end of each chapter and important calculations are shown in the text. A summary of the mathematical relations which might cause difficulty are included in the appendix, with values (1951) of constants (showing uncertainties) and various conversion factors. Atomic weights are based on the oxygen-16 scale (1956–57). The index seems comprehensive. The book is well produced with clear diagrams. The lucid arguments based on a logical development of the subject make the second edition of Daniels and Alberty most welcome among the modern texts giving a broad coverage of physical chemistry at university-level.

B. J. MOODY

Annual Review of Physical Chemistry, Vol. 11

Edited by H. Eyring, in association with C. J. Christensen and H. S. Johnston. Pp. vii + 587. (Palo Alto, Calif.: Annual Reviews, Inc., 1960.) 7 dollars.

THE current volume of this valuable publication reviews the literature, in most cases, to the end of December 1959. Most of the articles are concise; the first one, for example, occupies a little more than twelve pages but has 347 references. Most of the topics covered in previous volumes reappear and are carried further. Some of the articles, such as one on the quantum theory of electronic structure of molecules, will be intelligible only to specialists; others are of a more general character. The very long article on statistical theory of transport is mathematical. There are short articles on chromatography and nuclear and electronic resonance.

The article on photosynthesis will be of interest to biologists. The subject is evidently not yet fully cleared up; in particular, recent work on the pathway of carbon has received some amount of criticism. It is stated that on long manned space flights the possibility of growing algae on human wastes and using them as food is being considered. In the article on combustion and flames account is taken of high temperatures, and it is emphasized that much information about the properties of substances is still required. As for detonation phenomena, it is said that the old Chapman–Jouguet theory remains a satisfactory approach but in spite of much recent research very much remains to be done. The volume is one which physical chemists will welcome.

J. R. PARTINGTON

A Guidebook to Mechanism in Organic Chemistry

By Dr. Peter Sykes. Pp. xiii + 247. (London: Longmans Green and Co., Ltd., 1961.) 21*s*. net.

THE teaching of organic chemistry has been undergoing a revolution, and this excellent paper-backed guide-book reflects the way in which a synthesis between the experimental, factual approach essential to organic chemistry and the application of electronic theories to bring an understanding of the mechanism of reactions involving carbon compounds is being achieved at Cambridge. Much experience of lecturing and supervising undergraduates has tempered this lucid exposition of the essentials of the subject. The author has had in mind the needs of the scholarship candidate, undergraduate, technical college student and professional chemist of classical upbringing. He is rather optimistic about the sixth-form boy who will not have the necessary background of atomic and molecular orbital theory, but undoubtedly there is much here to stimulate and excite the intellectual curiosity of the more able boy.

After a brief description of valency theory in relation to organic compounds, reactivity is discussed in terms of relative electron density and the operation of steric factors. The energy changes associated with reactions and the principles underlying the investigation of their mechanism are clearly explained. The relative strengths of organic acids and bases are considered at length in terms of electronic theory and separate chapters are devoted to each of the more important types of organic reaction in such a way as to make this a most useful reference work. In addition, the printers of this most attractive book have produced (to quote the author), "that rare phenomenon, structural formulæ that are both clear and æsthetically satisfying". B. J. MOODY

Introduction to Mechanics, Matter and Waves

By Prof. Uno Ingard and Prof. William L. Kraushaar. Pp. xv + 672. (Reading, Mass., and London: Addison-Wesley Publishing Company, Inc., 1960.) 53*s*.

IN general the standard of this work, which deals with the mechanics of solids and fluids, heat and wave motion is that of the first year of an honours-degree course in physics. There is, unfortunately, a lack of balance in that two-thirds of the book is devoted to mechanics with the inevitable consequence that the remaining two topics are highly condensed and, indeed, the section on heat suffers also from an incompleteness of presentation. While applauding the determination of the authors to bring out the experimental aspects of mechanics, it seems likely that the student who possesses the knowledge of calculus and vector algebra which is required later will find the earlier discussion of force, motion and momentum frustrating, and that his interests would be better served by the omission of the more elementary illustrations in favour of an extended discussion of heat and wave motion. This apart, the treatment of mechanics is excellent, and the basic concepts of energy, and of both linear and angular momentum, are illustrated by numerous examples which range from the motion of projectiles and oscillating bodies to planetary orbits and spinning symmetrical bodies.

In the section devoted to heat the macroscopic and microscopic points of view of thermodynamics have been dovetailed into a united whole, but this commendable approach has been marred by omissions, such as the failure to present the modern definition of the absolute scale of temperature and by several errors, particularly in connexion with specific heats and with relationships involving the laws of thermodynamics. The final three chapters comprise a study of wave pulses in solids and gases, and include a treatment of both progressive and standing waves.

H. STEEPLE

Microwave Ferrites

By Dr. P. J. B. Clarricoats. Pp. xi + 260. (London: Chapman and Hall, Ltd., 1961.) 50*s*. net.

THE class of non-reciprocal microwave components which has emerged as a result of the use of magnetized ferrites has given the microwave circuit designer a new degree of freedom. Even a casual perusal of recent literature on microwave work will indicate the impact which these devices have already made, and Dr. Clarricoats's book on the subject is valuable and timely.

The subject-matter of the book includes three main topics: ferrite materials, ferrite-loaded waveguides, and ferrite components. After a brief introductory chapter, the second is mainly devoted to the structure and fundamental magnetic properties of ferrites. In the third chapter an account of the microwave properties of ferrites is given, and the fourth chapter gives a more detailed account of the loss mechanism on a physical basis, rather than phenomenologically as in the third chapter.

Chapter 5, which represents more than a third of the book, deals clearly with the very complicated problems associated with the propagation of electromagnetic waves in ferrite-loaded waveguides. Although this chapter is primarily an account of the fundamental theory, to which Dr. Clarricoats has himself made some important contributions, it is written with applications in mind, and for this reason will be particularly useful to the microwave engineer.

A final chapter gives a useful review of various non-reciprocal microwave ferrite components. In such a rapidly developing field, any review is inevitably a little out of date by the time it is published. but examples of most of the important devices developed by the end of 1959 are included. The chapter forms a very useful introduction to the extensive literature on the subject.

The book is warmly recommended as a valuable addition to the literature of microwaves.

A. L. CULLEN

Elements of Modern Physics

A Course in those aspects of Modern Physics that underline important Engineering Developments. By Paul L. Copeland and William E. Bennett. Pp. x + 507. (New York and London: Oxford University Press, 1961.) 68*s*. net.

THE publication of a volume such as this is sure evidence of the tremendous and rapid growth of atomic physics since the days of J. J. Thomson and Prof. E. Rutherford, and as the results of the more recent work done in this field are becoming increasingly incorporated into engineering practice, this book must certainly be welcomed by engineering students and also by other students of science who wish to keep abreast of modern scientific thought and developments.

The book is divided into three parts. Part 1 concerns itself with the rise of atomic physics, and develops the particular aspects of the structure of those media which seem continuous on casual inspection; it includes such topics as the kinetic theory of gases, relativity, the quantum theory and the structure of atoms. Part 2 introduces some of the ideas of wave-mechanics, while Part 3 is an introduction to nuclear physics.

To get the fullest advantage of this excellent book it is necessary that the student should have a good knowledge of classical physics and also should be well groomed with calculus and differential equations.

For those students who may wish to extend their knowledge beyond the scope of this book a useful set of references is provided at the end of each chapter, where there is also a summary and a set of searching questions.

The authors have certainly made a notable contribution to the furtherance of knowledge in this important field of modern physics, and one would hope that the book would be extensively used by many students of engineering, and others who may be interested in the phenomenal strides made in so short a time; it most surely deserves to be.

T. M. Yarwood

Proceedings of the International Conference on Theoretical Aspects of Very High-Energy Phenomena

Held at CERN, 5–9 June, 1961. Edited by J. S. Bell, F. Cerulus, T. Ericson, J. Nilsson and H. Rollnik. (Sponsored by the International Union of Pure and Applied Physics.) Pp. v+426. (Geneva: CERN—European Organization for Nuclear Research, 1961.) n.p.

The CERN conference on "Theoretical Aspects of Very High-Energy Phenomena" was held in connexion with present thinking on possible super high-energy accelerators for the post-1970 period. The report comprises about 40 papers mostly devoted to extrapolations of present-day ideas to higher energies than are at present available, but not, in general, directed to specific accelerators. The present report is not too rich in theoretical reasons for proceeding to higher energies, but this is perhaps not to be expected—in his summary lecture Prof. van Hove refers to the well-known drastic limitation of our power of imagination.

The report comprises the following sections: weak interactions; electromagnetic interactions; experimental results (including cosmic ray and CERN *PS* results); statistical model; diffraction theory; one-meson exchange; dispersion theory and Mandelstam representation; accelerators. The section on electromagnetic interactions is largely concerned with the electron storage rings building and projected at Frascati and Stanford; in addition to electron–electron colliding-beam experiments providing a sensitive test of quantum electrodynamics at high energies, electron–positron annihilation gives a large field of interesting and significant events as set out in the paper by Gatto. Higher-energy protons should provide tests of theories of weak interactions (particularly by giving neutrino and muon beams) and should help to elucidate diffraction scattering and the asymptotic behaviour of cross-sections at high energies, on which so much theoretical speculation is at present bent.

R. G. Moorhouse

Symposium of Plasma Dynamics

Edited by Francis H. Clauser. (Sponsored by the Air Force Office of Scientific Research. Prepared under the auspices of the National Academy of Sciences.) Pp. ix+369. (London: Pergamon Press; Reading, Mass.: Addison-Wesley Publishing Company, 1960.) 84*s*. net.

The symposium at Woods Hole, Massachusetts, in June 1958 boasted an impressive list of participants almost exclusively from the United States. No formal papers were given, but rather a series of discussions with introductory talks at the beginning of each session.

The chapters on the experimental investigation of plasma properties show the beginnings of many studies on waves in plasmas, the interaction of electron beams with ionized media, shock-waves in conducting media in the presence of a magnetic field in addition to diagnostic work on transient plasmas in a variety of geometries.

The theoretical contributions discuss instabilities with great clarity, and the heating and confinement of plasmas. Continuum and particle approaches are reviewed and compared, and the limits of their applicability considered.

A short chapter on aerodynamic effects when a plasma streams through a magnetic field together with longer contributions on extra-terrestrial magneto-hydrodynamics conclude the symposium. Interesting accounts of such phenomena in the solar system appear, and cosmic magnetic fields are reviewed also. Their influence on interstellar material has repercussions over a wide range of astrophysics. A vigorous debate on the simulation of galactic phenomena in the laboratory is reported, in which one conference aim—the frank expression of conflicting views—is realized to the full. This book presents clearly the concepts of modern plasma dynamics, forming a good introduction to the subject, complete with a valuable bibliography. It cannot, naturally, be taken as a summary of the present state of the art.

P. F. Little

Yearbook of Astronomy 1962

Edited by J. G. Porter, in association with Patrick Moore. Pp. viii+216. (London: Eyre and Spottiswoode (Publishers), Ltd., 1961.) 15*s*. net.

This book is designed not so much for the amateur with his own telescope and observing programme but for the very many who can observe only with the naked eye, binoculars or small telescopes. To this end it succeeds admirably, and we look forward to seeing the *Yearbook* maintaining the high standard of this issue for many years to come.

The *Yearbook* may be divided into two sections. The first section provides a set of "Monthly Notes", mainly concerned with planetary observations and a set of star charts. The "Monthly Notes" are augmented by notes on various phenomena of interest, such as meteor showers, zodiacal light, etc. The star charts are arranged so that the stars move from the positions in one chart to the positions in the following chart in a period of 2 hr. This section is completed by notes on eclipses, occultations, comets, meteors, minor planets and selected events for 1963.

The second section contains thirteen short articles on various aspects of astronomy. Each article is written by an author experienced in the field covered by the article. The shortness of the articles is compensated by including a fairly comprehensive reading list so that if any reader is sufficiently stimulated by a given article he can carry on his reading by choosing a suitable book from the list.

A list of astronomical societies, a comprehensive glossary of astronomical terms and a list of selected interesting telescopic objects conclude the book. The *Yearbook* should form a very useful addition to the shelves of any amateur astronomer.

D. McNally

Relativity for Engineers and Science Teachers

By Laurence H. A. Carr. Pp. vi+52. (London: Macdonald and Co. (Publishers), Ltd., 1960.) 12*s*. 6*d*. net.

THIS little book is a commendably brave, and, one must add, successful attempt by the author to convince students, hitherto perhaps a little timorous of getting to grips with the theory of relativity, that the principles, when shorn of the complex mathematical treatment, are not so formidable after all.

Although the full subject of relativity consists of two separate theses, the special theory dealing with two bodies in uniform relative motion and the general theory which concerns the movement of bodies under the influence of a permanent accelerating force, it is only the former which is dealt with here.

The opening chapter is given to recapitulating the basic theory of what is regarded as the classical mechanics, while the rest of the book is devoted to the modifications introduced by the theory of relativity and which are necessary to bring experimental data and theory into harmony.

The well-known Michelson–Morley experiment was, of course, the crisis in physics which sparked off the theory of relativity by Einstein in 1905, and the author of *Relativity for Engineers and Science Teachers* deals with this very well in a separate chapter, in which he brings out clearly the value of the "contraction formula" of Lorentz–Fitzgerald, namely,

$$\sqrt{1 - \frac{v^2}{c^2}},$$

which occurs throughout the theory of relativity.

At the end of the book there is an extremely useful summary of the conclusions reached, and this should be most helpful.

Altogether the book is well thought out and excellently done, and students and teachers alike should find it stimulating, informative and understandable.

T. M. Yarwood

Electron Microscopy in Anatomy

Proceedings of a Symposium held by the Anatomical Society of Great Britain on the Ultra-Structure of Cells. Pp. viii+288. (London: Edward Arnold (Publishers), Ltd., 1961.) 50*s*. net.

THIS is an interesting and genuinely useful symposium. The importance of the various chapters varies considerably, but there are several contributions of outstanding value, especially those on the myelin sheath of nerves, the 'unit membrane', and the secretory processes of the pancreatic exocrine cell. These chapters provide very convenient summaries of the present state of knowledge.

Twenty years from now it will be interesting to look back on such a book as this. Has the early work in electron microscopy proceeded along the most fruitful lines? It is too soon to judge. The techniques used in the preparation of tissues seem to be rather empirical. It is almost as though we had not profited from the work of such men as Alfred Fischer, W. B. Hardy, and Gustav Mann, who sought, six decades ago, to place the technical methods of light microscopy on a firmer, less empirical basis. There is a scramble to be the first to 'see' new things, but scarcely enough effort to ensure that what is exhibited in our micrographs bears any close relationship to what was present in life. Perhaps this phase was inevitable: perhaps we are now emerging from it into a more hopeful future. This book gives a useful indication of how far we have gone and where we are likely to go next.

J. R. Baker

Industrial Research in Britain

Advisory Editor: A. W. Haslett. Fourth edition. Pp. 461. (London: Harrap Research Publications, 1962.) 84*s*. net.

IN the fourth edition of *Industrial Research in Britain* Mr. A. W. Haslett succeeds Dr. Percy Dunsheath as advisory editor, and additional sections for sponsored research organizations, scientific attachés, data-processing services, translations of Soviet technical and scientific periodicals and technical libraries have been introduced. The existing sections have been completely revised and some are presented in a new format. Sir Harry Melville outlines the part which the Government plays in civil research and development and in the technical information services of Britain. Mr. A. F. H. Baylis contributes an article on careers in professions associated with industrial research which is both admirable in itself and supported by an excellent bibliography.

In the sixteen years since the book was first published its character has been transformed, and although the price has been quadrupled it now supplies what the first edition could scarcely claim to do—a comprehensive reference book on industrial research in Britain generally. Such points of criticism as may be advanced are in matters of detail, doubtless due to oversight, and chiefly in relation to publications. The section dealing with research activities of Government departments and statutory bodies fails to give the complete picture of Government organization that might have been expected; the Nature Conservancy is the only one of the Research Councils not mentioned and even in a volume dealing with industrial research the omission is invidious as well as unjustified. Although the pamphlet *Government Organization in the Civil Field* is out of date, its existence might have been mentioned. Similarly, the section on industrial research in universities should fittingly have mentioned the publication put out by the British Council for the Department of Scientific and Industrial Research entitled *Scientific Research in British Universities*. Surprisingly, the section dealing with periodicals omits *The New Scientist* although including older periodicals of the same general scientific or technical character.

R. Brightman

(*Continued from page* 42)

over large regions of the temperate zone ionosphere as well as the auroral zones. Such heat input can modulate[5] the temperature at 150 km. by hundreds of degrees K. For the following calculation put $\delta T_0 =$ 300° K. and $T_0 =$ 500° K. originally. Then n_0 changes by 60 per cent due to change in scale height at the 0 level. The percentage change in n will increase with height until it is more than 200 per cent at 650 km. This compares favourably with the increase by a factor of 2·5 of the density at 656 km. deduced from the orbital acceleration[1] of Satellite 1958 β2 (cf. ref. 3).

It is concluded on the basis of the crude analysis above that joule heating of the lower reaches of the ionosphere (100–250 km.) is a major factor in modulation of thermospheric densities. Other factors would be the absorption of radiation from the Sun giving an additional day–night effect[6] superimposed on the *Sq* variation, and the generation of heat by corpuscular radiation impinging on the ionosphere from the magnetosphere. While the energy for the *Sq* variation is thought to be derived from tidal oscillation of the upper atmosphere, the energy for the magnetic disturbance variation is due to that portion of the radiation of the Sun which takes a day or so to come to the Earth. The magnitude of the density fluctuations dependent on magnetic disturbance should increase with height and with proximity to auroral regions. It is suggested that thermospheric densities as deduced from the orbital acceleration of a satellite should correlate well with the local magnetic *K* index at the latitude and longitude of its perigee.

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A Source of Energy for the Ionosphere

THE heat requirement of the equatorial and temperate zone ionosphere is normally about 2 × 10^{10} cal. sec.$^{-1}$ (cf. Johnson[1]). This is because of the loss of heat downwards due to a temperature gradient of about 10° K. km.$^{-1}$ between 100 and 200 km. altitude. It is the purpose of this communication to suggest that joule heating by ionospheric electric currents can supply heat in this quantity. Current of density (j) causes joule heating at the rate Q given by j^2/σ_3, where σ_3 is the Cowling conductivity.

Consider first those ionospheric currents causing the quiet solar daily variation (*Sq*) of the geomagnetic field. Their intensity[2] is about 10^4 amp. per degree of latitude. Assuming they flow in a height range of 50 km. in a quiet ionosphere in which $\sigma_{3(\text{average})} \approx 4 \times 10^{-14}$ E.M.U., $Q(Sq)$ is about 10^{-8} erg cm.$^{-3}$ sec.$^{-1}$. Over the whole globe this amounts to about 10^{10} cal. sec.$^{-1}$.

Secondly, there are the currents which contribute to the disturbance daily variation (*Ds*) of the geomagnetic field. Average currents in the auroral zone during the Second International Polar Year[3] taken in conjunction with a ten-fold increase of ionospheric conductivity implies a peak $Q(Ds$, auroral) value of 10^{-5} erg cm.$^{-3}$ sec.$^{-1}$ at 150 km. altitude and averaging about 10^{-6} erg cm.$^{-3}$ sec.$^{-1}$ throughout the 100–200 km. height-range[4,5]. An estimate of $Q(Ds$, low latitude) is difficult to make, for the appropriate ionospheric conductivity during disturbance is not known. However, $Q(Ds$, low latitude) is assumed to be of the same order as $Q(Sq$, low latitude). Assuming the auroral heating region extends 10,000 km. around the two auroral zones at any given instant, the total average flux from them is about 2 × 10^{10} cal. sec.$^{-1}$.

Thirdly, there is probably a contribution from the storm time (*Dst*) variation of the geomagnetic field supposed here to be due to currents flowing in the ionosphere with an average strength of about 10^4 amp. per degree of latitude (cf. Figs. 23. 24 of Chapman and Bartels[2]). Assuming no great change in average conductivity over the whole globe, $Q(Dst)_{\text{av}} \approx Q(Sq)$.

There will be a flux of heat from auroral regions to other parts of the ionosphere. Johnson[1] has shown that convection is far more important than conduction in this process. Heating of the auroral ionosphere will cause distortion of the constant pressure surfaces so that at 200 km. altitude such a surface can change height by as much as 50 km. (refs. 4 and 5). The horizontal gradient of pressure is balanced by the Lorentz force $j \times H$, where H is the (near vertical) geomagnetic field. This Lorentz force was not taken into account in ref. 1. This pressure gradient can have winds of more than 100 msec.$^{-1}$ associated with it[4]. Such winds could convect heat from the auroral zones to distances of 10,000 km. in times of order 10 hr., and could produce all the heat requirements of the lower latitude ionosphere[1]. Any conduction of heat from the auroral zones would proceed most rapidly at the higher ionospheric levels, to come downwards in other regions of the globe.

Now $Q(Ds)$, and $Q(Dst)$, may range over some orders of magnitude. However, this point is uncertain for it is not known whether an increase in j is accompanied by an increase of σ_3 to leave $Q(Ds)$ substantially unaltered. Since magnetic disturbance (δH) is approximately proportional to j, an increase of a factor 10 of δH above average implies a 100-fold increase of σ_3 (that is, 1,000 times a quiet low latitude value) to keep $Q(Ds)$ constant. The implied electron densities are then 10^8 cm.$^{-3}$, that is, a factor of 50–100 larger than can reasonably be expected over large regions. Thus it is reasonable that $Q(Ds)$ increases at least by a factor 10 above average for the very large magnetic disturbance. Moreover, magnetic disturbance is generally more widespread when it is more intense. Hence at these times the flux available to the global atmosphere is greatly enhanced, perhaps by a factor 100 above normal.

A major source of heat for the ionosphere is joule heating by those electric currents flowing in it to cause geomagnetic variations (separated above into *Sq*, *Ds*, *Dst*). It is suggested that heat from this source is normally sufficient to account for the heat flux in the low-latitude ionosphere. At times of very large magnetic disturbance the available heat, due to *in situ* heating and flux from the auroral zones, can be at least a factor 10 above average.

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PHYSICS

A Simple Low-Noise Low-Frequency Transistor Amplifier

The properties of the amplifier to be described are entirely due to the type of transistor used, and the purpose of this communication is to report on its suitability for the amplification of electro-physiological wave-forms, particularly those originating in the brain. Of the commonly recorded physiological potentials, the electroencephalogram (EEG) presents the most difficulties because of the low voltage (10–1,000 μV.) and low frequency (1–50 c./s.) of its components. Recordings are usually obtained from electrodes on the scalp which give an output impedance of the order of 10,000 ohms. Hitherto, transistors have been unsuitable for amplifying these potentials direct because their intrinsic noise at low frequencies was too high and because the input impedance of the simpler circuit configurations was too low—about 1,000 ohms in the common emitter mode.

However, a new type of planar silicon transistor (Texas Instruments Inc., Type No. 2*N*929), designed to have low noise, has recently become available. Furthermore, because of the low currents at which it can be operated, it has a high input impedance when used in the common emitter mode. The circuit of Fig. 1 probably does not represent the best that can be devised for this transistor, but it gives a useful performance as an amplifier, providing a gain of about 30 and presenting an input impedance of about 200,000 ohms for a transistor power consumption of 10 μamp. at 3 volts.

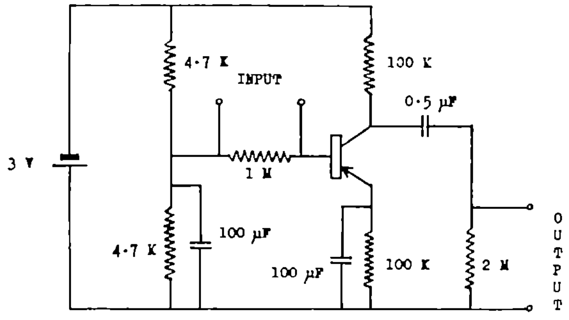

Fig. 1

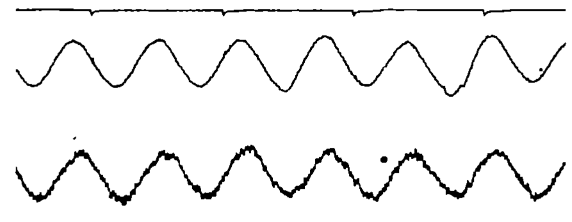

Fig. 2. Upper trace, 1-sec. time marker; middle trace, 5-μV. peak-to-peak input at 1·5 c./s. from a source impedance of 100 ohms, via transistor amplifier; lower trace, same input via EEG amplifier

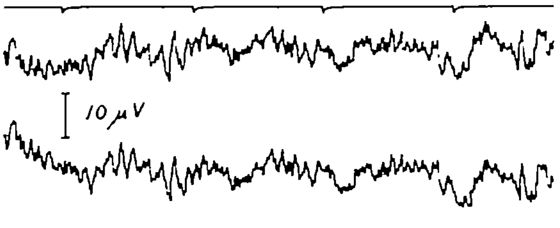

Fig. 3. Upper trace, 1-sec. time marker, middle trace, EEG signal via transistor amplifier; lower trace, same signal via standard EEG amplifier

The noise-level is illustrated in Fig. 2. The middle trace shows the output from the transistor amplifier (further amplified by a conventional EEG channel working at low gain) for an input of 5 μV. peak-to-peak at $1\frac{1}{2}$ c./s. derived from a source impedance of about 100 ohms. The lower trace shows the same signal amplified by another channel of the same EEG machine. The transistor amplifier has a noise-level of appreciably less than 1 μV. referred to the input. This is better than that obtained by an average EEG amplifier of which the lower trace is a typical example. Fig. 3 shows a low-voltage EEG signal derived from a pair of standard padded electrodes on the scalp and recorded by both methods at a sensitivity of 10 μV./cm. These traces suggest that the noise-level of the transistor amplifier is not sensibly worsened by the higher output impedance of ordinary EEG electrodes—about 8,000 ohms in this case.

A floating single-ended amplifier stage of this kind, with one side of the power supply earthed, has an in-phase rejection ratio of zero. However, the low-power requirements of these devices, which make it possible to use power supplies completely isolated from earth, open up new possibilities for the attainment of very high in-phase rejection ratios. Work is in hand to devise practical arrangements.

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X-Ray Yields from Elements in the Range Beryllium to Aluminium

Absolute measurements have been made, it is believed for the first time, of the *K* X-ray quantum yields resulting from electron bombardment of solid targets containing the elements beryllium, boron, oxygen and fluorine. For beryllium and boron 99 per cent pure targets were used while beryllium oxide and lithium fluoride were used for the oxygen and fluorine yields; corrections were applied for electron and X-ray absorption in the beryllium and lithium respectively. The yields from carbon (graphite) and aluminium targets have also been measured.

The X-ray count-rate was determined by pulse height analysis of the output from a gas-flow proportional counter filled with argon plus 2·5 per cent carbon dioxide. An essential feature of this counter was the provision of a very thin nitrocellulose entrance window capable of withstanding a pressure difference of one atmosphere: for the beryllium measurements this was only about 1000 Å. thick. After subtraction of the continuous radiation from the pulse height distribution, the measured *K* radiation count-rate was corrected for instrumental effects, such as the variation of counter efficiency with X-ray wave-length. A range of incident electron energies between 500 and 30,000 eV. was used on each target; the current was independently controlled and was varied between 5×10^{-10} and

8×10^{-7} amp. The diameter of the irradiated area of the target was less than 50μ.

The observed variation of the X-ray yield with electron energy is in excellent agreement with Archard's[1] theoretical predictions. The absolute values of the yields, other than that from beryllium, are 5–15 per cent higher than those given by Archard's theory, provided that the latter is modified by using Burhop's[2] formula for the fluorescence yield; the beryllium yield is about half that predicted. The carbon and aluminium yields are about half those reported by Dolby[3].

Apart from their significance as a test of the theory of X-ray emission from solid targets, the results indicate that X-ray microanalysis of all elements of atomic number higher than 3 is now theoretically and technically feasible. A full account and discussion of the work is being prepared for publication elsewhere.

Thanks are due to Dr. T. E. Allibone, director of the Laboratory, for permission to publish this communication.

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Errors involved in using the Rayleigh Equation for Interpretation of Light-Scattering Data beyond its Normally Accepted Limits

FOR a non-absorbing, spherical particle which is very small compared with the wave-length of the incident light, the intensity of the scattered light may be calculated from the Rayleigh equation, which assumes that the particle may be represented by a single vibrating electric dipole. The limit normally associated with its use is a radius, r, of $0{\cdot}05\lambda$, where λ is the wave-length of the radiation in the medium surrounding the particle. This corresponds to a value of the size parameter $\alpha = 2\pi r/\lambda$ of 0·3 approximately. When the particle is comparable in size to the wave-length of the incident light, account must be taken of the electric multipoles and the induced magnetic multipoles. The solution of this problem was originally proposed by Mie[1] for any values of α and m, where m is the refractive index of the particle relative to that of the medium, and involves complicated expressions which are laborious to evaluate.

However, in certain cases this can be avoided[1]. When the scattering functions calculated from the Rayleigh equation for $\alpha > 0{\cdot}3$ are compared with published values obtained using the Mie formulæ, it is found that the errors involved in using the Rayleigh equation are not significant for values of m in the region of 1·5 and for $\alpha < 1{\cdot}0$. The limit beyond which use of the Rayleigh equation introduces an appreciable error is dependent on the value of m as illustrated for the scattering coefficient K in Fig. 1. (Similar calculations, but in a more restricted range of m values, have been reported earlier[8].) This is also shown for the total intensity at 90° in Table 1 using the limited number of reliable Mie values available.

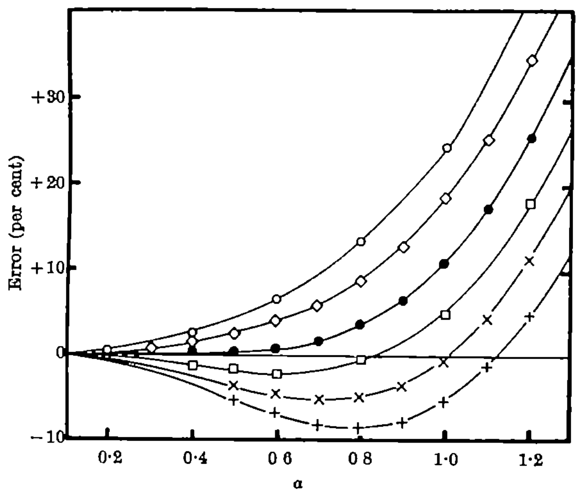

Fig. 1. Percentage error involved in using the Rayleigh equation to evaluate the scattering coefficient K

(Reference is given to the sources of Mie values used in the calculations.) ○, $m = 1{\cdot}25$ (ref. 3); ◇, $m = 1{\cdot}38$ (refs. 4 and 5); ●, $m = 1{\cdot}44$ (refs. 4 and 5); □, $m = 1{\cdot}55$ (refs. 4 and 6); ×, $m = 1{\cdot}65$ (ref. 7), +, $m = 1{\cdot}75$ (ref. 7)

Table 1. PERCENTAGE ERROR INVOLVED IN USING THE RAYLEIGH EQUATION TO EVALUATE THE TOTAL INTENSITY OF SCATTERED LIGHT AT 90°

(Reference is given to the sources of Mie values used in the calculations)

α	$m = 1{\cdot}33$ (ref. 4)	$m = 1{\cdot}44$ (ref. 4)	$m = 1{\cdot}55$ (ref. 4)	$m = 1{\cdot}75$ (ref. 8)
0·5	+ 2·68	+ 0·37	− 1·69	—
0·6	+ 4·41	+ 1·28	− 1·74	− 6·37
1·0	+22·5	+15·1	+ 8·78	− 1·26
1·2	+45	+37	+29	+17·2

Clearly the limit to which the Rayleigh equation can be usefully applied in the range $1{\cdot}3 \leqslant m \leqslant 1{\cdot}75$ is higher than that usually quoted. Fortunately, many of the colloid systems of current interest lie in this range.

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GEOLOGY

Study of Natural Glasses through their Behaviour as Membrane Electrodes

THE low-temperature chemical alteration of natural glass occurs in two stages: an initial stage in which it remains glassy but absorbs as much as 6 per cent water[1], and a final stage in which devitrification to clay minerals, with release of silica, occurs[2,3]. During the first stage the composition of the glass may change, with gain of K_2O and water and loss of Na_2O (Smith, R. L., personal communication). This change is due to ion exchange.

The traditional method of examining ion exchange is to equilibrate the powdered material with a series of solutions containing the ions of interest, and to analyse the chemical changes in the glass and/or solution. A new method is described here which is rapid and accurate for suitable materials, such as the natural glasses. This method consists of forming a membrane electrode of the ion-exchanger and determining the electrical potential of this against a reference electrode, in solutions of the exchangeable ions.

The potential of a glass membrane electrode in an aqueous solution of two salts has been shown by Nikolsky[4] to be dependent on the activities of the (univalent) exchangeable ions $[A^+]$ and $[B^+]$ according to equation (1).

$$\text{e.m.f.} = C_{AB} + \frac{RT}{\mathcal{F}} \ln \left([A^+] + K_{AB}[B^+]\right) \qquad (1)$$

Here K_{AB} is the mass action exchange constant for the exchange

$$AX + B^+ = BX + A^+ \qquad (2)$$

and X^- denotes a negative site on the glass. Equation (1) was later shown to be true only when the adsorbed ions form an ideal solution on the glass. More general equations are, first, that suggested by Eisenman *et al.*[5]:

$$\text{e.m.f.} = C_{AB} + \frac{RT}{\mathcal{F}} \ln \left([A^+]^{1/n_{AB}} + K_{AB}{}^{1/n_{AB}} [B^+]^{1/n_{AB}}\right)^n \qquad (3)$$

where n_{AB} is an empirical constant near unity, and, secondly, that given by Garrels *et al.*[6]:

$$\text{e.m.f.} = C_{AB} + \frac{RT}{\mathcal{F}} \ln \left(\frac{[A^+]}{\lambda_{AX}} + K_{AB} \frac{[B^+]}{\lambda_{BX}}\right) \qquad (4)$$

In equation (4), λ_{AX} and λ_{BX} are the rational activity coefficients of the adsorbed ions. Garrels *et al.*[6] further state that glass and mineral solid binary solutions frequently form 'regular solutions', so that the activity coefficients of the binary solution are related to the mole fractions (N) by the simultaneous equations:

$$\lambda_{AX} = \exp\left(\frac{B_{AB}}{RT} N^2_{BX}\right) \qquad (5a)$$

$$\lambda_{BX} = \exp\left(\frac{B_{AB}}{RT} N^2_{AX}\right) \qquad (5b)$$

where B_{AB} is an empirical constant.

These same authors have also derived equations for the bi-ionic potential resulting from the combination of one univalent and one bivalent cation, and from two bivalent cations. These equations are:

$$\text{e.m.f.} = C_{CD} + \frac{RT}{2\mathcal{F}} \ln \left(\frac{[C^+]^2}{\lambda_{C_2X_2}} + K_{CD} \frac{[D^{++}]}{\lambda_{DX_2}}\right) \qquad (6)$$

and

$$\text{e.m.f.} = C_{EF} + \frac{RT}{2\mathcal{F}} \ln \left(\frac{[E^{++}]}{\lambda_{EX_2}} + K_{EF} \frac{[F^{++}]}{\lambda_{FX_2}}\right) \qquad (7)$$

A simple and accurate means of determining both the ion exchange constant K and the type of solid solution that the adsorbed ions form is given by these equations.

To make an electrode, a 1-cm. × 1-cm. chip of a natural glass was ground flat on one side and finished with -900-mesh abrasive. This side was cemented with Canada balsam to a glass slide and the chip was ground to about 0·05 mm. thickness and finished with -900-mesh abrasive. The slide was heated and the glass membrane gently slid off into a small beaker. Successive rinsings with xylene removed the adhering balsam and the chip was dried and transferred to a flat paper surface. A 10-cm. length of 6-mm. glass tubing was ground flat on one end. This end was heated and coated with thermoplastic cement; while still hot the coated end was lowered on to the glass membrane so that the membrane was sealed to the tube around the edge without allowing the plastic to coat the centre of the membrane. The tube was then filled with 0·01 M hydrochloric acid; a silver wire coated with silver chloride was inserted, and the open end of the tube was sealed. It is necessary to seal the membrane carefully in order to make the membrane itself the lowest resistance electrical path between the internal solution and whatever external solution surrounds the electrode. This method is similar to that of C. E. Marshall[7] except for the use of considerably thinner membranes.

The completed electrode is placed in a solution with a saturated potassium chloride–calomel, or a silver–silver chloride reference electrode that includes a saturated potassium chloride salt bridge. The potential between these electrodes was measured with a high impedance electrometer. The solution is maintained at 25·00 ± 0·05° C. and stirred magnetically. The electrode is first placed in a solution of known concentration of a single cation, as the chloride, and the potential measured as soon as equilibrium has been attained. Anions have no effect on the potential except for a contribution to the ionic strength which influences the activity coefficients of the cations. Aliquots of successively more concentrated standard solutions of a second chloride are added in order to vary the cation ratio as widely as possible. After each addition the equilibrium electrical potential is measured and recorded along with the composition of the solution.

The activities are calculated from the known concentrations of the cations in solution by using single ion activity coefficients from the Debye–Hückel equation for solutions more dilute than 0·01 molar. For more concentrated solutions the activity coefficients are derived on the assumption that $\gamma_{\pm\mathrm{KCl}} = \gamma_{\mathrm{K}^+} = \gamma_{\mathrm{Cl}^-}$, and therefore that:

$$\gamma_{M^+} = \frac{\gamma^2_{\pm M\mathrm{Cl}}}{\gamma_{\pm\mathrm{KCl}}}, \text{ and } \gamma_{M^{++}} = \frac{\gamma^3_{\pm M\mathrm{Cl}_2}}{\gamma^3_{\pm\mathrm{KCl}}}$$

From the activities and the electrical potential measurements the appropriate electrode equation (4), (6) or (7) is solved in combination with equation (5) by the curve-fitting method shown in Garrels *et al.*[6]. The solution yields the constants of the electrode equation, as, for example, in equation (4), C_{AB} and K_{AB}, and the value of B_{AB} of equation (5).

The exchange constants found by this method for two rhyolytic obsidians and a tektite are shown in Table 1. The obsidians differ in that one is a lava, the other a welded tuff.

Chemical changes due to the reaction of water with natural glass in two extreme situations may be calculated. In case A the mass of glass reacting is negligible compared with the mass of the water. In case B the mass of water is negligible. These are approximated in the geochemical history of water flowing through a freshly deposited bed of vitric tuff. Initially the water composition will be affected by the glass as in case B, but as large volumes of water continue to flush through the final composition of the glass shards will approach that in case A. For

Table 1. EXCHANGE CONSTANTS AND B_{AB} VALUES (KCAL./MOLE) FOR SOME NATURAL GLASSES (25° C.)

	Obsidian	Welded tuff	Tektite
K_{NaH}	3·2	3·5	3·0
B_{NaH}	−0·24	−0·47	−0·36
K_{KH}	1·3	1·4	1·5
B_{KH}	−0·59	+0·41	0·0
K_{NaK}	2·5	2·4	2·0
B_{NaK}	−0·18	nd	nd
K_{NaCa}	0·042	0·050	0·047
B_{NaCa}	−0·18	nd	−0·41
K_{KCa}	0·014	0·020	0·023
B_{KCa}	+0·20	−0·53	−0·53
K_{MgCa}	1·0	1·0	1·55
B_{MgCa}	nd	0·0	0·0

Obsidian from Puerto de Abrigo, Valles Caldera, New Mexico; welded tuff from American Falls, Idaho; tektite from Marulas, Philippine Islands. nd, Not determined.

the obsidian in Table 1, the relevant equations are (considering only Na^+, K^+ and H^+):

$$\frac{[HX]\ [Na^+]}{[NaX]\ [H^+]} = 3{\cdot}2;\quad \frac{[HX]\ [K^+]}{[KX]\ [H^+]} = 1{\cdot}3;$$

$$\frac{[KX]\ [Na^+]}{[NaX]\ [K^+]} = 2{\cdot}5$$

The results given in Table 2 are in good agreement with analyses of hydrated glass in tuffs (4) and with compositions of ground waters in beds of vitric tuff[8].

Table 2. CHANGES IN SODIUM, POTASSIUM AND HYDROGEN CONTENT OF AN OBSIDIAN DUE TO ION EXCHANGE WITH A GROUND WATER. CASE *A*, MASS OF WATER ≫ MASS OF GLASS; CASE *B*, MASS OF WATER ≪ MASS OF GLASS

	Glass composition (mole per cent)		
	Initial	Final, case *A*	Final, case *B*
Na_2O	4·07	2·13	4·07
K_2O	3·77	5·34	3·77
H_2O as H^+ on exchange sites	0·001 (est.)	0·68	0·001

	Water composition (molal)		
	Initial (assumed)	Final, case *A*	Final, case *B*
Na^+	1×10^{-4}	1×10^{-4}	$1{\cdot}6 \times 10^{-4}$
K^+	1×10^{-4}	1×10^{-4}	$5{\cdot}2 \times 10^{-5}$
H^+	1×10^{-5}	1×10^{-5}	$1{\cdot}2 \times 10^{-8}$

The technique described can also be used to study ion exchange on minerals and as a quick way of determining the usefulness of artificial glasses for glass electrodes without changing the composition by heating during the blowing of bulbs. Since the chemical durability of a glass is related to the ease of cation–hydrogen ion exchange, this technique also could be used as a test of durability.

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CRYSTALLOGRAPHY

New Compound in the System $CaO–Al_2O_3–CaF_2$

I WISH to report a new compound in the system $CaO–Al_2O_3–CaF_2$. The compound is thought to be $3CaO.3Al_2O_3.CaF_2$ and was discovered simultaneously in separate but related fields of work. One investigation was concerned with the effect of additions of calcium fluoride to the system $CaO–Al_2O_3$. The other investigation was concerned with the effect of calcium fluoride on the important cementitious compound monocalcium aluminate. Quench-furnace techniques, X-ray methods, and optical methods were used in this work.

The compound has also been identified in aluminous slags obtained from phosphorus-production furnaces.

Chemical analysis of the compound for calcium, aluminium and fluorine are compared in Table 1 with theoretical figures. Difficulties of analysis were encountered with the fluorine determinations.

Table 1. CHEMICAL ANALYSIS OF THE NEW COMPOUND

	Found (per cent)	Theoretical (per cent)
CaO	39·73	40·63
Al_2O_3	53·81	55·39
F	6·05	6·88

Weissenberg and rotation X-ray photographs were taken of a single crystal of the compound. From these the unit cell was determined as being hexagonal with:

$$a = b = 17{\cdot}29\ \text{Å}.,\ c = 7{\cdot}01\ \text{Å}.$$

The density, measured by the flotation method, was found to be 2·96 gm./cm.^{3}; the theoretical density, ρ_c = 3·03 gm./cm.^{3}. Powder photographs, taken using a Guinier-type focusing camera, were indexed using the data obtained from the single crystal work and the results are shown in Table 2.

Fig. 1 shows a thin section of the compound viewed with crossed nicols at a magnification of 35 times. It was found to be uniaxial negative with refractive indices ω = 1·628, ε = 1·618.

Besides the melting point, which is 1,507 ± 2° C., a few measurements of its cementitious properties have been made. The compound was ground to a specific surface of 2,716 cm.^{2}/gm.; compressive strengths of

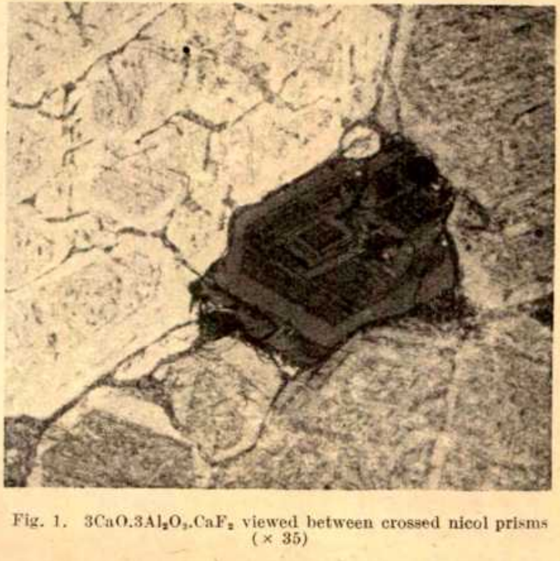

Fig. 1. $3CaO.3Al_2O_3.CaF_2$ viewed between crossed nicol prisms (× 35)

Table 2. Indexed Powder Photograph Lines of the Compound $3CaO.3Al_2O_3.CaF_2$

Plane	Calculated value (Å.)	Observed value (Å.)	Strength
121	4·403	4·40	*M*
220	4·322	4·32	*W*
311	3·573	3·57	*MS*
102	3·410	3·39	*W*
141	3·303	3·30	*VW*
410	3·268	3·27	*W*
022	3·174	3·17	*M*
231	3·085	3·08	*VS*
212	2·980	2·96	*VW*
330	2·882	2·88	*S*
501	2·754	2·75	*W*
132	2·679	2·67	*S*
421	2·624	2·62	*MS*
402	2·558	2·55	*MW*
151	2·511	2·50	*MW*
600	2·497	2·49	*MW*
322	2·450	2·44	*W*
341	2·323	2·32	*W*
052	2·277	2·27	*W*
113	2·256	2·24	*W*
242	2·202	2·19	*MW*
440	2·161	2·15	*W*
512	2·133	2·13	*M*
303	2·116	2·11	*M*
071	2·046	2·04	*M*
531	2·046	—	—
710	1·983	1·978	*S*
162	1·913	1·910	*MW*
413	1·900	1·894	*MW*
451	1·845	1·848	*VVW*
702	1·826	1·822	*MS*
352	1·826		
801	1·811	1·805	*MW*
333	1·815		
622	1·787	1·782	*VVW*
721	1·770	1·769	*VW*
603	1·706	1·698	*M*
641	1·668	1·666	*M*
082	1·651	1·649	*VVW*
371	1·638	1·635	*VVW*
821	1·634	1·635	*VVW*
314	1·614	1·606	*W*
443	1·586	1·581	*VW*
740	1·552	1·551	*M*
462	1·542	1·538	*W*
911	1·532	1·529	*M*
561	1·532		
732	1·519	1·515	*W*
713	1·512	1·508	*W*
504	1·513		
633	1·468	1·463	*MW*
291	1·444	1·442	*W*
660	1·441		
192	1·433	1·430	*W*
652	1·433		
10,1,0	1·421	1·419	*M*

S, Strong; *M*, medium; *W*, weak; *V*, very

Table 3. Compressive Strength of Vibrated Mortar Cubes

Age (days)	1	3	7
Strength (lb./sq. in.)	7,300	8,125	10,425

Table 4. Setting Times using Different Water to Cement Ratios

Water/cement	Initial set		Final set	
	Hr.	Min.	Hr.	min.
0·22	0	20	19	0
0·24	2	35	23	55
0·26	2	35	—	—
0·28	3	0	28	0
0·30	7	20	30	10
0·35	29	45	48	0
0·50	64	0	—	

vibrated mortar cubes and setting times were then determined by the methods laid down in British Standard 915, 1947.

It is interesting to note that although the final set was 19 hr. when the water to cement ratio was 22 per cent, the compressive strength after 24 hr. was sufficient to pass the British Standard for aluminous cement. X-ray measurements have shown that hydrates $4CaO.Al_2O_3.13.H_2O$ and $CaO.Al_2O_3.10H_2O$ are formed.

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RADIATION CHEMISTRY

Electron and Corona Bombardment of Polyethylene

The adverse effects of corona discharges on polyethylene have been known for some years. Damage to the polymer has variously been ascribed to ionic bombardment[1], electron bombardment[2] and to chemical and thermal effects.

It has recently been observed that corona discharges in moist air can produce oxalic acid crystals on a polyethylene surface[3]. When a charged steel sphere at 20 kV. (r.m.s., 60 cycle a.c.) was placed on a polyethylene surface for 20–120 hr., a ring of oxalic acid crystals was formed around the point of contact; the radius of the ring could be varied by changing the applied voltage. It was suggested that electrons from the sphere travelled along the lines of force of the applied field, and calculations based on this concept gave ring diameter values in accordance with the experimental results.

In experiments with stretched polyethylene films[4], electrical stresses of 200 V./mil were applied in ambient atmospheres of air at rather high humidities. Oxalic acid crystals formed in every case in a ring surrounding the electrodes and were more sharply defined in areas where corona intensity was least severe. The acid was not formed in a nitrogen atmosphere, nor in dry air. Corona resistance was greater at high humidities through the formation of a conducting surface layer which graded the electric field.

Because of the increasing interest in the use of radiation cross-linked polyethylene for electrical purposes, it seemed desirable to show whether the high-energy electrons used for cross-linking had the same effects as a corona discharge in producing oxalic acid. Studies of the γ-ray and neutron irradiation of polyethylene have shown that oxalic acid is not formed[5].

Moulding powder granules of low-density polyethylene (type *M*101, Monsanto Chemicals, Ltd.) were irradiated in moist laboratory air with electrons from a Van de Graaff electrostatic particle accelerator. The usual cross-linking dose of 20 Mrads was delivered in 1-Mrad increments to prevent excessive rise in temperature, and an initial electron energy of 2 MeV. was used to ensure virtually uniform dosage through the granules. In another experiment, using less-severe conditions, a dose of 2 Mrads was delivered in 60 increments using 0·5 MeV. electrons.

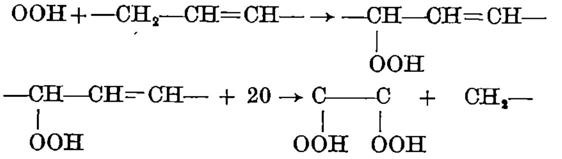
Extraction of the granules with hot water was followed by evaporation to dryness, and the residue in each case was warmed with a small crystal of diphenylamine, which forms a condensation product with oxalic acid. This can be detected by the blue colour of its alcoholic solution, giving a detection sensitivity of 10 μgm. However, in no experiment was any acid found on the polymer surface; attempts to detect acid in the bulk of the polyethylene were unsuccessful as diphenylamine is apparently destroyed when warmed with the polymer and no colour is formed even in the presence of large amounts of added oxalic acid.

It is possible that oxalic acid was formed and as rapidly destroyed through scission of the C—C bonds by electrons[6]. No reference to the effect of electrons on solid oxalic acid has been found, but such scission would yield entirely volatile products. A 2-gm. sample of the solid acid dihydrate lost only 0·3 per cent of its weight on receiving a 20-Mrads dose of 2-MeV. electrons, and was apparently unaffected by the treatment apart from acquiring a light buff colour.

The following mechanism has been proposed[3] for the formation of oxalic acid by a corona discharge in moist air:

$$O_2 + e \rightarrow O_2^{-}$$

$$O_2^{-} + H_2O \rightarrow OH^{-} + .OOH$$



$$OOH + \text{—CH}_2\text{—CH=CH—} \rightarrow \text{—}\underset{\substack{|\\ OOH}}{\text{CH}}\text{—CH=CH—}$$

$$\text{—}\underset{\substack{|\\ OOH}}{\text{CH}}\text{—CH=CH—} + 2O \rightarrow \underset{\substack{|\\ OOH}}{\text{C}}\text{——}\underset{\substack{|\\ OOH}}{\text{C}} + CH_2\text{—}$$

It depends on an initial combination between molecular oxygen and electrons of low energy produced by ionization in the polymer. It is probable that under the bombardment conditions of the present experiments such low-energy electrons were produced in insignificant numbers. As the mechanism also relies on the presence of transvinylene groups in the polymer, it is suggested that polyethylenes with a small number of such groups might be more corona-resistant than others. It has been found that 'Rigidex *R*101/2' (British Hydrocarbon Chemicals, Ltd.) and 'Marlex 50' (Phillips Petroleum Co.), for example, have 10–20 transvinylene groups per 10^6 carbon atoms, while other polyethylenes made by the Ziegler process may have up to 600 such groups. In view of the low-energy transfer in some corona discharges, it would seem unlikely that appreciable unsaturation could be produced in this way, although this mechanism has been suggested[3].

The absence of surface cracking or checking in fast electron-irradiated polyethylene further differentiates this type of treatment from that of corona discharges.

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Radiolysis of Aqueous Solutions of Hydrogen Sulphide and an Interpretation of Radiolytic Thiol Oxidation

The radiolysis of dilute aqueous solutions of the thiols cysteine[1–3], cysteamine[4] and mercaptoethylguanidine[5,6] has been investigated by various workers, who have shown that in oxygenated systems a short chain mechanism occurs yielding the corresponding disulphide as the major product. Swallow[1] suggested the following mechanism:

$$RSH + \cdot OH \rightarrow RS\cdot + H_2O \qquad (1)$$

$$RSH + HOO\cdot \rightarrow RS\cdot + H_2O_2 \qquad (2)$$

$$RS\cdot + RSH \rightarrow RSSR + H\cdot \qquad (3)$$

$$H\cdot + O_2 \rightarrow HOO\cdot \qquad (4)$$

Littman, Carr and Brady[7] have shown that reaction 5:

$$RSH + H\cdot \rightarrow RS\cdot + H_2 \qquad (5)$$

occurs readily. Consequently, on Swallow's mechanism a chain reaction should occur in the absence of oxygen. The fact that it does not[1,2,6], and that reaction 3 is approximately 17 kcal. per mole endothermic, makes this mechanism unlikely.

To investigate further this type of reaction the radiolysis of aqueous hydrogen sulphide solutions has been examined in greater detail than previously reported[3,8].

On irradiating an oxygen-free solution of 0·0175 *M* hydrogen sulphide with cobalt-60 γ-rays product yields $G(S)$, $G(H_2)$ and $G(H_2O_2)$ of 3·4, 3·9 and 0·5 molecules per 100 eV. respectively were found. These yields agree satisfactorily with those predicted from radical and molecular product yields generally accepted for the radiolysis of dilute aqueous solutions where all radicals are scavenged[9] assuming the following mechanism:

$$H_2S + \cdot OH \rightarrow HS\cdot + H_2O \qquad (6)$$

$$H_2S + H\cdot \rightarrow HS\cdot + H_2 \qquad (7)$$

$$2\,HS\cdot \xrightarrow{H_2O} H_2S + S \qquad (8)$$

Similarly a 0·070 *M* hydrogen sulphide solution in 0·1 *N* sulphuric acid gave initial yields, $G(S)$ and $G(H_2O_2)$ of 3·4 and 0·8 respectively, again agreeing satisfactorily with predicted values for an acid solution. A slow reaction between H_2S and H_2O_2 was detected at this lower *p*H.

Table 1. Initial Yields of Sulphur from 10 ml. of a 0·0003 *M* Oxygen Solution*

$[H_2S]$ (molar)	Temperature (° C.)	Dose-rate eV. ml.$^{-1}$ min.$^{-1}$ ($\times 10^{-15}$)	$G(S)$ Molecules per 100 eV.
0·0035	ambient	8·5	8·2
0·0175	,,	8·5	13·9
0·0525	,,	8·5	16·8
0·0875	,,	8·5	18·9
0·0875	25	8·5	19·5
0·0875	0	8·5	16·6
0·0875	ambient	0·136	23·3

* Partial pressure of oxygen = 200 mm. mercury.

In the presence of oxygen the initial yield of sulphur was greatly increased, the variation of $G(S)$ with hydrogen sulphide concentration, temperature and dose rate being shown in Table 1. No hydrogen peroxide was found until the dissolved oxygen had been used, while sulphate was formed prior to oxygen depletion with an initial yield $G(SO_4^{=})$ of 6 ± 1·5 from a 0·0875 *M* hydrogen sulphide solution. These results suggest a short-chain mechanism probably involving a reaction between hydrogen sulphide molecules and reactive peroxy intermediates in the relatively slow propagation step 10:

$$HS\cdot + O_2 \rightarrow HSOO\cdot \qquad (9)$$

$$HSOO\cdot + H_2S \rightarrow HSOOH + HS\cdot \qquad (10)$$

The peroxide intermediates then probably react rapidly with themselves, with hydrogen sulphide and with hydrogen peroxide to yield finally sulphur and sulphate. Sulphur yields were the same in 1 *N* sulphuric acid solutions as in the sulphuric acid-free solutions mentioned above.

From the yields of radical and molecular products from water, and from the measured yields of sulphur and sulphate from a 0·0875 *M* hydrogen sulphide solution it follows that $G(-O_2)$ is approximately 21. It is suggested from this work that a comparable mechanism, involving the intermediate radical $RSOO\cdot$, might occur in oxygenated solutions of thiols, and that their ability to use up oxygen may partly explain their mode of protection against harmful radiation effects.

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CHEMISTRY

Bond-Length Alternation in Heterocyclic Ring Systems

THERE has been considerable interest recently in the problem of C—C bond alternation in polyenes[1-3]. The preparation by Chapman, Paddock, Paine, Searle and Smith[4] of a series of cyclic phosphazenes $(PNF_2)_n$, where $n = 3, 4 \ldots 17$, raises the same question for heterocyclic systems $(AB)_n$ containing π-electrons. On the basis of simple molecular orbital theory, it has been shown for cyclic polyenes that, as the number of atoms in the ring N tends to infinity, the stable configuration has C—C bonds unequal in length[2]. The critical value of N for which the bonds first become unequal is uncertain; but there is evidence for bond alternation[3] in $C_{18}H_{18}$, $C_{24}H_{24}$ and $C_{30}H_{30}$.

A discussion of the problem for cyclic phosphazenes depends on the type of structure postulated[7-9]. As Craig[10] has shown, there are only two different types of bonding possible in an $(AB)_n$ ring. I shall call the type in which the orbitals on A and B have the same site symmetry, $p\pi$—$p\pi$ bonding; and the type in which they have different site symmetries, $p\pi$—$d\pi$ bonding. I use superscripts $p\pi$ and $d\pi$ to distinguish them in the argument here, which is based on the simple molecular orbital theory.

Let δ be the difference between the coulomb integrals for the orbitals on atoms A and B, and let r_1, r_2 and β_1, β_2 be the lengths and resonance integrals of alternate AB bonds. Let E be the total energy of the system, then E may be written in terms of the σ- and π-energies, E^σ and E^π respectively, as:

$$E = E^\sigma + E^\pi \qquad (1)$$

E^σ is assumed to be the sum of σ-bond energies, thus:

$$E^\sigma = n_1E_1^\sigma + n_2E_2^\sigma \qquad (2)$$

where E_1^σ, E_2^σ are the σ-bond energies of the two different A–B bonds, and n_1,n_2 the numbers of each bond. E^π cannot be written in this way.

To discuss equilibrium, we require the first and second derivatives of the energy with respect to an increase in bond-length x, evaluated at $x = 0$. I write these E_x and E_{xx} respectively. For stable equilibrium:

$$E_x = E_x^\sigma + E_x^\pi = 0 \qquad (3)$$

and

$$E_{xx} = E_{xx}^\sigma + E_{xx}^\pi \qquad (4)$$

must be positive.

For $p\pi$—$p\pi$ bonds

$$E^{p\pi} = \sum_l |[\delta^2 + 4(\beta_1^2 + \beta_2^2 + 2\beta_1\beta_2 \cos 2\pi l/n]^{\frac{1}{2}}|,$$

$$l = 0, \pm 1, \pm 2, \ldots \pm (m - 1), m$$

where $m = n/2$ for n even, and $\pm (n-1)/2$ for n odd.

For $p\pi$—$d\pi$ bonds,

$$E^{d\pi} = \sum_l |[\delta^2 + 4(\beta_1^2 + \beta_2^2 - 2\beta_1\beta_2 \cos 2\pi l/n)]^{\frac{1}{2}}| \qquad (6)$$

Now β_1 and β_2 may be written[2],

$$\beta_1 = \beta_0\, e^{ax}, \qquad \beta_2 = \beta_0\, e^{-ax} \qquad (7)$$

where $r_1 = r_0 - x$, $r_2 = r_0 + x$, a is a constant, and β_0, r_0 are the resonance integral and bond-length for equal A–B bonds.

From equations (5), (6) and (7),

$$E_x^{p\pi} = E_x^{d\pi} = 0 \qquad (8)$$

and

$$E_{xx}^\pi = \sum_l \frac{-16\,\beta^2a^2}{|[\delta^2 + 8\,\beta^2\,(1 \pm \cos 2\pi l/n)\,]^{\frac{1}{2}}|} \qquad (9)$$

with the positive sign for $p\pi$—$p\pi$ bonds and the negative sign for $p\pi$—$d\pi$ bonds. With $\delta = 0$, this reduces to the expression given by Longuet-Higgins and Salem[2].

For heterocyclic systems $\delta \neq 0$, and the denominator in equation (9) can never be zero. Thus E_{xx}^π remains finite for all values of n. E_{xx}^σ, however, tends to infinity as $n \to \infty$, as Longuet-Higgins and Salem[2] have pointed out. Thus E_{xx} must be positive as $n \to \infty$, and the equilibrium at $x = 0$ is stable. This means that, for the infinite heterocyclic ring $(AB)_n$, the stable configuration has equal A–B bonds, the opposite result to that for homocyclic rings. For heterocyclic rings there may be a critical value of n below which the bonds are unequal in length.

It would be interesting to discover if, in the cyclic phosphazenes, this critical value of n exceeds 3. To discuss this, values are needed for the parameters in equation (9), and an expression is required for E_{xx}^σ. E_{xx}^σ may be written[5]:

$$E_{xx}^\sigma = nk \qquad (10)$$

where k is the force constant for the P—N bond. I have made a rough calculation with $k = 8$ md/Å. (ref. 11), $\beta_0 = 25$ kcal./mole, and $a = 0{\cdot}4$ Å.$^{-1}$. This indicates that if δ is greater than about $\beta_0/10$, $E_{xx}^\sigma > E_{xx}^\pi$ for all values of n, and the ring with equal P—N bond-lengths is stable. If $\delta < \beta_0/10$, and n is small. E_{xx}^π might be greater than E_{xx}^σ, and a ring with unequal P—N bond-lengths would be favoured. In general, it seems probable that $\delta > \beta_0/10$, and that the P—N bonds in all cyclic phosphazenes should be equal in length.

Experimental determinations of bond-lengths and angles in cyclic phosphazenes show that the substituents have a considerable effect on the structure[12]. There is, however, no evidence for unequal bond-lengths. One of the effects of substituents is to alter δ. and the argument given above shows how a variation in δ can affect the relative importance of σ- and π-bonds in determining the structure of cyclic phosphazenes. At the present time there seems to be satisfactory agreement between theory and experiment.

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Heat Pulses at Initiation of Liquid Explosives

The hydrodynamic theory of detonation has proved to be very fruitful for the discussion of steady detonation. According to the theory an intense shock wave is a prerequisite for the accomplishment of the chemical reaction during the short space of time that is available. The theory does not, however, explain how the shock wave is generated at initiation.

Cook *et al.*[1] assume that 'heat pulses' in some cases play an important part in the initiation process. In the present investigation the possibility of a heat pulse which propagates at a great speed without being directly associated with a shock front is discussed. It is preliminarily assumed that the temperature in a layer of nitroglycerine suddenly rises to such a height that the chemical reaction is completed by a few impacts. Owing to the relaxation of the rotational and vibrational energy the released explosion energy will be concentrated on the translational degrees of freedom[2-4] of the newly formed molecules. After some tens of collisions the molecules have approximately acquired the Maxwellian distribution of velocities and this corresponds to a 'translational temperature' T^x, which is considerably higher than the equilibrium values. The transfer of the energy to other degrees of freedom proceeds comparatively slowly. The number of collisions required for the development of rotational energy is about 10^3 and of vibrational energy about 10^5. For T^x:

$$E_e/n = 3\,RT^x/2 \qquad (1)$$

applies. E_e is the explosion energy, n the number of molecules in the detonation gas per molecule of explosive and R the gas constant. For nitroglycerine[5] $E_e = 340{,}000$ kcal./kmol., $n = 7{\cdot}25$ and $T^x = 15{,}800°$ K. The fraction of impacts which leads to decomposition is .

$$q = e^{-E_a/RT^x} \qquad (2)$$

Insertion of Roginsky's[6] value for the activating energy, $E_a = 48{,}000$ kcal./kmol , gives $q = 0{\cdot}22$. According to this $n_s = 1/q = 4{\cdot}5$ impacts are sufficient for the decomposition of the nitroglycerine molecule $C_3H_5(ONO_2)_3$, and due to the high density the reorganization of the atoms to CO_2. H_2O, N_2 and O_2 is in all probability a very rapid process. The result goes to prove that it is possible for a decomposition process of this kind to continue once it has got well away.

The mean velocity of the newly formed molecules can be obtained from the relation:

$$Mv^2/2 = E_e/n$$

in which

$$\bar{v} = 0{\cdot}92\sqrt{v^2}$$

This gives the following velocities in metres per second:

$$3\ CO_2 : \bar{v}_1 = 2{,}750;\ \ 2{\cdot}5\ H_2O : \bar{v}_2 = 4{,}300;$$
$$1{\cdot}5\ N_2 : \bar{v}_3 = 3{,}440;\ \ 0{\cdot}25\ O_2 : \bar{v}_4 = 3{,}220.$$

The figure before the gas indicates the number of molecules per nitroglycerine molecule. The arithmetical mean value for all molecules is $\bar{v} = 3{,}440$ m./s. This high molecular velocity corresponds to a high velocity for the propagation of disturbances.

For liquid nitrogen at − 199° C. the ratio between the velocity of sound and the molecular mean velocity is approximately $m = 4$, and according to Rice and Walsh's[7,8] results for water the ratio between the shock velocity and the molecular velocity lies between $m = 4{\cdot}2$ at 8 kbar. and 50° C. and $m = 5{\cdot}6$ at 250 kbar. and 1,670° C. At atmospheric pressure and room temperature the velocity of sound in water is only 2 5 times the molecular velocity, but this may be due to molecular association which makes the estimated molecular velocity too high. For organic liquids the ratio between sound and molecular velocity at atmospheric pressure and room temperature is, as a rule, between 4 and 6.

According to Campbell, Davis and Travis[9] we have the following process in transmission of detonation from a donor charge through an inert layer to a receptor charge of a liquid explosive. Behind the front of the incoming shock wave the receptor is heated by shock wave compression. If the temperature is above a certain level an accelerated rise in temperature by self heating is started. The maximum of this rise lies close to the impact surface similarly to what Selberg and Johansson[10] have shown for the environment of a wire suddenly heated. There should probably be prerequisites here for a 'heat pulse'. Campbell, Davis and Travis observed a frontal velocity of 10,400 m./s. when the particle velocity was 1,600 m./s. $\bar{v} = 3{,}440$ m./s. and $m = 5$ gives a velocity of disturbance in nitroglycerine of 17,200 m./s. In calculating the propagation velocity of the heat pulse the time for dissociation of the explosive molecules must be taken into consideration. Since a self-heating process is in progress in the receptor charge it seems reasonable to assume that $n_s < 4{\cdot}5$.

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Use of Di-(2-ethylhexyl) Orthophosphoric Acid for the Separation of the Elements Lanthanum–Gadolinium by Reverse Phase Partition Chromatography

INVESTIGATIONS into the use of alkyl phosphoric acids as metal extractants have shown that there are relatively large differences between the stabilities of the complexes of individual rare earths with di-(2-ethylhexyl) orthophosphoric acid (HDEHP), the separation factor for any adjacent pair being about 2·5[1]. This figure suggests that it should be feasible to devise a comparatively simple scheme for rare earth separations based on a multi-stage extraction procedure utilizing HDEHP as extractant. If the organic reagent can be immobilized on a solid phase the effect of a number of successive extractions may be achieved by eluting the metals chromatographically from a column of the extractant, and it has already been shown that complexing agents may be incorporated into solid supports to provide column materials of this type[2-6]. Investigations into the behaviour of a number of support materials retaining HDEHP have indicated that a poly-(vinyl chloride/vinyl acetate) copolymer ('Corvic', Imperial Chemical Industries, Ltd.) will hold the complexing agent firmly and will give elution curves with the rare earths which show relatively little tailing. Using columns packed with 'Corvic' retaining HDEHP it has been found possible to separate small quantities of the elements lanthanum–cerium–praseodymium–neodymium–promethium–samarium–europium and gadolinium, using normal chromatographic techniques.

Column material was made up by mixing the poly-(vinyl chloride/vinyl acetate) copolymer with a solution of chloroform (5 ml.), toluene (3 ml.) and purified HDEHP[1] (2 ml.) in the ratio of 10 gm. of solid to 10 ml. of solution. Organic solvent was evaporated off until the total weight of 10 gm. dry weight of 'Corvic' plus the adsorbed liquid was 12·5–12·7 gm., when the resulting powder was first slurried in $N/500$ perchloric acid, then made up into a column of the required size in the usual way. The temperature of the column was thermostatically controlled at 60° C., column size was 1 cm. diameter × 30 cm. and the flow-rate was approximately 1·0 ml./cm.^{2}/min. Between 0·01 and 0·1 mgm. of the irradiated trivalent elements lanthanum-gadolinium (with the exception of promethium) were added to the top of the column, as required, in very dilute acid solution, and the column then washed with $N/500$ perchloric acid. In the initial experiments, when the behaviour of adjacent pairs of rare earths was being considered, the extracted rare earths were eluted with perchloric acid solution of constant strength, but for later experiments, when a more comprehensive separation was required, the acidity of the influent was gradually increased. For the determination of the activity in the column effluent the perchloric acid solution leaving the bottom of the column was either passed through a γ-scintillation flow counter connected to ratemeter and chart recorder, or collected in fractions of 1 ml. or less, and the activity of each fraction assayed separately. The nuclides giving rise to the measured radiation were characterized by γ-ray spectrometry or from half-life curves.

The effectiveness of HDEHP retained on 'Corvic' for the separation of the elements lanthanum–gadolinium may be gauged from Fig. 1, in which the elution of the metals, as recorded by the flow counter, is shown, together with the acidities at peak maxima.

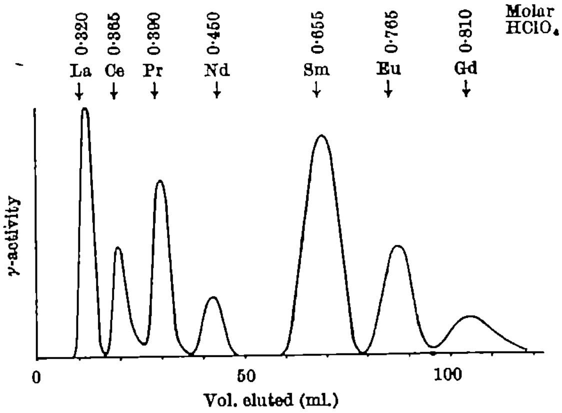

Fig. 1. Separation of the elements La–Gd by gradient elution from a 'Corvic'–HDEHP column

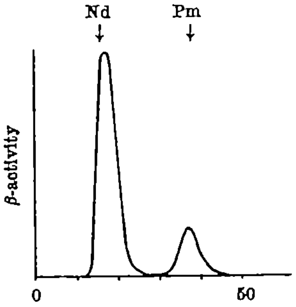

Fig. 2. The elution of Nd and Pm from a 'Corvic'–HDEHP column by 0·39 M perchloric acid

The γ-counter used was not sensitive to the largely β-active promethium, but the separation of promethium from neodymium is shown in Fig. 2. It can be seen that a good resolution of the elements is achieved by a simple gradient elution technique which relies only on the differences in the stabilities of the individual metals with HDEHP, and does not require the addition of complexing anions to the eluting phase.

The separation of cerium from praseodymium and europium from gadolinium proved troublesome when perchloric acid of constant acidity was employed as influent to the column, but gradient elution greatly reduced overlap of the elements in the effluent, and permitted a separation to be achieved, even for the europium–gadolinium pair, which has been previously shown to be difficult to isolate by different techniques[3,7].

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A Simplified Method for Preparation of Very Pure Silicochloroform

A NUMBER of methods are available for the preparation of trichlorosilane ($SiHCl_3$), in the laboratory, for example, by the action of dry hydrochloric acid gas on silicon[1,2] and by the reaction of hydrochloric acid gas and metallic silicides[3-7], such as magnesium silicide, iron silicide, or 25 per cent copper silicide. Trichlorosilane has also been prepared by passing a mixture of halosilanes and excess of hydrogen or hydrogen-halide over aluminium, zinc, or magnesium[8], or by halogenation of monosilane (SiH_4), with hydrochloric acid gas in presence of aluminium chloride as a catalyst[9].

In all these methods the amount of formation of trichlorosilane is low. This is due to the fact that, along with the main reaction, which leads to the formation of trichlorosilane, side reactions also occur. These side reactions give rise to the formation of $SiCl_4$, SiH_2Cl_2, SiH_3Cl, etc. At increasing temperature the reaction leading to the formation of silicon tetrachloride occurs more rapidly[7]. The yield of silicochloroform, calculated from the weight of the reacting silicon, is about 50 per cent[4]. This low yield of silicochloroform is also due to inadequate cooling of the gaseous reaction products. The concentration of silicochloroform in the gaseous reaction products is low, and hence a very good cooling system for condensing trichlorosilane is essential. Unfortunately, liquid air cannot be used for this purpose because unreacted hydrochloric acid gas would also condense along with trichlorosilane. In methods for preparation of trichlorosilane in which silicides of metals such as magnesium, copper, or zinc are used as starting materials, instead of silicon, and hydrochloric acid gas, the process of preparing these metallic silicides is neither simple nor suitable for obtaining pure trichlorosilane. The process of preparing these metallic silicides involves first intimately mixing silicon with at least 10 per cent by weight of one of these foreign metals. After this, the mixture is usually sintered at a high temperature, normally 1,000° C., to activate the silicon. Silicon activated in this way is then subjected to the action of hydrochloric acid gas in order to produce silicochloroform. This operation of mixing silicon with another metal and then sintering at a high temperature leads to the contamination of silicon with many impurities. Therefore silicochloroform obtained by this method is not pure. To produce pure trichlorosilane it would be more practical if, instead of activating silicon, which involves contamination of silicon with impurities, the other reagent, hydrochloric acid gas, could be activated by some process.

Considering the drawbacks of these available methods, we have been able to develop an improved simple method, which can be used on a laboratory or production scale for the preparation of pure trichlorosilane. The over-all reaction is:

$$Si + 3HCl \longrightarrow SiHCl_3 + H_2$$

In this method for the preparation of pure trichlorosilane, instead of activating silicon, which is normally accomplished by mixing it with some other metal such as magnesium, copper, or iron, and sintering the mixture at a high temperature, which results in the contamination of silicon with impurities, hydrochloric gas molecules are activated. The activation of hydrochloric acid gas molecules takes place, possibly due to the presence of $HCuCl_2$-type complexes. These $HCuCl_2$-type complexes are formed because of the presence of very small amounts of cuprous chloride, etc., in the reaction tube. The quantity of copper therefore required is very small, being less than 0·1 per cent of the weight of silicon in the reaction tube. These activated hydrochloric acid molecules react with silicon to produce pure trichlorosilane, while the impurities which are present in silicon are left behind. The gaseous reaction products from the reaction tube when condensed in our specially developed condenser, as in Fig. 1, give an average silicochloroform yield of about 90 per cent, calculated from the amount of reacted silicon.

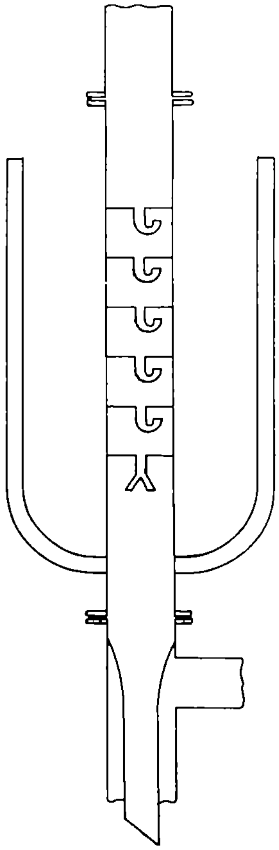
Fig. 1

The apparatus used in its simplest form consists of a horizontal 'Pyrex' tube. A weighed quantity of small crystals of silicon, 1–5 mm. in size, is placed in a quartz boat. This boat containing silicon is introduced into the reaction tube, which is 500 mm. long and has an inner diameter of 25 mm. The inner wall of the middle part of the reaction tube, where the boat containing the silicon lies, is fitted with a copper spiral. This copper spiral weighs approximately 0·1 per cent of the weight of silicon in the boat. The copper spiral is so arranged that it cannot possibly come in contact with the silicon and lies quite apart from it. A chromium–nickel resistance wire is wound around the middle portion of the reaction tube to heat it to the required reaction temperature in conjunction with an ordinary 10-amp. and 0–250 V. 'Variac'. At one end of the reaction tube is an inlet for the incoming gases while its other end is joined to a specially developed condenser (Fig. 1) for condensing silicochloroform vapours. This condenser in turn is connected to a collecting flask. for collecting trichlorosilane, which has an outlet allowing the uncondensed gases to escape into the atmosphere. A thermo-couple, nickel/nickel–chromium, is placed in such a way that it lies just on the surface of the silicon for measuring the reaction temperature. After arranging the apparatus, a current of hydrogen is passed through it to drive out all the air. The reaction tube is next heated to the reaction temperature of 400° C. by passing a current through the resistance wound around it. At the same time, the condenser is cooled by means of alcohol and dry ice (– 78° C.). Next hydrochloric acid gas is led into the reaction tube at the rate of 105 litres per hour, while the flow of hydrogen is stopped. The reaction starts, the pure silicochloroform forming in the condenser and collecting in the storing flask. The relationship between the rate of overall reaction, $Si + 3HCl \longrightarrow SiHCl_3 + H_2$, and the reaction temperature and the rate of flow of hydrochloric acid gas was investigated. The results obtained from these investigations are given in Tables 1 and 2.

Table 1. RELATIONSHIP BETWEEN THE RATE OF REACTION AND THE REACTION TEMPERATURE

Reaction temperature (° C)	Hydrogen chloride rate of flow (l./hr.)	Weight of silicon reacting (gm./hr.) In absence of copper spiral	Weight of silicon reacting (gm./hr.) In presence of copper spiral
300	105	0 80	2 1
350	105	2 3	5·1
400	105	3 1	8 0

Table 2. RELATIONSHIP BETWEEN THE RATE OF REACTION AND THE RATE OF FLOW OF THE HYDROCHLORIC ACID GAS

(The investigation was carried out only in presence of a copper spiral)

Hydrochloric acid gas rate of flow (l./hr.)	Reaction temperature (° C)	Weight of silicon reacting (gm./hr.)
43	400	5 9
72	400	8 0
105	400	8 0

From trichlorosilane, obtained by this method, silicon was produced. The results of the spectroscopic analysis of this silicon and that used as the starting material for the preparation of trichlorosilane are given in Table 3.

Table 3. COMPARISON OF THE SPECTROSCOPIC ANALYSIS OF RAW AND PURE SILICON

Elements present as impurities	Raw silicon concentration (per cent)	Pure silicon concentration (per cent)
B	0·002	—
Fe	0 0005	—
Mg	0 0002	—
Al	0 002	—
Cu	0·0002	—
Ti	0 0005	—
Ni	0 0005	—
Mn	0 001	—
Ca	0 002	0 002

—, Not detectable by spectroscopic analysis.

We thank Mr. Biert and Miss Reindle for carrying out the various spectroscopic analyses.

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BIOCHEMISTRY

Calculated Composition of a 'Messenger' Ribonucleic Acid

A SPECIAL type of ribonucleic acid, the 'messenger' RNA, has been postulated recently, which is assumed to specify the sequence of the amino-acid constituents of a given protein[1]. This RNA is said to be made under the control of deoxyribonucleic acid and to mirror the composition and the regularities (base-pairing, etc.)[2] of the latter, with uracil substituting for thymine[3–6]. The existence, in DNA, of a non-overlapping, 'degenerate' code, composed of nucleotide triplets that are 'read' from a fixed starting point, has also been put forward[7]. These advances have been paralleled by the equally recent elaboration of what would seem to be the complete amino-acid code in the form of a series of nucleotide triplets[8–11].

If all this be accepted, a way towards the indirect establishment of the nucleotide sequence of 'messenger' RNA would seem to be open. The composition of many proteins is known precisely, and in several instances also the specific sequence of the amino-acids. The determination of the nucleotide arrangement in RNA, on the other hand, is beset with great difficulties.

I have attempted to reconstruct the composition of the supposititious 'messenger' RNA that would code for bovine pancreatic ribonuclease, in order to see whether a plausible structure would emerge. The results are peculiar. The computation is based on the well-established composition of this protein[12] and on the triplet 'letters' proposed in recent papers. Thirteen of the required nineteen coding units were taken from Speyer *et al.*[10]; those for glutamic acid and alanine came from Martin *et al.*[11]; with regard to four components, aspartic acid (UAG), methionine (UAG), asparagine (UAA) and glutamine (UCG), only information published in the daily Press was available[13].

The following approximate composition of the 'coding' RNA results: $A_{63}\,G_{59}\,C_{77}\,U_{173}$; or, as mole per cent. A, 16·9; G, 15·9; C, 20·7; U, 46·5. (A, G, C, U, stand, respectively, for adenylic, guanylic, cytidylic and uridylic acids.)

A ribonucleic acid of this composition must create some surprise. If it exists, it has not been seen. The strikingly high content in uridylic acid, nearly a half of the hypothetical molecule, is in contrast to almost all available information[14]. Also other features of the constructed molecule are most unusual: the ratio of A to U is 0·36; the purines account for only one-third of the nucleotides; the ratio of 6-amino to 6-keto nucleotides[15] is 0·60. Moreover, the inspection of the proposed code. with its baffling richness in uridylic acid, makes it unlikely that many proteins would be found to demand less implausible 'messengers'. If the assignment of coding units really proved to be correct, auxiliary hypotheses would be required, in order to explain the discrepancy between the composition of the 'coding' RNA under discussion and that of 'messenger' RNA exhibiting the regularities of DNA. The most obvious, but still unwarranted, assumption may surmize the production of the 'messenger' in the form of two polynucleotides, complementary with respect to base-pairing, of which only one determines the protein; a postulate that leaves the function and the fate of the other half very much in doubt. Here, I wish to limit myself, however, to pointing out certain perplexing elements.

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In vivo and *in vitro* Incorporation of Amino-Acids by Melano-Protein Particles of the Harding–Passy Mouse Melanoma

ELECTRON microscopic evidence indicates that the formation of melanin granules in melanocytes occurs in a region of the cell associated with the Golgi membranes[1,2]. Although the characteristic flattened sacs and vesicles of this structure are evident in many types of vertebrate and invertebrate cells, the vesicular profiles are particularly apparent in cells concerned with secretion and absorption[3], and differ in several morphological respects from the endoplasmic reticulum[4]. The biochemical composition and role of this membrane structure in the formation of melanin granules are not yet established.

This communication reports a procedure for the isolation of a particle fraction rich in melano-protein and lipid, and a comparison with other cell particulate fractions of the capacity of the melano-protein particles to incorporate amino-acids *in vivo* and *in vitro*.

Harding–Passey melanoma tissue was homogenized with a loose-fitting Potter–Elvehjem glass homogenizer in 2·3 vol. of a medium containing 0·15 *M* sucrose, 0·01 *M* magnesium chloride, 0·025 *M* potassium chloride and 0·035 *M tris* buffer, *p*H 7·8. The melano-protein particle fraction and the mitochondria were isolated by an initial low-speed centrifugation at 600*g* for 5 min. to sediment nuclei and broken cells, followed by a higher speed centrifugation at 10,000*g* to collect mitochondria and melano-protein particles. (All centrifuged forces are calculated from the bottom of the tube.) Tumour microsomes were obtained after centrifugation for 1 hr. at 144,700*g* in a 'Spinco' preparative centrifuge. The pellet obtained at 10,000*g* was suspended in homogenizing medium and placed as the top gradient in a 5-ml. centrifuge tube layered in advance with 1·0 ml. 1·7 *M* sucrose and 1·0 ml. 1·5 *M* sucrose. The tubes were centrifuged in a swinging bucket rotor *SW L* of the 'Spinco' centrifuge for 1 hr. at 173,000*g*. Under these conditions melano-protein particles separate from lighter tumour mitochondria and are collected as a pellet at the bottom of the tube together with some heavier tumour mitochondria. Other less-dense melanin-rich particles can be observed, and these appear to be related to the age and degree of melanization of the tumour. The melanin-rich proteins are referred to as melano-protein particles in Table 1 and constitute 6–9 per cent of the total protein of the tumour.

Table 1. INCORPORATION OF ALGAL PROTEIN HYDROLYSATE *in vivo* INTO CELL FRACTIONS OF THE HARDING–PASSEY MOUSE MELANOMA. CONDITIONS DESCRIBED IN TEXT

Cell fraction	Time (hr.)	C.p.m./cm.^{2} (protein)	C.p.m./cm.^{2} (NA)	Percentage total (protein)	Percentage total c.p.m. (protein)	Mgm. RNA/mgm. protein
Nuclei cells broken cells	0·5	336	48	22·1	19·2	0·002
	3·0	495	143	34·0	9·5	0·006
Light mitochondria	0·5	360	52	9·9	9·0	0·016
	3·0	970	100	5·5	6·2	0·011
Microsomes	0·5	580	43	12·1	18·2	0·042
	3·0	1,630	111	16·8	25·0	0·032
Cell sap	0·5	405	164	49·5	51·5	0·008
	3·0	820	390	41·5	39·0	0·009
Melano-protein particles	0·5	119	49	6·1	0·7	0·019
	3·0	935	21	9·0	9·8	0·021

NA, Nucleic acid. C.p.m are expressed at infinite thickness

Table 1 shows the distribution of labelled amino-acids in protein and nucleic acid of the isolated tumour cell fractions, and the protein and RNA content of each cell fraction following an intraperitoneal injection of 0·5 µc. algal protein hydrolysate per gm. of mouse tissue. 100 µmol. of non-labelled carrier tyrosine was injected at the same time as the hydrolysate to dilute radioactive tyrosine present in the hydrolysate. The activity of the proteins recorded in Table 1 is therefore indicative of the rate of synthesis of the protein component of the melano-protein particles. After 0·5 and 3·0 hr. following injection, the melano-protein fraction accounts for 0·7 and 9·8 per cent of the total activity incorporated by the tumour *in vivo*.

Fig. 1 illustrates the time-dependent incorporation of L-valine-^{14}C *in vitro* into protein of three cell fractions obtained at 600*g*, 10,000*g* and 173,000*g*. The complete reaction mixture consisted of 0·5 µc. L-valine-^{14}C (1·54 µmol./µc.), 3·0 mgm. of the particulate tumour protein, 5·0 µmol. magnesium chloride, 10 µmol. potassium phosphate, 10 µmol. glucose, 75 µmol. sucrose, 12·5 µmol. potassium

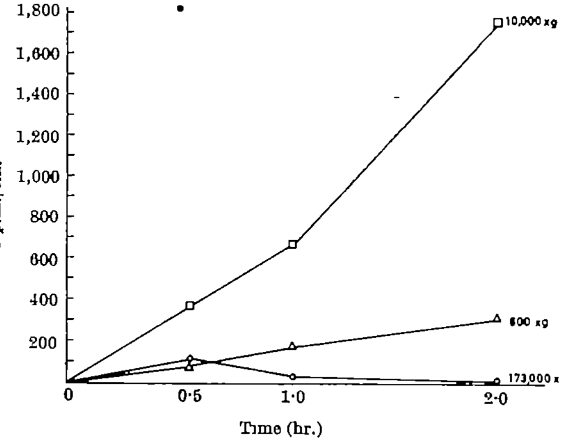

Fig. 1. Incorporation of L-valine-^{14}C *in vitro* into cell fractions of the Harding–Passey mouse melanoma. Conditions described in text. C.p m are expressed at infinite thickness

chloride, 0·1 μmole diphosphopyridine nucleotide, 1·0 μmole adenosine triphosphate, 10 μmol. carbamyl phosphate, 15 μl. carbamyl phosphokinase (0·61 μmol. ATP/min./μl.) and 20 μmol. *tris* buffer, *p*H 7·8 in a final volume of 1·0 ml. Under these conditions the particles obtained at 10,000*g* have the highest specific activity and continue to incorporate L-valine at an increasing rate for 2 hr. Further purification of this pellet by sucrose gradient centrifugation shows that essentially all the radioactivity sediments with the melanin-rich particles. Tumour microsomes reach a maximum rate of incorporation at or before 0·5 hr., and show a decreasing rate of incorporation after this time-interval. The activity of the melano-protein particles is not dependent upon an external supply of ATP. The omission of ATP, carbamyl phosphate and carbamyl phosphokinase does not reduce the incorporation, nor does the addition of ribonuclease inhibit the reaction. In a subsequent communication the evidence that suggests that the particles meet the energy requirement for the incorporation from ATP synthesized during anaerobic glycolysis will be presented. In addition to a high content of melanin, the particles contain 35 per cent lipid of which 5 per cent is phospholipid. Electron micrographs of the isolated particle fraction reveal vesicles with enclosed melanin granules in various stages of synthesis. There are no characteristic ribonucleoprotein particles visible, although RNA is present in the particles (Table 1). The radioactive proteins of the particles show solubility characteristics expected of that of melano-protein.

This work represents an attempt to extend to the biochemical level the reactions involved in melano-protein synthesis, and agree with the electron microscopic evidence obtained by others[1,2] that biologically active membrane structures exist in melanocytes which differ biochemically and morphologically from tumour microsomes and which may participate directly in the biosynthesis of specialized cell proteins.

This work was supported in part by a grant from the U.S. Public Health Service and the Swedish Cancer Society.

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Rapid Procedure for Monitoring Steroid Transformations

THE Δ^1 dehydrogenation of 12α-fluoro,11β-hydroxytestosterone by *C. simplex* is an extremely fast reaction. Peak conversion to product may occur within 1 hr. after addition of the substrate. Furthermore, the product is quickly degraded after the reaction is completed. A reaction of this type requires a rapid method of determining harvest time.

Several paper chromatographic solvent systems using descending solvent-flow were examined at 22° C. Bush-type systems[1], such as benzene–ethanol–water, were unsatisfactory because of the time required for development of the chromatogram plus the resulting poor separation. A Zaffaroni-type system[2], toluene–propylene glycol, gave good resolution but was unsatisfactory as a monitoring system since a 15-hr. solvent development was required.

The combination of a horizontal solvent-flow and an elevated temperature has been used successfully to achieve a rapid separation (1–4 hr.) of carbohydrates[3], volatile fatty acids[4], and amino-acids[5-7] that normally require 16–40 hr. by the classical room temperature descending techniques.

Based on the ability of horizontal paper chromatography at an elevated temperature to achieve such rapid separations, the technique was applied to the present problem with rewarding success. A horizontal solvent development at 60° C. using toluene–propylene glycol separates 1-dehydro,12α-fluoro,11β-hydroxytestosterone from 12α-fluoro,11β-hydroxytestosterone in 60 min.

The developing solvent is prepared at 22° C. in a temperature-controlled room by equilibrating equal volumes of toluene and propylene glycol. The propylene glycol-rich lower layer is discarded. The toluene-rich upper phase is placed in the trough of a stainless steel chromatographic chamber, measuring 10½ in. square and 2 in. deep. The design of the chamber is identical to that previously reported[3], except that the size has been increased. The bottom of the chamber is covered with toluene in order to maintain a toluene atmosphere within the chamber. The glass-covered chamber is housed in a mechanical convection oven at a temperature of 60° C.

Ethanol-washed Whatman No. 1 filter paper, 17 cm. × 22 cm., is used. The origin is 4·5 cm. from one end. A 2·5-cm. tab, folded at a right angle to the paper, is inserted into the solvent trough. The opposite end of the paper is serrated using pinking shears and a 1·0-cm. tab is folded at right angles to the paper to permit the easy flow of solvent off the paper strip. The manner in which the filter paper strip is supported in a horizontal position has been described previously[3].

After spotting, the paper is impregnated with propylene glycol by dipping it into a 10 per cent solution of propylene glycol in chloroform. The origin end of the paper is dipped first, just up to the origin, and blotted. The remainder of the strip is then passed through the dipping solution with a rapid pass over the origin.

Following the dipping step, the strip is hung in a hood until the odour of chloroform can no longer be detected. The strip is then placed in the chamber and treated with the developing solvent for 60 min. at 60° C.

After development with the solvent, the strip is dried for 10 min. at 90° C. The positions of the steroids are located by examining the chromatogram with a zinc silicate coated paddle over an ultra-violet light source[8]. The following $R_{\text{substrate}}$ values were obtained: 1-dehydro,12α-fluoro,11β-hydroxytestosterone 0·4; 12α-fluoro,11β-hydroxytestosterone 1·0; and 1-dehydro,12α-fluoro,11β-hydroxyandrostenedione 1·8.

The Δ^1 dehydrogenation of 12α-fluoro,11β-hydroxytestosterone by *Corynebacterium simplex*[9] was conducted in a 100-gal. stainless steel fermentor. The medium contained 0·10 per cent 'Yeastamin', 0·11 per cent 'Cerelose', 0·10 per cent monopotassium phosphate, and tap water to volume. The *p*H of the medium was adjusted to 7·5 with sodium hydroxide before sterilization. The fermentation was conducted

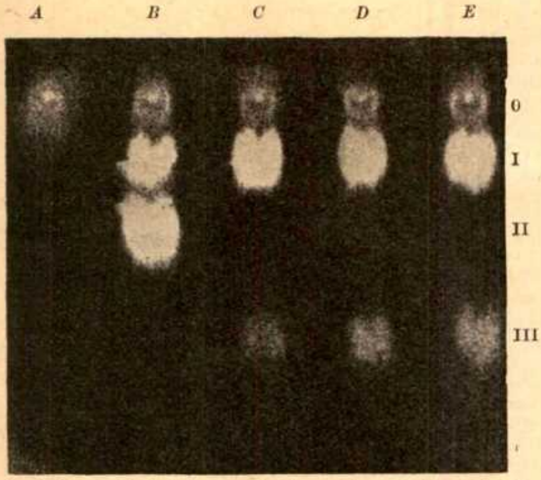

Fig. 1. Ultra-violet photocopy of a chromatogram after a 60-min. solvent development at 60° C. in the solvent system toluene–propylene glycol: *A*, broth sample before steroid addition; *B*, 15 min. after substrate addition; *C*, plus 1 hr.; *D*, plus 2 hr.; *E*, plus 3 hr.; O, ultra-violet-absorbing impurities at the origin, I, 1-dehydro,12α-fluoro,11β-hydroxytestosterone; II, 12α-fluoro, 11β-hydroxytestosterone; III, 1-dehydro,12α-fluoro,11β-hydroxy-androstenedione

at 25° C., using an aeration equal to a superficial air velocity of 1·0 ft./min. at 10 lb./sq. in. and agitation equal to 0·2 horse-power/100 gal. of broth. The sterilized medium was inoculated with 5 per cent by volume of suitable germinator inoculum propagated in the usual manner. The steroid substrate (100 μgm./ml.) was added after 24 hr. of growth as a sterile solution in methanol.

The conversion of the substrate to the product was monitored at intervals of 1 hr. 8-ml. aliquots of whole broth were extracted in cork-stoppered test tubes with 1·5 ml. of methyl isobutyl ketone by shaking for 5 min. on a reciprocating shaker operated at 360 two-in. strokes per min. The contents of the tubes were centrifuged at 2,000 revs. per min. for 5 min. after which the steroid-rich methyl isobutyl ketone layers were spotted and chromatographed.

A composite chromatogram, Fig. 1, of the individual hourly samples shows that the conversion of 12α-fluoro,11β-hydroxytestosterone to 1-dehydro,12α-fluoro,11β-hydroxytestosterone was completed within 1 hr. after substrate was added. Prolonging the reaction past the point of peak conversion results in the increase in concentration of a secondary product, 1 - dehydro,12α - fluoro,11β - hydroxyandrostenedione (Fig. 1, III). This secondary product appears in the 1-hr. sample and slowly increases in concentration with time.

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Photometric Method for Estimating Amylase

METHODS for estimation of amylase are legion. H. Wildner[1] has cited some 150 methods. Furthermore, it seems that many workers apply their own modifications. Generally speaking, the majority of methods used are based on one of the following principles: (1) measurement of the decrease in the viscosity of the fluids during the enzymatic hydrolysis of starch; (2) measurement of the increase in reducing sugars; (3) observation of the change in the colour reaction with iodine going from strictly blue through violet and red to pale yellow or colourless. The colour-reaction method would be a very simple and suitable one, were it not complicated by the fact that the colour changes in a double manner, qualitatively (blue-violet-red, etc.) and quantitatively (decrease in colour intensity) at the same time.

In applying the colour reaction, different methods for obtaining an estimate have been suggested ranging from visual observation of the colour[2–4] to comparison with some suitable colour standard[5–7] and various colorimetric readings[8–10]. During a work on fungal and bacterial amylases it was observed, in this laboratory, that photometric measurement in monochromatic light, for example, at a wave-length near the maximum absorption of the initial pure blue colour, is not suitable, owing to a rather abrupt decrease in the absorption at that particular wave-length. On the other hand, if the colour reactions given by a series of samples drawn from an enzyme reaction mixture at definite time-intervals are read in polychromatic light in a photometer supplied with a neutral grey filter, it turns out that the light absorption, when plotted in an extinction-time diagram, falls off in a way closely resembling a logarithmic-type curve. Accordingly, when $\log E$ is plotted against time, a perfectly straight line is, indeed, obtained.

A procedure for determining amylase activity, based on this straight-line correlation, has been used in this laboratory with good results.

To 3 ml. of a 1 per cent solution of starch (soluble starch, Merck) in 0·1 *M* Sörensen phosphate buffer *p*H 6·9 containing 0·01 *M* sodium chloride is added a suitable sample of the enzyme solution to be tested, and the volume made to 6 ml. with distilled water. The mixture is placed in a water bath at 39° C., and the degradation of the starch is followed thus: at certain time-intervals, depending on the enzymatic activity, but easily learnt by experience, 0·50 ml. samples are transferred into test tubes containing 2 ml. 0·2 *N* sulphuric acid followed by addition of 0·2 ml. of a 0·015 *N* iodine solution. When the colour, successively, has shifted to suitable degrees, which are, likewise, observed by experience, 4–5 samples, 0·50 ml. each, are taken from the reaction mixture and transferred to test tubes containing 5 ml. 0·2 *N* sulphuric acid. The appropriate times for the withdrawal of these samples are exactly noticed and the test tubes placed in an ice bath for addition of iodine and photometric reading later on.

When the last sample has been taken, measurements are made as follows (the procedure may be interrupted at this point for some hours, if the temperature is kept low):

The acidified samples are allowed to rise to room temperature, and 0·20 ml. 0·015 *N* iodine solution is added. The colour fades somewhat with time, and the photometric reading is, therefore, made

Table 1. ESTIMATION OF AMYLASE ACTIVITY (A)

Exp.	Amount of enzyme* (mgm.)	Sample No.	t (sec.)	E	1 + log E	$t_{0 \cdot 8}$	A
I	0·307	1	1,392	1·082	1·0342	2,044	4·89†
		2	1,710	0·844	0·9263		
		3	1,958	0·681	0·8331		
		4	2,370	0·4725	0·6744		
		5	2,952	0·2849	0·4547		
II	0·614	1	695	1·115	1·0472	1,036	9·66†
		2	836	0·880	0·9445		
		3	966	0·710	0·8513		
		4	1,200	0·478	0·6794		
		5	1,476	0·297	0·4728		
III	0·921	1	410	1·24	1·0934	676	14·79†
		2	490	1·01	1·0043		
		3	629	0·716	0·8549		
		4	770	0·490	0·6902		
		5	970	0·293	0·4669		

* 0·1–0·2 and 0·3 ml. respectively of a solution of takadiastase containing 3·07 mgm./ml.

† A fairly good agreement is observed between the proportion $A_{\rm I} : A_{\rm II} : A_{\rm III} = 1 : 1{\cdot}98 : 3{\cdot}03$, as obtained by experiment, and the theoretical value 1 : 2 : 3.

exactly 2 min. after the addition of iodine. For reference, a blank is made identical with the samples, except that no starch is present. A Zeiss model Elko II spectrophotometer, supplied with a neutral grey filter of a suitable optical density, has been successfully used.

Results from a typical experiment are recorded in Fig. 1 and Table 1.

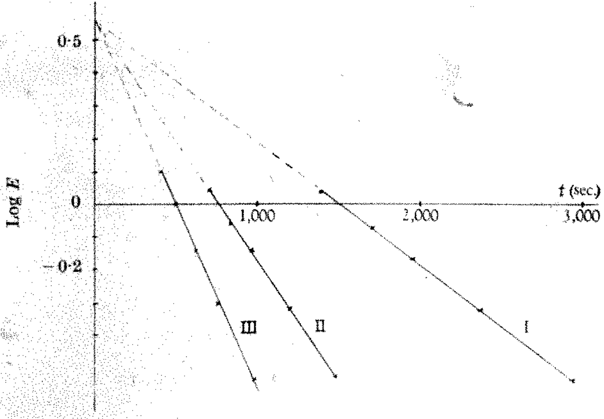

Fig. 1

It is seen that the points follow straight lines. The lines are, further, converging toward the same point on the y-axis, indicating the light absorption at zero time. The slopes of the lines are directly proportional to the amounts of enzyme. Accordingly, the time-interval corresponding to any definite light absorption is inversely proportional to the enzyme activity. Within the limits of minor experimental errors the experimental values are in good agreement with the theoretical ones.

For convenience, we have in our laboratory established the following expression for amylase activity as measured under the above conditions: amylase activity $= A = 10^4 \times t_{0 \cdot 8}^{-1}$, where $t_{0 \cdot 8}$ is the time, expressed in seconds, corresponding to $\log E = -0{\cdot}2$ or $1 + \log E = 0{\cdot}8$ or $E = 0{\cdot}6310$.

A simplification in calculating the activity is achieved as follows: strictly speaking, one needs only two photometric measurements for calculation of $t_{0 \cdot 8}$ by interpolation. If $E_1 > 0{\cdot}6310 > E_2$, one gets:

$$t_{0 \cdot 8} = t_1 + \frac{1 + \log E_1 - 0{\cdot}8}{\log E_1 - \log E_2} \times (t_2 - t_1)$$

Quite another question is: Why are log E, E being in itself a logarithmic function, and the time interconnected by a linear function of that sort? I can offer no explanation for this. Whatever it may be, the principle has proved useful as a basis for a reliable and simple estimation of amylase.

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Development Techniques for the Separation of Amino-acids on DEAE Cellulose Paper

THE term 'development' is used here to describe the process of chromatographic separation. Anion exchangers based on a cellulose matrix have been found to be fundamentally more useful for the separation of amino-acids than anion exchangers based on a resin matrix of the polystyrene type[1,2].

Additional utility is conferred on any medium when it is available in the form of a paper. Techniques have been developed which are peculiar to the paper form of an ion-exchange material and which increase the scope of ion-exchange chromatography. One such technique, in which ion-exchange and conventional type separations are combined on a two-dimensional chromatogram, has already been described[3-5].

The present communication is concerned with the use of DEAE cellulose paper in conjunction with techniques of development which further emphasize the convenience and flexibility of the ion-exchange paper and which are concerned mainly with the ability of a paper to offer a preformed bed of dry ion-exchange material with very high mechanical stability.

A wet-start development technique. The usual procedure in column techniques is to equilibrate the column with the developing solvent prior to the application of the mixture under test. This technique has been simulated on the paper form of DEAE cellulose in the chromatogram illustrated in Fig. 1. Equilibration with 0·001 M sodium chloride was carried out by means of a descending chromatographic wash for 24 hr. The paper was then carefully and evenly blotted to remove as much of the capillary liquid as possible and the amino-acids were applied in slightly alkaline solution (pH 8) to the damp paper and the chromatogram developed with 0·001 M sodium chloride solution for 3 hr. The solvent front was not, of course, visible during development. The separated amino-acids were revealed on this and all other chromatograms by spraying with a 0·2 per cent solution of ninhydrin in acetone containing 10 per cent acetic acid.

Dry-start development techniques. The technique of dry-start development which has been described in

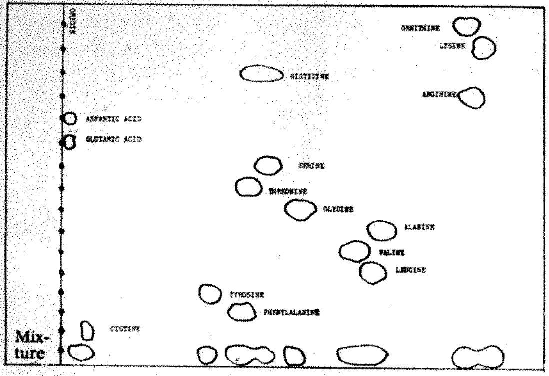
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Fig. 1

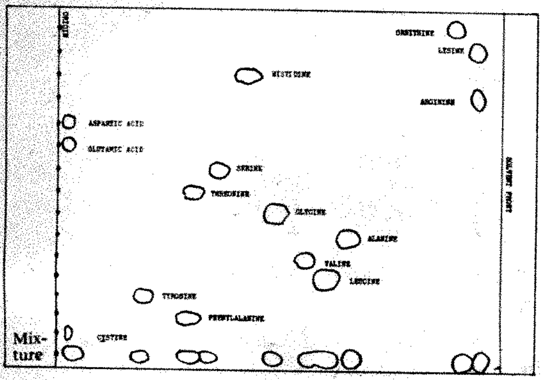
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Fig. 2

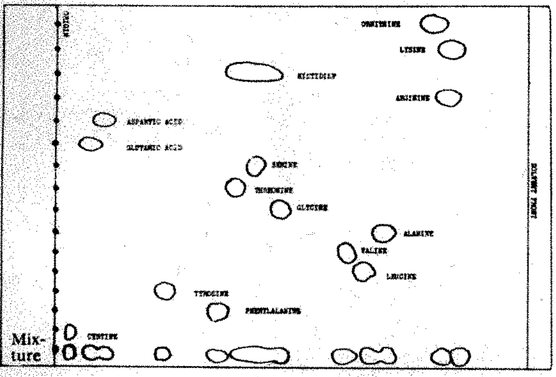
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Fig. 3

connexion with strong cation exchange papers[6] cannot be practised on the weak exchangers because of hydrolysis which occurs as the equilibrating electrolyte is removed by a water wash. Because of this, dry-start techniques used on DEAE cellulose paper are generally characterized by a *p*H gradient from solvent front to solvent source. In this technique, which is peculiar to paper media, the amino-acids were applied to the dry, untreated, free base form of the paper. Fig. 2 illustrates a chromatogram developed in this manner with 0·001 *M* sodium chloride. The resolution obtained is higher than that shown in Fig. 1, where the same solvent was used in conjunction with the wet-start technique.

The effect of the induced *p*H gradient in such a system is added to by the gradient in capillary liquid distribution from solvent front to solvent source, which assists resolution and which characterizes paper chromatograms irrigated in an initially dry state[7].

The chromatogram illustrated in Fig. 3 is a further example of a dry-start development technique using a *p*H gradient. The DEAE-cellulose paper was again used in its dry, free-base form and the developing solvent was a 0·01 *M* *p*H 4·5 buffer. The gradient operating in this chromatogram covered a lower region of the *p*H scale and an improved resolution was obtained among the slower-moving amino-acids relative to that shown in Fig. 2.

When these results are considered in the light of the fact that the particular DEAE-cellulose used for these experiments was an uncross-linked, relatively low capacity (1 mequiv./dry gm.) exchanger, they promise considerable future potential for this type of anion exchanger as a high-resolving chromatographic medium.

I thank the directors of W. and R. Balston, Ltd., for permission to publish this work.

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Structure of Lathyrine

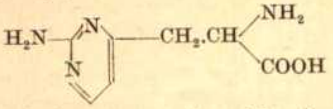
In a preliminary report[1] the isolation of a new amino-acid, giving a red colour with ninhydrin, from seeds of *Lathyrus tingitanus* was described. The molecular formula was given as $C_7H_{10}O_2N_4$ and the apparent pK_a values of its one carboxyl and two proton accepting groups as 2·4, 4·1 and 9·0. The compound showed an absorption maximum, varying with *p*H between 285 and 300 mμ, which disappeared when an aqueous solution of the amino-acid sulphate was hydrogenated over Adam's catalyst. The reduced compound contained a guanidino or substituted guanidino group and gave a purple colour with ninhydrin.

It is now reported that lathyrine absorbs two molecules of hydrogen when reduced under these conditions, and that the reduced compound has been isolated as a monohydrate m.p. above 250° (decomp.) $[\alpha]_D^{22°}$ + 64·11 in water (c., 1·1). Found: C, 40·87; H, 7·91. $C_7H_{14}O_2N_4.H_2O$ requires C, 41·18; H, 7·84 per cent.

This finding suggests that the guanidino group detectable in the reduced compound is part of a reduced ring and that lathyrine itself is a substituted 2-amino-pyrimidine. The second pK_a value of lathyrine and its absorption maximum correspond closely to the values of 2-amino-4-methyl-pyrimidine (pK_a 4·15 (ref. 2) or 4·25 (ref. 3); $\lambda_{max.}$ 289–299 mμ varying with *p*H (ref. 3)).

Both lathyrine and the reduced compound reacted as α-amino mono-carboxylic acids when treated successively with cupric nitrate and ninhydrin[4], indicating that the three remaining carbon atoms are arranged together as an alanyl group or separately as glycyl and methyl groups.

The nuclear magnetic resonance spectrum at 56·4 Mc./sec. for a solution of lathyrine in deuterium oxide showed four bands (in p.p.m. from internal *tert.*-butanol); a doublet (1·97) and a triplet (2·88) each having some fine structure and two equally split (5 c./sec.) doublets (5·46 and 6·96 respectively). The multiplicity of the high-field doublet–triplet pattern was characteristic of a $.CH_2.CH:$ grouping confirming the presence of an alanyl side-chain. The positions, separation and splitting of the two low field doublets indicate the presence of vicinal protons in an amino-pyrimidine nucleus[5] according with the presence of a single (alanyl) group in the 4 position and β-(2-amino-pyrimidine-4-yl) alanine as the structure of lathyrine.



This conclusion was confirmed by the study of the nuclear magnetic resonance spectrum of 2-amino-4-methyl-pyrimidine in deuterium oxide solution. This spectrum showed a singlet (1·02) due to the methyl group and two doublets (5·34 and 6·81) with a splitting of 5 c./sec.

It may be noted that the fine structure of the high field doublet and triplet in the spectrum of lathyrine disappeared when the spectrum was measured in acid solution but developed into a twelve-line *ABX* pattern[6] in alkaline solution.

We thank Profs. W. Robson and H. Harris for their interest in this work, Dr. J. Walker for a gift of 2-amino-4-methyl-pyrimidine, and Mr. J. I. H. Rees, of the University of London Botanic Supply Unit, for growing the seed.

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PHYSIOLOGY

Relative Effects of a Deficiency in Calcium or Phosphorus and Vitamin D on the Clinical Appearance and Composition of the Skeleton and the Teeth of the Sheep

ALTHOUGH it is generally agreed that the teeth are less sensitive than the skeleton to the effects of dietary deficiencies of calcium, phosphorus or vitamin D (ref. 1), observations made during a recent investigation of sheep suggest that much depends on the criteria by which the effects of the deficiencies are judged. The present work also provides evidence on the relationship between tooth quality and the carbonate to phosphate ratio of the mineral phase, discussed by Sobel[2].

Three groups of Blackface sheep were fed from 6 to 18 months of age on diets deficient in phosphorus and vitamin D (group *C*), or in calcium (group *B*), or on a diet containing adequate quantities of all three nutrients (group *A*). At the end of the treatment period, 6 animals from each group were killed, and the remaining 7–9 animals in each group were fed on diets adequate in these constituents for a further 12 months.

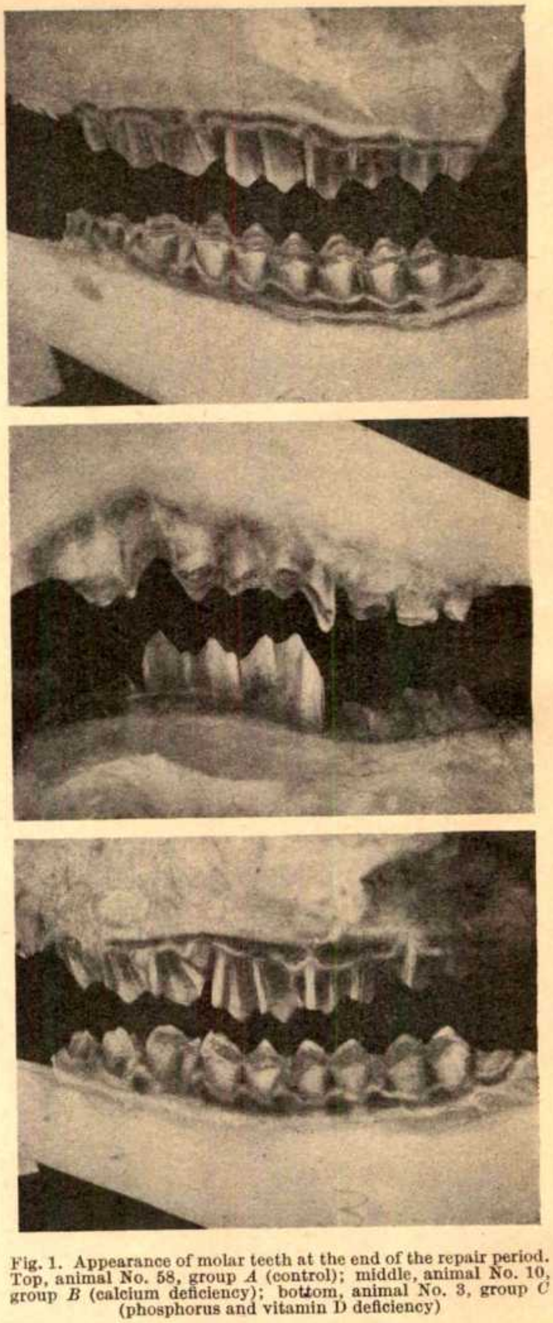

Fig. 1. Appearance of molar teeth at the end of the repair period. Top, animal No. 58, group *A* (control); middle, animal No. 10, group *B* (calcium deficiency); bottom, animal No. 3, group *C* (phosphorus and vitamin D deficiency)

After 12 months on the deficient diet, the sheep in group *C* had leg deformities that were compatible with rickets. In group *B* no rachitic signs were observed in radiographs of the radii (Table 1). Thus, clinically, the skeletons of group *C* animals were defective while those of group *B* animals were not. In contrast, marked clinical abnormalities were observed in the teeth of most of group *B* animals, but practically none was observed in those of groups *A* and *C*. During the 12-month period on deficient diets and during the subsequent 12-month repair period, the eruption of permanent incisors was markedly delayed in group *B* animals compared with those of group *A*, while in group *C* the permanent incisors erupted slightly earlier than those of group *A*. Furthermore, abnormal wear, primarily of the permanent incisors, third molars and third permanent premolars, had occurred in most animals of group *B* (Fig. 1*B*) when they were killed at 30 months of age, while comparable changes were not observed in animals of groups *A* and *C* (Figs. 1*A* and *C*). From these clinical observations of the skeleton and the teeth, the diet deficient in calcium had a greater effect on the teeth than on the skeleton, while the diet deficient in phosphorus and vitamin D had a greater effect on the skeleton than on the teeth. A somewhat similar conclusion was reached by Gaunt and Irving[3] from earlier work at this Institute in which histological and chemical observations were made on the teeth of rats.

The generalization stated in the first paragraph of this communication regarding the relative effects of mineral deficiencies on the skeleton and the teeth was derived largely from ash analyses of teeth and bone, and similar results from the present experiment (Table 1) would also support this view.

Table 1. RADIOLOGICAL VALUES AND CHEMICAL COMPOSITION OF BONES AND TEETH OF GROWING SHEEP AT THE END OF A TWELVE-MONTH PERIOD ON DEFICIENT DIETS

	Group		
	A	*B*	*C*
Radius (radiological assessment)*	8·8	7·6	2·2
Mandible (ash weight, gm.)	26·0	15·8	7·9
Mandible (percentage of ash)	68·8	65·2	50·9
Third molar			
Enamel (percentage of ash)	94·9	94·5	94·4
Dentine (percentage of ash)	78·7	77·5	78·7
CO_3/PO_4 (whole tooth)	0·078	0·085	0·106

* Method described by Benzie, Boyne, Dalgarno, Duckworth, Hill and Walker (ref. 4).

Sobel[2] discussed evidence indicating greater solubility of apatite with a high carbonate to phosphate ratio than when the ratio is low, and linked a high ratio with poor quality of the tooth with respect to caries. There are numerous ways of judging quality, but in the present experiment with sheep the teeth of highest carbonate to phosphate ratio were those of animals deficient in phosphorus and vitamin D (Group *C*) (Table 1), and as judged by clinical observations of wear, their teeth were of good quality, while those of animals deficient in calcium (group *B*) that had a lower carbonate to phosphate ratio were of poor quality. The ratio for teeth of animals on an adequate diet (control group *A*) was very similar to that for group *B*; and so no evidence of a relationship between carbonate to phosphate ratio and tooth quality was found.

This investigation was carried out during the tenure of a predoctoral fellowship from the National Institute of Dental Research, U.S. Public Health Service.

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Antigen-dependent Stimulation of Synthesis of Deoxyribonucleic Acid in Spleen Cells from Immunized Rabbits

PREVIOUS work[1] has indicated the importance of cell division for antibody production in an *in vitro* spleen cell system. These findings prompted an investigation of the factors affecting the synthesis of deoxyribonucleic acid (DNA) in these preparations. The experiments described here demonstrate that the addition of antigen to the incubation medium stimulates the synthesis of DNA in spleen cells from previously immunized rabbits. The stimulation is antigen-specific.

The rabbits were immunized by a month course of intravenous injections of alum-precipitated antigen. One or more months after the last injection the spleen was removed and a cell suspension prepared as previously described[2]. Samples of the suspension containing 1 × 10^7 cells were incubated with 0·2 μc. of thymidine labelled with carbon-14 (at 18·3 mc./m.mole) in a total volume of 1·7 ml. of a modified Eagle's medium. At the end of incubation the cells were washed twice in 0·85 per cent saline and dissolved in 0·4 *N* sodium hydroxide. The DNA was precipitated by the addition of salmine as described by McIntire and Sproull[3]. The precipitate was washed and dissolved in 6 *N* hydrochloric acid, for sample preparation. The radioactivity was determined and the total counts were taken as a measure of synthesis of DNA. The nature of the labelled precursor and the method of precipitation have been considered as an adequate guarantee of the specificity of the method for DNA.

The effect of adding antigen to the incubation medium is shown in Fig. 1. The synthesis of DNA

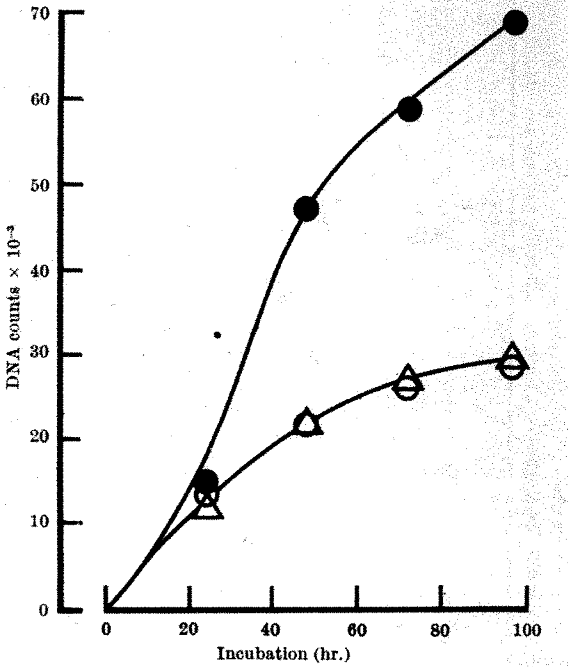

Fig. 1. Antigenic stimulation of DNA synthesis. The rabbit had been previously immunized with human serum albumin. DNA synthesis was measured by the incorporation of ^{14}C-thymidine into DNA. 1 × 10^7 cells were incubated with the following additions to the medium. △, No addition; ●, human serum albumin, 0·5 mgm./ml.; ○, egg albumin, 0·5 mgm./ml. For practical reasons, DNA synthesis was measured for the 24-hr. period prior to collecting individual cultures. The figures, therefore, represent the sums of the appropriate 24-hr. values

Table 1. EFFECT OF ANTIGEN ON THE SYNTHESIS OF DNA IN SPLEEN CELLS FROM PREVIOUSLY IMMUNIZED RABBITS

Incorporation of thymidine into DNA. Total counts

	Protein added to medium				
Antigen used in immunization	None	Egg albumin	Human γ-globulin	Human serum albumin	Bovine serum albumin
None	398	379	453	453	Not done
Egg albumin	331	2,377	735	753	452
Human γ-globulin	4,080	6,350	16,100	12,000	Not done
	775	959	3,040	1,300	Not done
Human serum albumin	10,500	9,560	18,100	32,700	Not done

0·2 μc. ^{14}C-thymidine was added to 1×10^7 cells which had been incubated for 24 hr. in medium containing 0·25 mgm./ml. of the proteins indicated. The cells were collected after a further 24 hr. and the activity in the DNA determined.

falls off in the control culture. In the presence of antigen, however, it increased initially and remained high throughout the period of observation. The 24–48-hr. period was chosen as the most suitable period for further measurements.

The antigen specificity of the stimulation is shown in Table 1. With cells from an unimmunized animal, none of the proteins used stimulated DNA synthesis. With cells from immunized animals there appeared to be some stimulation with other proteins but this was small compared with the stimulation observed with the protein used for immunization. The cross-reaction shown between human γ-globulin and human serum albumin was accounted for by the fact that the preparations had components in common.

The effect of antigen concentration is shown in Fig. 2. Stimulation was observed with less than 1 μgm./ml. In other experiments stimulation appeared to be maximal at 0·1–1 mgm./ml.; but it is not yet known whether this figure is significant or merely reflects some limitation of the incubation conditions. It can be observed that 100 times more human serum albumin was required to give a comparable stimulation to that observed with human γ-globulin.

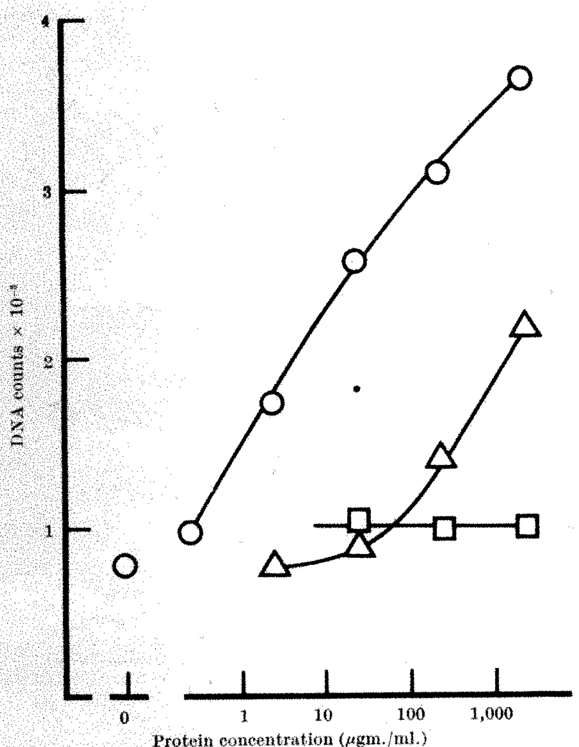

Fig. 2. Effect of antigen concentration on DNA synthesis. The rabbit had been previously immunized to human γ-globulin. The incorporation of ^{14}C-thymidine into DNA was measured over the period 24–48 hr. after the addition of protein. 1×10^7 cells were incubated with the concentrations of protein indicated. ○, Human γ-globulin; △, human serum albumin; □, egg albumin

In these experiments more than 12×10^3 counts may be incorporated into the DNA of stimulated cells incubated with 0·2 μc. of thymidine ($5\cdot3 \times 10^5$ counts in our assay system) at 18·3 mc./m.mole. This represents the utilization of $2\cdot47 \times 10^{-4}$ μmole thymidine for DNA synthesis. If the DNA of one cell is taken to contain 5×10^{-9} μmole of thymidine this incorporation is equivalent to DNA replication by 5×10^4 cells or by 0·5 per cent of the cells in a culture of 1×10^7 cells. This figure represents a minimum value as no account has been taken of the contribution made by unlabelled endogenous thymidine.

Parallel measurements of the incorporation of tritiated thymidine into cells were made on stripping film autoradiographs of smears prepared after incubation. These showed that the increased synthesis of DNA was primarily due to the incorporation of thymidine into an increased number of cells (Table 2).

It is thus clear that the addition of antigen *in vitro* stimulates DNA synthesis and cell division in cell suspensions from rabbits immunized some months previously to the same antigen.

Table 2. COMPARISON OF INCORPORATION OF ^{14}C-THYMIDINE INTO DNA AND ^{3}H THYMIDINE INTO CELL NUCLEI

	^{14}C-Thymidine into DNA (total counts)	Cells labelled (per cent)	Total grain count per 5,000 cells
Control incorporation	3,960	1·1	2,950
Antigen-stimulated incorporation	8,350	2·8	5,370
Ratio stimulated/control	2·1	2·5	1·8

Cells were incubated with 0·25 mgm./ml. antigen for 24 hr. before the addition of thymidine. The cells were collected after a further 24 hr. incubation. Smears and autoradiographs were made of cells incubated with ^{3}H-thymidine and the DNA isolated from cells incubated with ^{14}C-thymidine.

It seems very likely that this response reflects the proliferation of antibody-forming cells which has been shown to follow antigenic stimulation *in vivo*[4] and therefore provides a suitable system for further study.

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Gonadotrophic Action of Aldosterone in *Xenopus laevis*

IT has been shown that 11-deoxycorticosterone[1] and related corticosteroids[2] such as corticosterone, 17-hydroxy-11-deoxycorticosterone, 11-dehydrocorticosterone, 17-hydroxy-11-dehydrocorticosterone, hydrocortisone and cortisone will cause ovulation when injected into *Xenopus laevis* in 5–10 mgm. doses.

It has now been found that aldosterone (5 mgm.) also induces ovulation. Like 17-hydroxy-11-dehydrocorticosterone this occurs 12–15 hr. after injection. With cortisone acetate and hydrocortisone acetate ovulation occurs 36–42 hr. after injection. All experiments were performed at 20°.

We thank Messrs. Ciba Ltd., Basle, for a supply of aldosterone.

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Distribution of Taste Sensitivity for Phenylthiocarbamide in Yugoslavia

INVESTIGATIONS into taste sensitivity for bitter as a particular hereditary characteristic in man have found their practical application in human, that is, population genetics. Phenylthiocarbamide (PTC) has been found the most reliable among the bitter substances used for discrimination between sensitive persons and insensitive. We believe that of the different methods described[1-3] the most advantageous one is that of Harris and Kalmus[4-7]. In addition to the testing and checking of modes of heredity, the establishment of frequency of tasters and non-tasters, a relation between the sensitivity for PTC and the thyroid diseases has been noted[7,8]. Recently there seem to have been new developments in the interpretation of this relation[9].

The sensitivity for bitter in certain other conditions and circumstances is also being tested[10,11].

However, all work of this kind is based on the data regarding the distribution of tasters and non-tasters in a healthy population of a certain age. We have tested 406 students in the Medical Faculty in Zagreb. The average age was twenty-one years, with equal proportion of sexes (male 201, female 205).

The method used was that of Harris and Kalmus[4-7]. The results are shown in the Table 1. From there it can be seen that sensitivity lies between the fourth and the fifth solution, 66·99 per cent of the tested persons are tasters, and 33·01 per cent are non-tasters, without a significant difference between the sexes. Although the result is within the frequency limits known in some European populations, the percentage of non-tasters is higher than even in the younger persons tested in Yugoslavia[12]. The analysis of the material shown, with regard to the place of birth of persons, shows an increased frequency of non-tasters among people from the area along the Adriatic coast. This has already been noted in the course of other work[13]. According to this analysis the frequency of non-tasters would be lower in the eastern parts of Yugoslavia. This will be the subject of further work.

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Table 1. THE FREQUENCY OF PTC THRESHOLDS AMONG 201 MALE AND 205 FEMALE STUDENTS

n = 406	PTC solution No.														Total
	<1	1	2	3	4	5	6	7	8	9	10	11	12	13	
	Non-tasters					Tasters									
Males	22	30	9	4	3	2	5	10	35	38	37	4	1	1	
Abs.	68					133									201
Percentage	33·83					66·17									49·57
Females	14	24	21	5	2	3	3	11	28	48	36	9	1	—	
Abs.	66					139									205
Percentage	32·20					67·80									50·49
Total	134					272									406
Percentage	33·01					66·99									100·00

PATHOLOGY

Interaction between Epithelial and Mesodermal Elements in Anthramine-induced Tumours of the Rat

IN a group of tumours arising in rat skin painted with 2-anthramine I have observed two examples of a compound tumour different in type from those described in previous similar experiments[1-3]. Their essential feature is that a sarcoma forms the stroma of an epithelial tumour. The natural history shows that the carcinoma and the sarcoma begin as separate growths and unite by collision. As a result the cytology and pattern of growth of the two elements are altered; the detail of the changes throws light on the relationship between epithelium and connective tissue, as well as on the process of carcinomatous invasion.

Twelve August rats, six weeks old, were painted twice weekly for 40 weeks with a 1 per cent solution in acetone of 2-anthramine (Aldrich Chemical Co., Inc., Wisconsin) using Lennox's method[2]. At each painting 0·2–0·3 ml. was applied. By the fortieth week the two rats concerned in this communication showed a number of pearly tumours 0·5–1·0 mm. in diameter studded over the almost hairless painted area on the back.

These tumours, microscopically basal-cell carcinomata, grew slowly for some weeks. Then in each rat a firm vascular dermal nodule developed which grew very rapidly, undermining several epidermal tumours and becoming ulcerated. The tumours and a rim of skin around them were excised when they had been present for several weeks.

First Rat. The greater part of the tumour is a fibrosarcoma composed of closely packed rather slender collagen fibres between which are numerous immature fibroblasts. There are very few argyrophilic reticulin fibrils and no fibres that stain with orcein. In the marginal skin a little distance from the fibro-sarcoma there are several basal-cell carcinomata. These arise from pilosebaceous ducts and are arranged in small rounded masses which show no tendency to invade. There are a few histiocytes around the masses and silver impregnation demonstrates reticulin fibrils in relation to them (Fig. 1). There is a distinct basement membrane of periodic acid–Schiff stained material at the margin. One segment of the sarcoma is highly cellular. It has been invaded by strands of basal-cell carcinoma composed of small elongated darkly staining cells. Around the strands the collagen of the sarcoma has been replaced by a mantle of rounded more lightly staining reticulum cells (Fig. 2) which have produced a dense network of reticulin fibrils (Fig. 3). There is a layer of material stained with periodic acid–Schiff reagent around the basal-cell carcinoma strands similar to the basement membrane at the dermo-epidermal junction. The invading epithelial cells penetrate to the lower limit of the sarcoma but do not pass into the normal tissue around it. The invasion and alteration of cytology do not affect the symmetry of the sarcoma.

Second Rat. The sarcoma is composed of large and very primitive cells, many being multinucleated or in a syncytium. They form no collagen or reticulin but lie in a rather amorphous matrix. This tumour is invaded extensively by strands of basal-cell carcinoma which tend to form a network. Many of the cells on the outer side of these strands are arranged in a low palisade, and elongated sarcoma cells are ranged along the border. The tissue in the meshes is myxomatous and the sarcoma cells in it are stellate. The basal-cell carcinoma strands do not invade beyond the limits of the sarcoma. There is no basement membrane or reticulin network at the junction of the carcinoma and sarcoma.

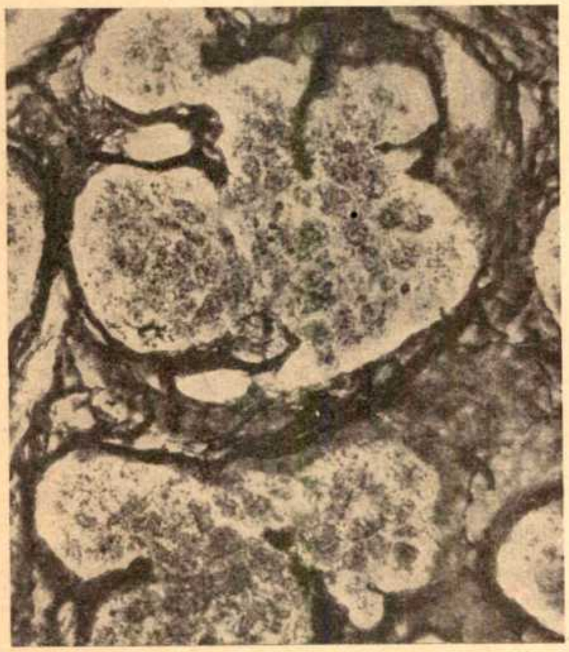

Fig. 1. Nests of basal-cell carcinoma in the dermis with reticulin fibrils surrounding the almost unstained tumour cells. (Robb–Smith silver impregnation. × *c*. 480)

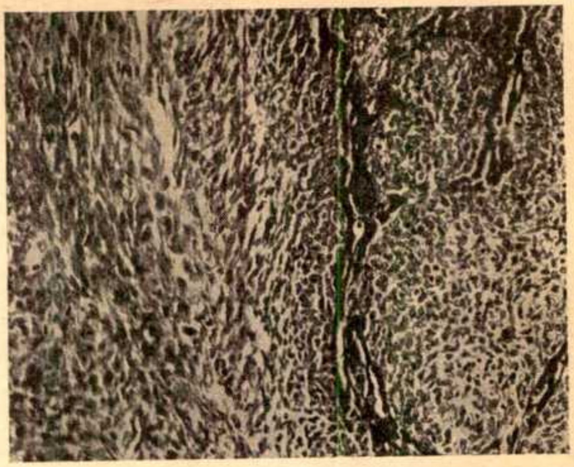

Fig. 2. Invading strands of darkly staining basal-cell carcinoma surrounded by a mantle of reticulum cells. On the left the structure of the fibrosarcoma is shown. (Hæmatoxylin and eosin. × *c*. 126)

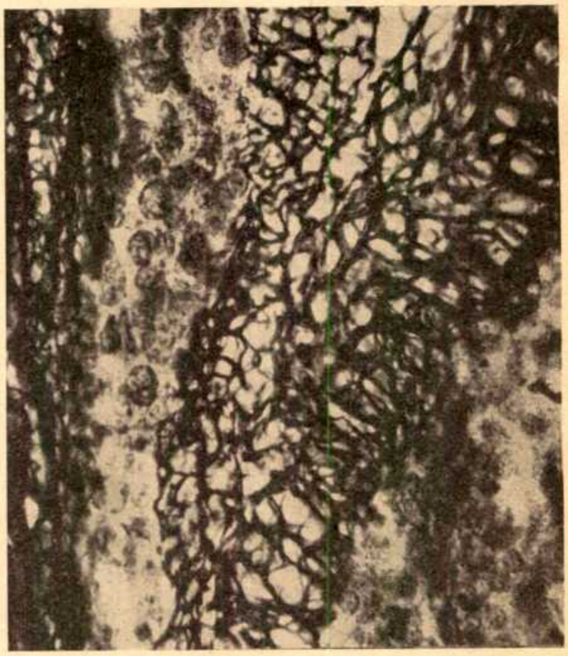

Fig. 3. The cells adjacent to the invading basal-cell carcinoma produce a dense network of reticulin. (Robb–Smith silver impregnation. × *c*. 480)

Tustanovskii and Vasiliev[4] have shown that immature fibroblasts dissolve the collagen around the mammary glands of pregnant mice and form a network of reticulin before the acini begin to proliferate. In the present experiment the same process is seen in the dermis around compact masses of anthramine-induced basal-cell carcinoma. In the fibrosarcoma this mechanism of stroma formation is greatly exaggerated and is associated with enhanced invasiveness of the carcinoma. The train of events is in some respects analogous to the invasion by epidermis of the young connective tissue in healing wounds[5]. In the second sarcoma the primitive cells react to the invading basal-cell carcinoma by forming a mesenchymal type of tissue. In both tumours the carcinoma spreads to the limits of the sarcoma but not out into the surrounding dermis.

Vasiliev[6] has reviewed the evidence relating connective tissue proliferation to invasive growth. He concludes that the formation of undifferentiated connective tissue is essential for the invasion of many malignant tumours, that cancer cells acquire an intrinsic ability to evoke its formation and that their invasive properties may depend on this function rather than on their degree of anaplasia. The behaviour of the two tumours is in keeping with these ideas and also with the view of Kligman and Pinkus[7] that the stroma influences the differentiation and pattern of growth of basal-cell carcinoma and allied tumours.

This work was carried out while I was Roussel Research Fellow.

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Sex Chromosomes as Markers in Transplanted Chicken Leukæmic Cells

THE transplantability of virus-induced chicken leukæmia has never been clearly established. If intact cells are transferred to a homologous recipient bird, a leukæmia may develop. This has been interpreted as a transplanted leukæmia rather than the ordinary virus-induced disease mainly on the basis of an unusually rapid course[1,2]. The proof of the ability of leukæmic cells to grow in a homologous host rests, however, on the recognition of donor cells in the recipient. This communication describes the use of sex chromosomes as reliable markers for such a purpose. The technique takes advantage of the fact that male chicken cells contain two sex chromosomes of equal size, whereas the female cells contain only one (Fig. 1).

A suspension of washed spleen cells from a leukæmic male chicken was injected intravenously into five non-inbred female recipients. Strain *R* erythroblastosis virus was used to induce leukæmia in the donor. Aspiration biopsies were taken from the femoral bone marrow of the recipients at the points of time indicated in Table 1. Suspensions of spleen cells were prepared from killed or dead animals. In order to obtain chromosome preparations, bone marrow and spleen cell suspensions were treated according to Moorhead *et al.*[3]. About 20 metaphases were classified as female or male in each preparation. Three of the recipients died from widespread leukæmia on the fourth day after transplantation; the other two showed no outward signs of disease and were killed after 35 days. The organs were grossly normal at autopsy. Table 1 summarizes the results.

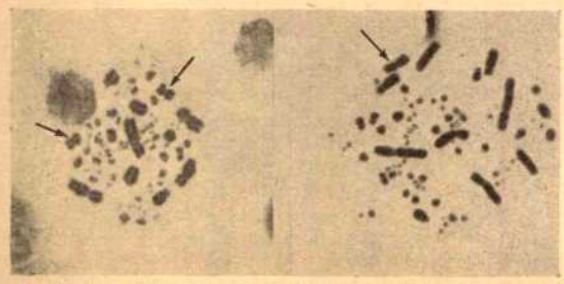

Fig. 1. One male (left) and one female metaphase. The arrows indicate the positions of the sex chromosomes

Table 1. PERCENTAGE OF DONOR (MALE) CELLS IN THE BONE MARROW AND SPLEEN OF FIVE FEMALE RECIPIENTS. $1\cdot2 \times 10^9$ CELLS WERE INOCULATED INTRAVENOUSLY ON DAY 0

	Percentage of donor (male) cells				
	Day 4		Day 21	Day 35	
	Bone marrow	Spleen	Bone marrow	Bone marrow	Spleen
(1) Died day 4 from leukæmia	79	82			
(2) Died day 4 from leukæmia	75	83			
(3) Died day 4 from leukæmia	92	58			
(4) Survived, killed day 35	100		0	0	0
(5) Survived, killed day 35	25		0	0	0

The results show that the transplanted leukæmic cells were able to survive and grow in the homologous hosts. Recipients 1, 2, and 3 succumbed to widespread leukæmia with a high percentage of donor cells in the bone marrow and spleen. Recipients 4 and 5 recovered in spite of the large cell dose used. They both showed a considerable proportion of donor cells in the bone marrow on the fourth day after transplantation. These cells eventually disappeared, presumably due to a homograft reaction.

The technique will be described in detail later. Besides being simple and reliable, it has the advantage of making serial sampling possible from the bone marrow. In this manner, a dynamic picture of the events in individual birds may be obtained.

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HISTOLOGY

Eosinophilic Cells as Possible Mast Cell Precursors

IT is commonly accepted that mast cells in the rat embryo originate from lymphoid-like cells by a process of deposition and numerical increase of cytoplasmic granules, which are basophilic throughout development[1,2].

From an observation on the adult rat which I have made, it seems that another mode of development is possible.

During work on pregnancy changes in the genital tract's connective tissue of the albino rat the presence of numerous peculiar cells was noted. They were seen in the loose connective tissue surrounding the vagina during the early hours after parturition. The cells are localized around small blood vessels (Fig. 1).

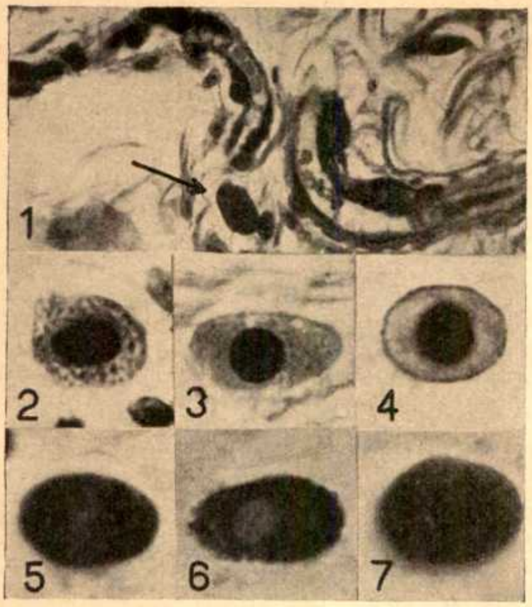

Fig. 1. Eosinophilic cell 4 hr. *post partum* in typical perivascular localization; hæmatoxylin–eosin (× 400)
Figs. 2–4. Eosinophilic cells 4 hr. *post partum* (× 1,200). (2) hæmatoxylin–eosin; the granules are brightly eosinophilic; (3) toluidine blue; the granules do not stain; (4) periodic acid–Schiff; the granules are negative; note the peripheral glycogen granules
Figs. 5–7. Corresponding cells 10 hr. *post partum* (× 1,200). (5) hæmatoxylin–eosin; the granules are bluish; (6) toluidine blue; the granules are intensely metachromatic; (7) periodic acid–Schiff; the granules are periodic acid–Schiff-positive

Their shape is regularly ellipsoid with a central round nucleus. Their most outstanding feature is the presence of numerous uniform, brightly eosinophilic cytoplasmic granules (Fig. 2). With toluidine blue the granules do not stain at all (Fig. 3). The granules have no affinity for the aldehyde–fuchsin stain. The periodic acid–Schiff method does not stain them either, though this technique makes apparent another component: localized at the cell border numerous small glycogen granules are seen. In cross-section these appear as fine strings of beads (Fig. 4). So long as these eosinophilic cells are present not a single mast cell is seen in the whole area.

10–24 hr. after delivery the cells with the above-mentioned characteristics can no longer be seen. Their places in the perivascular spaces seem to be taken by cells which have exactly the same morphology, but have other staining characteristics: in the hæmatoxylin–eosin sections the granules vary from reddish to bluish (Fig. 5). They further exhibit strong metachromasia with toluidine blue (Fig. 6), are periodic acid–Schiff-positive (Fig. 7) and can also be stained with the aldehyde–fuchsin technique.

The description of the second (later) cell type fits well to mast cells.

There are strong reasons for assuming that the two cell types are identical; their morphology and localization are highly similar. Their occurrence is mutually exclusive.

It seems probable that the cells synchronously go through a process of maturation possibly under the influences of endocrinological changes connected with parturition and genital tract involution. During this process the metachromatic granules are formed in two phases: first an eosinophilic (protein) nucleus is laid down; then follows some sort of coating with polysaccharide.

Whether the observed glycogen supplies material for acid mucopolysaccharide synthesis is a matter of conjecture.

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IMMUNOLOGY

Immunization against *Schistosoma japonicum* in Rhesus Monkeys produced by Irradiated Cercariæ

In schistosome infection, it has been generally accepted that the antigens for stimulating the production of immune antibodies are the results of the production of eggs in the hosts[1,2]. Recently, we[3] demonstrated that inoculations of rhesus monkeys with the cercariæ of the non-human (zoophilic) strain of *S. japonicum* gave marked protection against a subsequent challenge with its human (pathogenic) strain. Since the non-human strain of *S. japonicum* is usually unable to develop into mature worms in rhesus monkeys, it indicates that the presence of schistosome eggs in the tissues is not necessary for the development of acquired resistance and that the antigens for stimulating the production of potent immune bodies may originate from schistosomulæ. This point of view was confirmed by Sadun *et al.*[4]. The question still remains whether this type of acquired resistance can be produced by the schistosomulæ of the human strain. This can be answered by inoculation of rhesus monkeys with irradiated cercariæ of the human strain before they are challenged with non-irradiated cercariæ.

The cercariæ of *S. japonicum* used were newly shed from the snail, *Oncomelania nosophora*, obtained from Japan. The cercariæ were irradiated in dechlorinated tap-water suspensions 10 mm. deep and 17 mm. in diameter; thus the volume was about 2·25 ml. The vessels containing the liquid were of glass with walls 1·6 mm. thick. The X-irradiation was delivered by a General Electric Maxitron unit. Radiation factors were 250 kVp., 30 m.amp., with 0·25 mm. copper and 1 mm. aluminium filtration. The half-value layer for the beam was 1 mm. copper. The distance from the target to the surface of the suspensions was 248 mm., which yielded an exposure dose-rate of 620 r./min. The total exposure dose given to the cercariæ for immunizing the monkeys was regulated so that the cercariæ were not devitalized but were modified in such a way that they did not develop into adults. It is especially important that female worms become sterile.

The preliminary experiments to determine the critical doses of radiation were performed on albino mice. Sex-determined cercariæ were used. After irradiation, they were transferred to cover glasses. Only the actively swimming cercariæ were used for inoculation, which was given by the cutaneous route with the cover-slip method. Each mouse (18–20 gm.) received 50 male and 50 female cercariæ. The mice were killed 35 days after the inoculation and examined for schistosome worms and eggs.

The rhesus monkeys used weighed 2·1–2·8 kgm. The immunizing inoculation for monkeys consisted of

Table 1. No. of Eggs of *S. japonicum* in the Stools of Immunized and Non-immunized Monkeys

	Inoculation						Eggs/gm. of stool/day	
	Immunizing			Challenge				
					No. of days after:			
Monkey No.	No.	X-ray exposure dose (r.)	No. of cercariæ	No. of cercariæ	First immunizing inoculation	First immunizing inoculation	Maximum	Mean, first 30 days
				Immunized monkeys				
76	3	1,700	8,000	400	154	18	0	0
77	3	1,700	6,000	400	154	40	80	36
82	3	2,000	6,000	400	154	18	30	3
78	4	2,000	9,000	400	154	54	38	17
79	4	2,000	9,000	400	154	18	12	3
				Non-immunized monkeys				
32				400			14,790	1,220
31				400			21,840	4,712
115				400			41,024	12,630
30				400			69,120	6,693
55				400			88,400	46,036

sex-undetermined cercariæ originating from ten or more snails. For the challenge inoculation of immunized monkeys and for the inoculation of control monkeys, only the sex-determined cercariæ were used, each monkey receiving 200 male and 200 female cercariæ. Beginning with the 30th day after the first immunizing inoculation, the stools of each animal were examined daily by the sedimentation method. Because the maximum number of eggs per gram of stool per day during the patent period and mean number of eggs of the first 30 days are usually reliable indices of the intensity of infection of non-immunized and immunized monkeys infected with *S. japonicum* these criteria were used as bases for comparing the two groups.

Albino mice. Six groups of 14 mice each were inoculated with irradiated cercariæ which had been exposed to 2,000 r., 10,000 r., 30,000 r., 45,000 r., and 60,000 r., respectively. Results at autopsy on the 35th day after inoculation showed that presence of immature worms in the group of mice which received 2,000 r., but no worms in the other groups. A second series of experiments followed. Three groups of 6 mice each were inoculated with irradiated cercariæ which were exposed to 500 r., 1,200 r., and 1,700 r., respectively. Results at autopsy on the 35th day after inoculation showed that in the 500-r. group, mature worms and eggs were found; in the 1,200-r. group, male worms with testes and female worms with ovaries but without eggs were found; and in the 1,700-r. group, all the mice harboured only immature worms except one mouse which harboured also one male worm with testes.

Rhesus monkeys. Judging from the results of our preliminary studies on the development of the irradiated cercariæ in mice, cercariæ exposed to 1,700 r. and 2,000 r. were used as the immunizing vaccine against *S. japonicum* in monkeys. The results as given in Table 1 show that in the immunized monkeys the maximum number of eggs per gram of stool per day in the patent period varied from 0 to 80, while in the non-immunized it varied from 14,790 to 88,400. The mean number of eggs of the first 30 days of the patent period in the immunized monkeys varied from 0 to 36, while in the non-immunized monkeys the differences ranged from 1,220 to 46,035. From these results, it may be concluded that inoculations of monkeys with irradiated cercariæ of the human strain of *S. japonicum* gave marked protection against a subsequent challenge with the same human strain.

Recently, Villella *et al.*[5] reported their successes with immunization against *S. mansoni* in albino mice inoculated with irradiated cercariæ. Thus, independently, workers in two laboratories were studying the immunizing effects of irradiated schistosome cercariæ at the same time. It is encouraging that the results of these two investigations closely agree, although they applied to different schistosome species and different hosts. It seems reasonable to infer that pre-inoculation of monkeys with either cercariæ of the non-human strain of *S. japonicum* or irradiated cercariæ of the human strain produce substantial protection against a subsequent challenge with the human strain. As we pointed out previously[3], the two immunization procedures, use of the cercariæ of the zoophilic strain and irradiation of the human strain, may eventually lead to the development of an effective way to combat schistosomiasis.

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Effect of Thymectomy on 'Delayed' Hypersensitive Reactions

The thymus is a lymphatic organ the role of which in immune reactions remains unknown. Thymus differs from other lymphatic tissues (lymph nodes and spleen) histologically and in a number of additional characteristics. One of the more interesting observations is that the mass and histology of this organ in germ-free animals are normal despite the markedly reduced mass of conventional lymphatic tissue[1]. The thymus, therefore, appears to develop independently of the antibody-producing organs. However, there is evidence to suggest that the antibody mechanism does not develop normally without thymus. Archer and Pierce[2] found considerable inhibition of antibody production at 8 weeks in rabbits thymectomized at birth. Similarly, there was marked inhibition of subsequent antibody production in chickens from which the bursa of Fabricius was excised at one week[3]. By contrast, bursectomy of 10-week-old chickens[3] or thymectomy of adult rabbits[4] failed to influence antibody production.

The effect of thymectomy on 'delayed' (cellular) hypersensitive reactions has not been investigated

Table 1. DELAYED REACTIONS TO BOVINE SERUM ALBUMIN

Group	Number of rats with reaction*		
	0–5 mm.	6–10 mm.	11–15 mm.
Thymectomized	20	4	1
Sham-operated	3	3	9
Unoperated	4	3	11

* Diameter of induration at 48 hr.

Table 2. EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Group	Lymphocyte count on day 0*	Number of rats with encephalomyelitis†			
		0	+	+ +	+ + +
Thymectomized‡ (0–50 mgm. residual mediastinal tissue)	4,700 (3,300–6,000)	5	1	0	0
Thymectomized‡ (50–200 mgm. residual mediastinal tissue)	8,800 (5,300–12,200)	4	0	0	2
Sham-operated	10,050 (4,500–15,600)	1	1	2	5
Unoperated	13,350 (11,200–15,300)	1	0	1	2

* Average count (range in parentheses).
† +, Minimal weakness; + +, partial paralysis; + + +, tetraplegia.
‡ Much of this residual tissue was not thymic.

Table 3. SKIN HOMOGRAFT REJECTION AT 10 DAYS

Group	Number of rats with graft*		
	Not rejected	Beginning rejection	Rejected
Thymectomized	4	5	1
Sham-operated	0	1	3
Unoperated	0	2	5

* Not rejected, homograft is indistinguishable from autograft and from normal skin; beginning rejection, minimal discoloration and swelling; rejected, varying degrees of discoloration and swelling up to total necrosis.

Papermaster *et al.*[5] found evidence that homograft rejection failed to occur in chicks injected on the fifth day of embryonic life with 19 nor-testosterone. This drug prevents development of the bursa of Fabricius, but also, unfortunately, inhibits development of lymphatic tissue throughout the body, and yields under-weight birds, suffering from chronic diarrhœa, which seldom reach adulthood[3]. We wish to present preliminary results showing a striking diminution of several types of 'delayed' reaction in rats thymectomized at birth.

Thymectomy or sternal splitting sham operation was performed on 1–4-day-old Sprague–Dawley rats. Post-operatively thymectomized and control animals gained weight equally, though tending to be slightly lighter than non-operated litter mates. All experiments were carried out in animals 7–10 weeks of age (150–250 gm.). Those failing to gain weight throughout experimentation were discarded.

In the first experiment, rats received crystalline bovine serum albumin (0·5 mgm. in 0·1 ml. complete Freund's adjuvant) in one hind foot-pad, and were tested with the same antigen (30 μgm. in 0·1 ml. saline) between days 8 and 13. Reactions were read at 24, 48 and 72 hr. There was a clear-cut reduction in the delayed response (Table 1), both in diameter and in the degree of induration (not shown in Table 1). Similarly, there was a marked reduction in experimental allergic encephalomyelitis, a disease believed to be mediated by 'delayed' sensitivity[6], in thymectomized rats (Table 2). These animals were injected in one hind foot-pad with 20-mgm. rat spinal cord in 0·1 ml. complete Freund's adjuvant, and observed daily for clinical disease up to 28 days. In another experiment (Table 3), thymectomy appeared to delay the onset and slow the tempo of rejection of skin homografts from Sherman strain rats. Grafts were inspected at 10 days and acceptance or rejection assessed by two observers. The level of circulating lymphocytes in the animals used for the experimental allergic encephalomyelitis experiment was found to be low at the time of inoculation (Table 2). This finding agrees with earlier observations showing both a lowered output of lymphocytes from the thoracic duct and a lowered blood-level of these cells following thymectomy[7].

These experiments emphasize the importance of the thymus in the initiation of delayed hypersensitive reactivity. Thymectomy in early life appears to impair seriously the ability of rats to develop delayed sensitivity to all the antigens thus far tested. The findings in the present study are in accord with immunological evidence, suggesting a relationship between delayed hypersensitivity and the circulating lymphocytes[8].

This work was supported by grants No. *B*-919 and No. *E*-1257 from the National Institutes of Health, and grant No. 268 of the National Multiple Sclerosis Society.

Note added in proof. Since this communication was submitted, Miller (*Lancet*, ii, 748; 1961) has presented preliminary results showing a failure of skin homograft rejection in mice thymectomized at birth, a finding in substantial agreement with our observations on homograft rejection in thymectomized rats.

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RADIOBIOLOGY

Radiation Protection of Tissue Culture Cells by Anoxia, Cysteamine and a Combination of Anoxia and Cysteamine

IN the recent literature[1,2] it has been emphasized that the problem whether the radioprotective action of a group of sulphydryl compounds as cysteine and cysteamine is based on the removal of oxygen or is caused by another mode of action on the cellular level is still not solved. In this communication we present evidence in support of the latter hypothesis.

Plating efficiency of cells from an established cell line of human kidney cells[3] was used as a parameter to measure sensitivity to X-irradiation. The cells were plated with Puck's 'feeder-layer' technique[4] in modified Petri dishes consisting of glass rings fitted with a 'Melinex' bottom[5,6]. The cells were allowed to become attached to the 'Melinex' bottom in the incubator before they were treated and irradiated at room temperature. The physical factors of the X-irradiation were 200 kV.; 20 m.amp.;

filtration 1·5 mm. copper; half value layer 1·80 mm. copper; dose-rate 200 r./min. Maximal back-scatter was used.

Cysteamine solutions of a concentration of 4 and 16 m*M* were prepared in tissue culture medium a few minutes before use. The culture medium consisted of 0·5 per cent lactalbumin hydrolysate in Hanks's solution with 5 per cent calf serum; penicillin and streptomycin were added to avoid bacterial infection. The normal medium was replaced by the cysteamine solution 30 min. before irradiation was begun. A few minutes after irradiation the cells were washed three times with normal culture fluid to limit the toxicity of cysteamine. Protection by 4 and 16 m*M* cysteamine is shown in Fig. 1 (curve *b* and *c*). A detailed report on the protection by different concentrations of cysteamine will be published elsewhere.

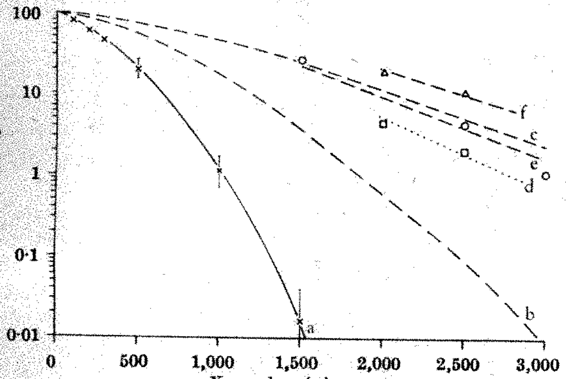

Fig. 1. *a*, Untreated controls; *b*, cysteamine 4 m*M*; *c*, cysteamine ,16 m*M*; *d*, anoxia; *e*, cysteamine 4 m*M* + anoxia; *f*, cysteamine 16 m*M* + anoxia

Anoxic conditions were obtained by flushing over purified nitrogen 10–30 min. before irradiation and during irradiation. For these experiments the glass rings were fitted with a 'Melinex' bottom and top and the rings were provided with a glass inlet and outlet for gas or culture fluid. Before nitrogen was flushed over, the culture fluid was removed. In a Hersch cell[7] connected with the outlet no oxygen could be demonstrated in the gas after the culture had been flushed over for 15 min. Protection by anoxia is shown in Fig. 1 (curve *d*).

The radioprotective action of a combination of anoxia and cysteamine was examined as follows: Cysteamine solution was introduced into a vessel as used for the experiments under anoxic conditions. The solution was removed after 20 min. and immediately afterwards the cells—with adhering fluid containing cysteamine—were flushed over with nitrogen 10 min. before and during irradiation. The results of this combined protection are shown in Fig. 1 (curves *e* and *f*).

In all experiments non-irradiated controls which received the same treatment were used to correct for an eventual slightly toxic action of the treatments.

Cysteamine in concentrations of 4 and 16 m*M* gave dose reduction factors of 1·8 and 3·1 respectively. Under anoxic conditions where no oxygen could be demonstrated a dose reduction factor of 2·5 was obtained. Since 16 m*M* cysteamine gave a higher dose reduction factor than complete anoxia the protective action of cysteamine cannot be explained as an effect caused by anoxia only. This is also shown by the fact that 16 m*M* cysteamine under anoxic condition is giving a better protection than 16 m*M* cysteamine only or anoxic condition only. A third demonstration that the radioprotective action of cysteamine is not caused by an anoxia induced by its autoxidation was found in an experiment in which air instead of nitrogen was flushed over the cells treated with cysteamine. In these circumstances the cysteamine afforded the same protection as under the usual conditions described earlier.

Our results are in close agreement with the results from Kohn and Gunter[8], who found that *l*-cysteine afforded a significant protection of irradiated *Escherichia coli B/r* in suspensions in equilibrium with 0, 5, 20 and 100 per cent oxygen at the time of irradiation.

We thank Dr. A. A. van Soestbergen for the measurements with the 'Hersch' cell.

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Phospholipids and the Reaction of Skin to Ultra-Violet Radiation

Investigations are being carried out in these laboratories into the primary photochemical changes in skin caused by ultra-violet irradiation. In the course of this work it was shown that the concentration of phospholipids in mouse skin was significantly decreased following prolonged *in vitro* irradiation at – 20° C.[1-3].

Extremely large doses of ultra-violet irradiation were used in these experiments, however, and to demonstrate the importance of the reaction in the physiological response of skin to such irradiation, a more sensitive technique was used. If phospholipids of the skin are broken down by irradiation *in vivo*, it may well be that following an exposure to ultra-violet there is an increase in the synthesis of phospholipids in the skin exposed. This has been demonstrated by measurements of the incorporation of phosphorus-32 into the phospholipid fraction of irradiated skin.

Albino mice (strain *A*) were used. One ear was exposed to ultra-violet irradiation from a Hanovia medium pressure mercury arc lamp (504/4 type arc tube), at a distance of 1 metre, for a period of 15 min. (dose 1·53 × 10^7 ergs cm.$^{-2}$). The other ear was shielded and used as a control for the subsequent assays. Immediately following the irradiation, 200 μc. of phosphorus-32 were administered to each mouse by intraperitoneal injection. After 24 hr. the mice were killed and the phospholipids extracted from each ear by Schneider's method[4].

The total phosphorus was determined by the method of Berenblum and Chain[5] after complete oxidation of the extracted lipid, and its activity was measured in a liquid counter. The mean specific activity of the phospholipid of the irradiated ear (10 experiments, control expressed as unity) was 1·27, standard deviation 0·165. This increase is highly significant.

The acid-soluble phosphates and the nucleic acids were also isolated and assayed; but the specific activity of these fractions in the irradiated ear was identical to that of the control. This appears to rule out the possibility of our findings resulting from a non-specific effect of irradiation on the metabolic turnover-rate, such as via capillary dilatation.

It is, therefore, suggested that phospholipids may play some part in the primary response of skin to ultra-violet irradiation, and further work is being carried out in this field.

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BIOLOGY

Territorial Behaviour in Flocks of Domestic Fowls

THE peck order, observed in domestic poultry flocks, has four main components—aggressiveness, submission, acceptance of submission and recognition. Intra-specific aggressiveness arises as a result of sexual selection for mates, territory and food[1], and submission signals and their acceptance are necessary to divert aggressiveness into harmless channels[2]. Recognition is necessary in a gregarious species to allow these other three forces to be integrated into a peck order. Aggressiveness is only lowered within a group; strangers are always attacked.

Poultry must be well integrated into a stable flock or production is affected[3]. In the large flocks used to-day, it seems unlikely that the birds can recognize and remember all their flock-mates. One way to avoid meeting strangers in a large flock is for individuals to use territorial behaviour to restrict the area of their movements. Each bird would then only meet those birds the territories of which overlap its own.

Regular observations were made on a flock of 80 birds at the University of Queensland Farm at Moggill to record the positions of birds over a period of 41 days in a shed 12 ft. × 36 ft. The areas were plotted on a plan and measured with a planimeter. The average area of movement of birds observed was 137 sq. ft. ± 41 sq. ft., that is, about one-third of the area available.

Further evidence of territorial behaviour was observed when the wire partition between two flocks was removed for three weeks before the flocks were sold. In the initial period many birds crossed the old boundary but were always defeated within a short time (up to ½ hr.) and returned to their own territory. The flock soon settled down with no hens established on the new territory though a few crossed the boundary occasionally. The cockerels of one flock advanced their boundary by about 4 ft. into the other pen, but the two groups did not intermingle freely in this new area.

The fact that birds take up these territories, the areas of which can be measured, suggests that it may be possible to develop a theoretical basis for poultry shed design. This territorial behaviour should be encouraged in large flocks since it reduces the numbers of conflicts when strangers meet. To encourage the tendency to restrict movements, it should be possible to define maximum distances between feeders, waterers and nests so that every bird will have these facilities within its territory.

The hypothesis proposed for this territorial behaviour pattern is based on the number of birds recognized by an individual. The territory size should then be inversely related to the density of birds. This is now being investigated.

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A New Type of Cardiac Parasite

A THOROUGH investigation of the ultramicroscopic structure of the frog's heart performed on animals suffering from red-leg disease led to the discovery of very small parasites in the blood-stream and also in the cardiac tissue of two such animals. They accumulated mainly in the sub-endocardial space, that is, the space between the ultra-thin layer of spreading endocardial cells and the sarcolem of muscle fibres. In this space, as previously shown, individual cells, collagen fibres and nerve axons are usually found. The parasites found there in great amounts are a hitherto unknown type of protozoa. The biggest individual specimen found to date measures about 10μ. There are mainly three forms: (1) a pyriform, the biggest of these specimens measured 3μ, having one cilium on its tip; (2) a spindle- or sausage-shaped appearance, having a size of $2–5\mu$ and one cilium on each end; (3) a bizarre type of amœboidal appearance, the biggest of these being up to 10μ in size. They have cilia in irregular distribution stemming from the body.

All these three types are characterized by a membrane surrounding the cytoplasm. This membrane has a thickness of 2–300 Å., and a parallel striation with a rhythmicity of about 500 Å., but no substantial sturdy pellicle is present. In the cytoplasm is an ovoid or spherical nucleus containing a dark karyosome. In the cytoplasm small vesicles and little dark spots can be seen. There is also a most striking parallel arrangement of finest lamellæ showing, at high magnification, a double lining and very small droplets between the lamellæ. The entire group of these lamellæ may be equivalent to a Golgi apparatus or an ergastoplasm or a so-called endoplasmic reticulum. It may also represent the most primitive type of mitochondrium, one lacking surrounding membrane, since in none of the innumerable images of these protozoa which was viewed was a mitochondrium of the usual type present.

The cilia show the typical inner structure of nine fibrils arranged in a circle around a central fibril. Sometimes the membrane surrounding the cilium shows a garland appearance surrounding the skeleton of the inner nine fibrils.

This parasite does not fit into any known species of protozoa. It seems to be the smallest—or at least among the smallest—known, and I therefore suggest for it the name *Ultrazoon ranarum*. It seems probable that further investigation may find that *Ultrazoon ranarum* is not the only representative of this type and that there may be others, too, belonging to this new class. What our investigations have also proved beyond doubt is the fact that part of these intruding parasites are destroyed through phagocytosis by the cells in the sub-endocardial space. It seems that this offers proof that the sub-endocardial cells of a vertebrate have phagocytic properties.

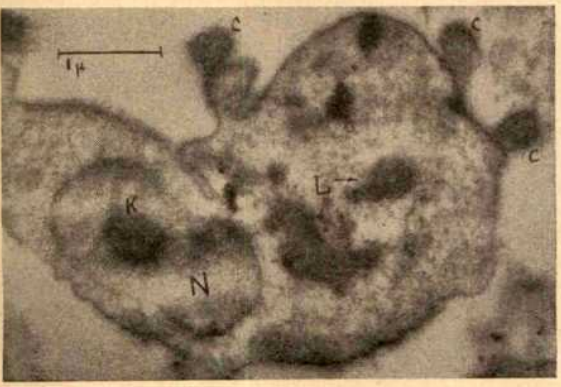

Fig. 1. *Ultrazoon ranarum*. *N*, Nucleus; *K*, karyosome; *L*, two groups of parallel lamels. Notice the small droplets in the cytoplasm and especially at the lamels. (× *c*. 15,000)

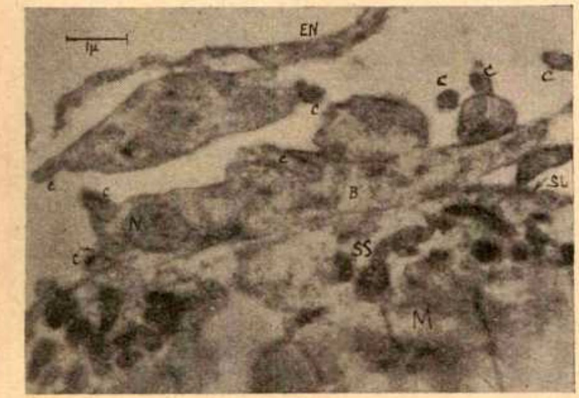

Fig. 2. Group of *Ultrazoa* between the endocard (*EN*) and the sarcolem (*SL*). In the subendocardial space *N*, nucleus, *C*, cilia, *B*, a group of dark dots surrounded by a light circle, as are frequently found near the origin of a cilium. They may be equivalent to the blepharoplast of other protozoa. *SS*, sarcosomes of the heart muscle. *M*, Myofibril. (× *c*. 8,000)

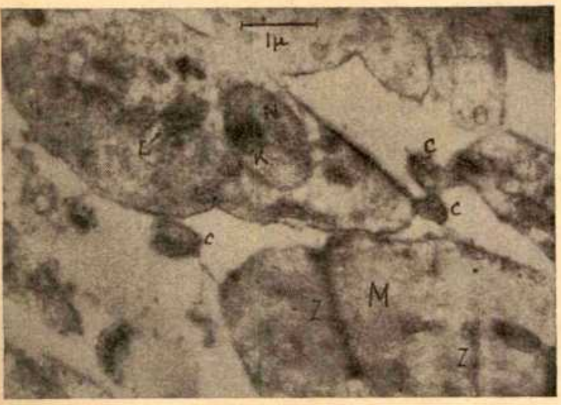

Fig. 3. Group of *Ultrazoa* next to a myofibril near the endocard. *N*, Nucleus; *K*, karyosome; *L*, group of parallel lamels; *C*, cilia; *M*, myofibril; *Z*, *Z*-band of the myofibril. (× *c*. 10,000)

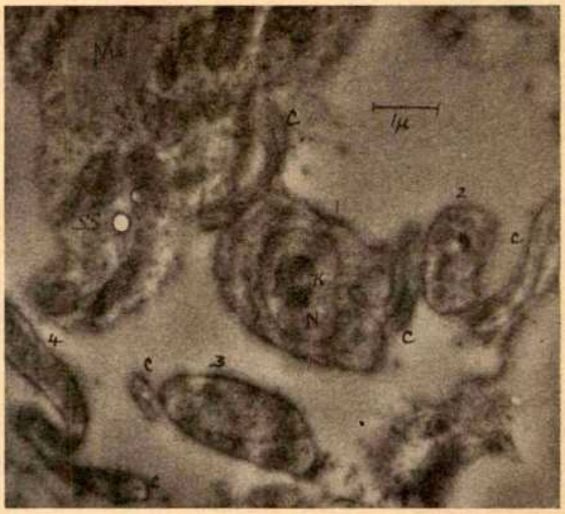

Fig. 4. Group of four *Ultrazoa* near a myofibril (*M*). On top of the myofibril a part of a *Z*-band can be seen. *SS*, Sarcosomes; *C*, cilia. The four *Ultrazoa* are indicated by Nos. 1–4

The findings here reported and Figs. 1–4 may become of interest to human cardiopathology. They should invite electronmicroscopic studies of endocarditis and also electronmicroscopic research into all the other protozoa-mediated diseases.

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Two Morphological Types of Placentæ in the Raccoon

THE literature on the placenta of the raccoon (*Procyon lotor* L.) is extremely sparse and, to the best of our knowledge, placentation in this carnivore is mentioned only by Watson[1], Rau[2], Mossman[3] and Amoroso[4]. The three last-named authors have all quoted aspects of the original contribution by Watson, and it seems, therefore, that knowledge of the raccoon placenta has been based on Watson's early observations. The material at his disposal was, unfortunately, limited to one gravid uterus which contained but one conceptus and, as Watson states, the material was not in the best condition. Observations, in this laboratory, on four gravid uteri involving 17 conceptuses have revealed certain understandable misinterpretations and omissions in Watson's paper. Furthermore, the discovery by one of us (R. F. S. C.)

of a hitherto unreported but prominent placental organ associated with the more commonly mentioned zonary placenta in the raccoon re-focuses attention on the so-called hæmatomata of the carnivore placenta.

The present material was obtained from the reproductive tracts dissected out of 4 pregnant raccoons which had been live-trapped in the spring of 1961 in Pennsylvania. The tracts were fixed in Bouin's solution at the time of killing and stored for later study. Table 1 shows how the conceptuses were disposed in the 4 uteri.

Table 1. DISTRIBUTION AND MEASUREMENTS OF THE EMBRYOS AND PLACENTÆ OF FOUR RACCOONS

(The ranges of the measurements are given in parentheses below each mean)

Animal No.	No. and distribution of embryos		Mean greatest length of embryos (mm.)	Mean width of annulus (mm.)		Mean length of hæmophagous organ (mm.)
	Left cornua	Right cornua		Mesometrial	Anti-mesometrial	
97	2	3	17·8 (16–19)	8·8 (8–10)	15·8 (14–17)	9·3 (8–12)
148	2	2	72·0 (69–75)	25·25 (23–28)	41·75 (36–46)	54·0 (40–62)
142	2	2	74·75 (71–79)	20·0 (19–22)	29·5 (28–31)	40·25 (36–45)
209	2	2	98·25 (94–104)	18·75 (15–21)	27·0 (21–29)	16·25 (0–34)

Subsequent measurements revealed that the material presented a developmental series (Table 1). Nos. 148 and 142 were assessed to have completed 2/3–3/4 of the gestation period while No. 209 was at a near full-term stage of development.

Removal of a window of tissue from the lateral aspect of each locular swelling (Fig. 1) revealed three obvious features within the contained cavity: (1) the embryo within its amnion, with its ventral side facing the mesometrium; (2) an uninterrupted zonary placenta; (3) a prominent and highly vascular organ emerging from a central and anti-mesometrial position of the placental annulus; this prominent structure, for reasons to be discussed here, we propose to call the 'hæmophagous' organ. These three features may be briefly dealt with as follows:

(1) *Amnion*. The amnion, particularly in the more advanced examples, invested the embryo fairly closely in these fixed specimens, and it seems clear that this is the structure which Watson interpreted as an epitrichium.

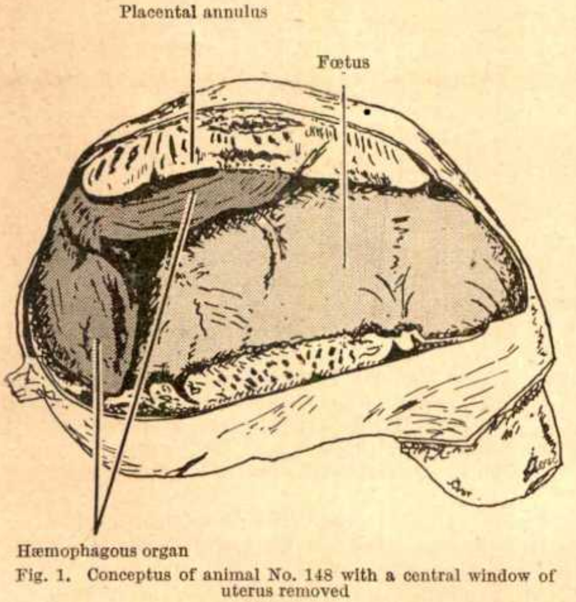

Fig. 1. Conceptus of animal No. 148 with a central window of uterus removed

(2) *Placental annulus*. The raccoon placenta has been described or figured as an incomplete zonary one[1-3]. In no single case of the 17 conceptuses examined in the present work was the annulus incomplete. Histologically, it forms a placental labyrinth, which according to the classification of Grosser would be described as endothelial-chorial. The branching labyrinthine lamellæ consist of fœtal trophoblastic cells surrounding maternal capillaries. These capillaries, which become sinusoid-like as development proceeds, are noteworthy for their atypical endothelium, which is formed of cuboidal cells and resembles the condition of the maternal capillaries of the ferret, the zorille[2] and the wolverine[5]. Fœtal capillaries in the interlamellar fœtal mesenchyme come to assume a sub- and intra-endothelial position with respect to the maternal sinusoidal walls. Thus the fœtal blood approaches very closely to that of the maternal side.

(3) *Hæmophagous organ*. Table 1 shows the developmental history of the hæmophagous organ with respect to size, and Fig. 1 illustrates its position in one of the conceptuses. Emerging from the annulus, its shape for the first half of its length is tubular, while its distal half is expanded and bulbous. The proximal portion runs anteriorly along the dorsum of the embryo, bends first to the right and then to the left over the head of the embryo, the bulbous region resting along the left side of its head and face. Table 1 suggests, however, that the organ grows and then regresses, almost disappearing by full-term. Thus in No. 209 the organ was greatly reduced and in one conceptus was represented by a mere plaque of tissue. Histologically, the hæmophagous organ consists of a highly complex series of branching lamellæ each possessing a fine core of fœtal mesenchyme on either side of which is a single layer of tall columnar fœtal epithelium. Maternal blood, which fills the interlamellar spaces and bathes the fœtal epithelium, gains access through expanded endometrial glands immediately below the organ. This is therefore a hæmochorial condition according to Grosser's classification. Our observations suggest that the cytotrophoblastic tips of the invading fœtal villi erode away maternal epithelium together with much connective tissue and glandular epithelium over the whole placental zone. Except in the region below the presumptive hæmophagous organ, this erosive action continues until the villus tips reach the bases of the glands. In the hæmophagous organ region, however, once the tops of the expanded portion of the glands have been eroded away, the tips of the villi cease their invasive action. This is followed by introversion and proliferation of the trophoblastic epithelium, resulting in the formation of the hæmophagous organ. These epithelial cells are highly columnar with protoplasmic processes on their free surfaces, are gorged with blood cells, granules and other inclusions and bear every sign of great phagocytic activity. A notable feature of these cells is the progressive accumulation within their cytoplasm of pigment material which is presumably a degradation product of hæmoglobin. Fœtal capillaries in the earliest stage (No. 97) have already assumed sub- and intra-epithelial positions with respect to this fœtal epithelium. Thus, in the hæmophagous organ, the maternal blood contents, phagocytosed and transformed by the activity of these

fœtal cells, are in close approximation to the fœtal capillaries.

It seems clear that the raccoon possesses two morphological types of placentæ, an endothelio-chorial one in the labyrinth, and a hæmochorial one in the hæmophagous organ. The terminology of Grosser, however, does not reveal the degree of proximity between maternal and fœtal blood, this proximity being closer in the hæmophagous organ than in the labyrinth in our earliest stage, and of equal closeness in Nos. 148 and 142. The hæmophagous organ in the raccoon may be similar to the "*blutbeutel*" of the otter[6] or the central hæmatoma of the wolverine[5], but development of the placenta in these animals has not been investigated. The term 'hæmatoma' has consistently been assigned in certain animals to regions of the placenta where vascularized chorioallantoic membrane comes into contact with extravasated maternal blood, and the general opinion is that these maternal blood spaces lie between the uterine epithelium and the chorion[3,4]. In the raccoon these blood spaces are not so disposed. Moreover, the term 'hæmatoma' has a pathological connotation which might be misleading when applied to such an 'unpathological' organ. Hæmatomata have also been called accessory placentæ, a term possibly suitable in certain animals but not in the raccoon, where this prominent organ may well be functionally predominant at certain stages of development. For these reasons we have proposed the name hæmophagous organ for a placental structure that consists predominantly of cells of great blood phagocytosing proclivities.

The work was supported by generous grants from the Lalor Foundation and the National Science Foundation (grant No. *NSF G*17658). One of us (R. F. S. C.) also wishes to acknowledge a Fulbright travel fellowship.

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Development of Reticulo-endothelial System in Avian Embryos

In spite of the wealth of investigations carried out on the reticulo-endothelial system practically nothing has been reported on its activity and morphology in the embryo. Various workers have noted phagocytosis in early embryonic tissue, but there is divergence of opinion as to the extent and definition of the cells concerned. Recently, Kent[1], using colloidal suspensions of 'Thorotrast' and of silver (silver-110), has made a valuable contribution to the knowledge of the appearance and phagocytic activity of macrophage cells in the chick embryo, and it is to support this evidence further that the present findings are reported.

Investigations were carried out on the chicken embryo using particulate carbon as the indicator of phagocytosis. This method has been previously reported by Nicol[2] as suitable for the assessment of macrophage activity in adult animals; it is dependent on the uptake of carbon of a uniform particle size (250 Å.) when presented in the bloodstream as a colloidal solution. Each egg was injected with the carbon introduced into one of the allantoic veins by means of a fine syringe. The amount of carbon to be injected was determined by the age of the embryo, and was calculated on the basis of 16 mgm. per 100 gm. of body-weight. The egg was incubated for 1 hr. following the introduction of the carbon, then the embryo was removed and sectioned for microscopical examination. Serial sections were obtained, using the cold microtome (cryostat) technique; the sections were stained with weak eosin, alternate slides being counterstained with hæmatoxylin.

These experiments show that the phagocytic activity of the reticulo-endothelial system begins early in embryonic development. At first there is a generalized distribution of cells capable of taking up particulate matter; they are found especially along the vascular pathways which become demarcated by the carbon stored in their lining cells. The liver then develops and takes over as the centre of the phagocytic elements and its sinusoids become clearly outlined as shown in Fig. 1. *a*, *b* and *c*. It is seen that there is a progressive concentration and localization of carbon in the hepatic macrophage cells as the embryo develops, and this without doubt indicates enhanced functional ability. The spleen, owing to its relative smallness, is not prominent as a phagocytic entity until later in embryonic life when its

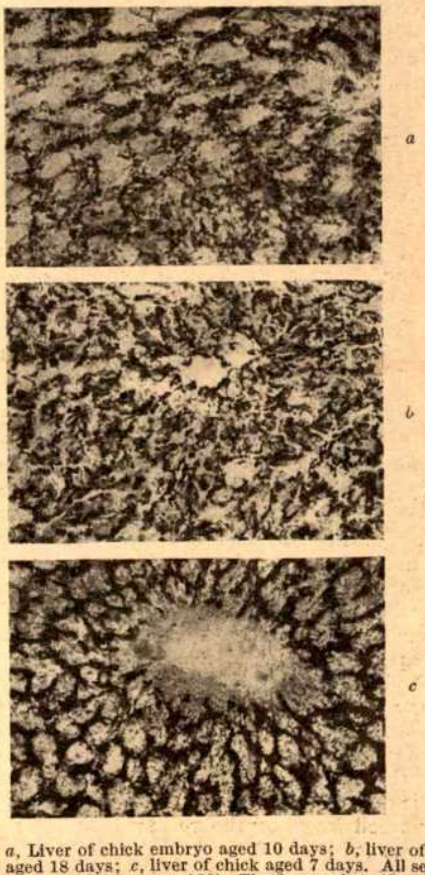

Fig. 1. *a*, Liver of chick embryo aged 10 days; *b*, liver of chick embryo aged 18 days; *c*, liver of chick aged 7 days. All section-stained with weak eosin (× *c*. 100). The figures show the appearance and distribution of the carbon-containing macrophage cells

activity becomes evident by the degree of increased carbon uptake in the phagocytic cells of the pulp. Other sites such as the bone-marrow, the lung and especially the primitive urinary elements also show phagocytic activity, but much less in amount than the liver and spleen, which have been shown to be the principal reticulo-endothelial organs in the adult animal[3].

Research on the physio-dynamics of the reticulo-endothelial system in the embryo is proceeding to associate the embryonic findings with reticulo-endothelial activity and antibody formation after hatching.

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Active Secretion of Oxygen into the Eye of Fish

BIOT[1] first described the active secretion of oxygen gas. He found the gas mixture in the swim-bladders of fish captured from great depths in the Mediterranean to consist of 85 per cent of oxygen. At the hydrostatic pressure of 100 atmospheres obtaining at these depths, the partial pressure of oxygen in the swim-bladder must have been 85 atmospheres. Biot announced that the oxygen had entered the swim-bladder through "secretion", and his discovery has been abundantly verified[2-4]. However, no other instances of oxygen secretion have been obtained despite the interest of a number of physiologists, notably Krogh and J. S. Haldane. We have now found large oxygen tensions in the eyes of marine teleosts.

W. N. F. Woodland[5], writing a full century after Biot, was the first to appreciate the essential nature of the gas-producing complex. A glandular area, the gas gland, formed by localized modification of the epithelial cells that line the swimbladder, is supplied with blood from a rete mirabile. This large, discrete organ consists of regular and intimate intercalations of arterial and venous capillaries, which follow parallel courses and carry blood to and from the gas gland. Although there is no proof, several authors[6-8] are of the opinion that the large oxygen tension found in the swim-bladder is built up by the active transport of oxygen from the venous to the arterial capillaries of the rete, and Marshall's[9] description of the secretory complex of abyssal fishes leaves very little doubt that this must be so. Indeed the rete does provide a very large area[10] for exchange of materials.

A similar rete mirabile is found in the choroid layer of the eye of teleost fishes and of the holostean fish, *Amia calva*[11-15]. The choroid rete mirabile supplies arterial blood to the choriocapillaris (the dense capillary network underlying the retina) and in turn receives the venous outflow from these capillaries. Barnett[15] has suggested that the rete might retain within the choriocapillaris some diffusible substance which is essential to vision. It appears to us that the pigment cell layer of the retina is at least formally analogous to the gas gland of the swim-bladder.

Table 1. OXYGEN TENSION IN THE EYES OF MARINE FISH

Species		No. of animals	Oxygen tension Average (range) (mm. mercury)	Rete
Sting ray	*Dasyatus centroura*	2	16 (7–34)	Absent
Skate	*Raja ocellata*	3	10 (6–17)	,,
Smooth dogfish	*Mustelus canis*	3	11 (9–11)	,,
Eel	*Anguilla rostrata*	2	18 (9–23)	,,
Conger	*Conger oceanica*	4	8 (2–12)	,,
Toadfish	*Opsanus tau*	4	18 (6–28)	Minute
Goosefish	*Lophius americanus*	4	93 (39–146)	,,
Sea bass	*Centropristes striatus*	2	161 (130–180)	Small
Tautog	*Tautoga onitis*	1	210	,,
Sea robin	*Prionotus carolinus*	3	468 (280–860)	Large
Fluke	*Paralichthys dentatus*	1	255	,,
Puffer	*Sphaeroides maculatus*	2	462 (365–575)	,,
Scup	*Stenotomus versicolor*	7	484 (272–770)	,,
Menhaden	*Brevoortia tyrannus*	2	255 (240–287)	,,
Goggle eye jack	*Trachurops crumenophthalmus*	4	416 (317–566)	,,
Cod	*Gadus morhua*	3	820 (575–1,180)	,,
Bluefish	*Pomatomus saltatrix*	4	454 (240–820)	Very large
Remora	*Echeneis naucrates*	3	775 (435–1,320)	,,

The structural resemblance between the swim-bladder and choroid retia suggested to us that their functions might be similar, and that the choroid rete mirabile–pigment cell layer complex might serve to create a large tension of oxygen behind the retina, which would provide a pressure head for diffusion to supply the vigorous oxygen demand of the relatively avascular retina. A small oxygen-sensing polarographic electrode (Beckman Spinco Division, No. 161–950 oxygen micro-electrode) was introduced into the front of the eye and passed between the lens and iris into the vitreous humour. The measurements were made on living fish submerged in sea water with a current of sea water passing over the gills. We believe that most of our measurements were made with the sensing tip of the electrode in the vitreous humor immediately in front of the retina. Very large tensions of oxygen were indeed found (Table 1). A variety of bottom-living marine fishes, sea robin, fluke, puffer and scup, and two pelagic fishes, jack and menhaden, exhibited oxygen tensions in the vitreous humor ranging from 250 to 800 mm. mercury. These tensions may be compared with the oxygen tension of arterial blood, which is presumably close to that of sea water, 160 mm. mercury. Two species, the bluefish and remora, deserve special comment. The choroid rete of these fast-swimming, sight-dependent, predaceous animals is so greatly developed that the rear of the eyeball bulges and the rete is rotated 90° from its usual symmetric position. The bluefish tolerates handling poorly, and measurements of the oxygen tension in the eye of this species were at best erratic. The remora, by contrast, is tough and resistant to laboratory insult. Three fish were captured. In two of these we measured oxygen tensions of 1,000 and 1,300 mm. mercury respectively, tensions greatly in excess of 1 atmosphere.

We were led to perform these experiments by the postulate that the choroid rete is concerned with oxygen transport. This postulate may be evaluated by comparison of several species having small retia with the already mentioned forms in which the rete is large. Two bottom-living species, sea bass and tautog, have relatively small choroid retia and exhibit oxygen tensions in the vitreous not too different from air. The goosefish and toadfish inhabit murky bottoms and find their food by chemical or tactile senses. The choroid rete of these species is minute, and the oxygen tension in the vitreous humour is correspondingly low, scarcely exceeding the probable venous oxygen tension. Two teleosts, eel and conger, lack choroid retia. The organ is also absent in elasmobranchs of which we have examined three representatives—smooth dogfish, skate, and sting ray. All five species, which lack retia, exhibit very low oxygen tensions at the retina, less than 30 mm. mercury. The observed correlation between the measured oxygen tension and the extent to which the choroid rete mirabile is developed suggests that the rete plays an essential part in establishing the large oxygen tension at the retina.

Two instances of transport of oxygen gas against a large concentration difference are now known. In each instance a rete mirabile is presumed to be the seat of transport, the gas gland or pigment epithelium playing an unknown but subsidiary part. The interesting question to be resolved is whether oxygen transport is a general property of cells magnified to easily measurable proportions by the rete mirabile acting as a counter-current multiplier[16], or is some special property of the rete-gland complex.

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Effect of Concentration of Dissolved Oxygen on Survival of Trout and Roach in Lethal Temperatures

IN the freshwater environment high temperature may be associated with low concentrations of dissolved oxygen, particularly in rivers receiving both heated and oxidizable effluents. Since the toxicity of some poisons is increased by lowering the dissolved oxygen concentration[1] and asphyxial levels of dissolved oxygen are increased by an increase in temperature[2], it is likely that lethal temperatures will be reduced by lowering the concentration of dissolved oxygen. Hitherto the lethal effects of high temperature on fish have been measured in the laboratory at concentrations of dissolved oxygen close to the air saturation value[3,4]; the present work was undertaken to evaluate the effect of low concentrations on such measurements.

Rainbow trout (*Salmo gairdnerii*) (length 4·5 cm. ± 0·4 cm.) and roach (*Rutilus rutilus*) (length 12·3 cm. ± 1·8 cm.) were taken from water at 16° C. in May and acclimatized to 18° ± 0·5° C. for at least three days before being tested. They were not acclimatized to low dissolved oxygen because a preliminary test showed that the survival-time of trout kept in a concentration of 4 p.p.m. and tested at 27° C. was similar (93 min.) to that (102 min.) of fish acclimatized to double this concentration.

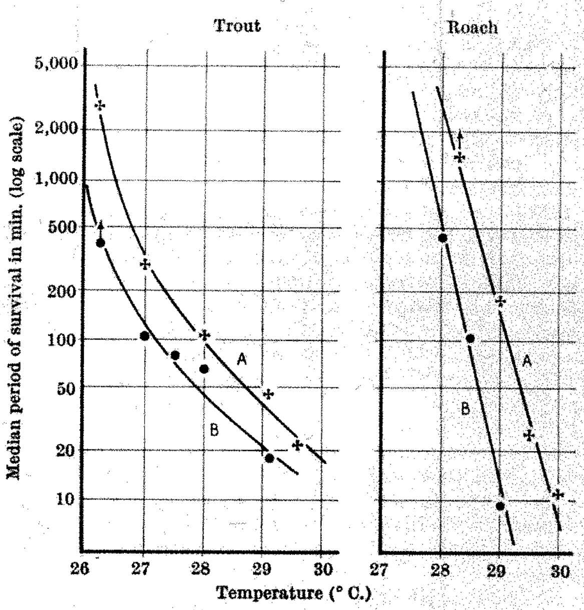

Fig. 1. Survival of trout and roach in lethal temperatures. *A*, Dissolved oxygen concentration, 7·4 p.p.m.; *B*, dissolved oxygen concentration, 3·8 p.p.m.

Tests were carried out in 40-l. aquaria supplied with 1 litre/min. of water at either 7·4 or 3·8 p.p.m. dissolved oxygen, which was obtained by mixing appropriate proportions of air-saturated and nitrogen-saturated water. Trout in batches of ten and roach in batches of five were transferred to the aquaria and the concentration of dissolved oxygen fell by as much as 0·5 p.p.m. and tended to rise again as the fish died. Test temperatures were controlled within 0·1° C. The times of survival of the fish were observed until they had all died or until the tests ended after 24 hr. The individual periods of survival were distributed approximately log normally, and since there was an incomplete kill in some tests the median period of survival was estimated graphically[5].

The relation between these medians and test temperature is shown for both levels of dissolved oxygen in Fig. 1. The survival time of trout and roach is lower at the low concentrations of dissolved oxygen than in the high ones, and though the difference is greater for roach than for trout the amount by which the temperature corresponding to a given period of survival is reduced by lowering the dissolved oxygen is similar for both species, amounting to 0·5° C. at 1,000 min. A fuller account of this and similar work being carried out will be given elsewhere.

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B_{12} Coenzyme Content of the Nodules from Legumes, Alder and of *Rhizobium meliloti*

It has been established[1] that cobalt is an essential element for the symbiotic growth of soybean plants and *Rhizobium japonicum* in a purified solution lacking combined nitrogen. It has been shown also that cobalt is needed for alfalfa inoculated with *Rhizobium meliloti*[2]. Legumes supplied with adequate fixed nitrogen do not respond to additions of cobalt[1,2]; but *R. japonicum*, *R. meliloti*, *R. leguminosarum*, *R. trifolii*, and *R. phaseoli* cultured in a purified medium containing both ammonium and nitrate nitrogen failed to grow normally without cobalt[3,4]. The vitamin B_{12} content of root nodules from soybean plants is positively correlated with the cobalt supply in the nutrient medium[1], suggesting that an important role of this element in nodule metabolism is related to that of the vitamin.

Recently, Barker *et al.*[5,6] have isolated a series of cobamide coenzymes which function in the conversion of glutamate to β-methyl aspartate, in the isomerization of methylmalonyl coenzyme *A* to succinyl coenzyme *A* (ref. 7) and in the conversion of 1,2-propanediol to propionaldehyde[8]. Since *Rhizobium* species and symbiotically grown legumes require cobalt for growth it is of obvious interest to determine whether or not these organisms contain the cobamide coenzymes.

Seedlings of alfalfa (*Medicago sativa*, variety Dupuis), soybeans (*Glycine max*, variety Chippewa), peas (*Pisum sativum*, variety American Wonder) and red clover (*Trifolium pratense*, common variety), inoculated with appropriate *Rhizobium* species, were grown in a greenhouse during the summer and autumn in 2-gallon vessels filled with 'Perlite'. Each vessel was supplied every other day with about 500 ml. of a nitrogen-free nutrient solution identical with that described previously[1] with the exception that cobalt, in the form of cobalt chloride, was added at a concentration of 0·05 p.p.m. Cultures were flushed with water on those days when the nutrient solution was not applied. When plants had reached the stages of growth indicated in Table 1, nodules were removed, washed with distilled water and stored at −15° C. until they were analysed.

The B_{12} coenzyme contents of nodules from legumes and alder and of cells of *R. meliloti* were determined by the enzyme assay method of Barker *et al.*[5]. For this procedure, 3–5 gm. of nodules were macerated in 3 vol. of 0·01 *M* potassium phosphate buffer at *p*H 6·5. The coarse material was removed by squeezing through cheese cloth. The liquid was placed in a boiling water bath for 5 min., rapidly cooled in ice and centrifuged at 16,000*g* for 10 min. The supernatant liquid was shielded from light and was used for coenzyme assays.

An extract of *R. meliloti* was prepared from 1 gm. of fresh cells obtained from a pure culture grown in a medium containing the following per litre: K_2HPO_4, 1·00 gm.; KH_2PO_4, 1·00 gm.; $MgSO_4.7H_2O$, 0·36 gm.; $CaSO_4.2H_2O$, 0·13 gm.; $FeCl_3$, 4·00 mgm.; $CoCl_2.6H_2O$, 20·19 mgm.; mannitol, 3·00 gm.; and yeast extract, 1·00 gm. The cells were suspended in 10 ml. of 0·01 *M* potassium phosphate buffer, *p*H 6·5, and prepared by the procedure used for nodules. The glutamate isomerase preparation, which was completely dependent on B_{12} coenzyme for activity, was prepared from *Clostridium tetanomorphum* by the procedure outlined by Barker *et al.*[5]. The coenzyme contents of the various extracts (Table 1) were determined by the glutamate isomerase assay[5] using adenylcobamide coenzyme isolated from *Clostridium tetanomorphum* as the standard. Concentrations of the adenylcobamide coenzyme were calculated, utilizing the molar extinction coefficient of 8·65 × 10^6 cm.^{2} per mole at 458 mμ given by Barker *et al.*[6]. The results in Table 1 are expressed as mμmoles of the dimethylbenzimidazolylcobamide coenzyme. The relative micromolar activity data supplied by Barker *et al.*[6] were used to convert coenzyme concentrations in terms of adenylcobamide coenzyme to concentrations of the dimethylbenzimidazolylcobamide coenzyme.

Table 1. VITAMIN B_{12} COENZYME CONTENT OF THE NODULES FROM LEGUMES AND ALDER AND FROM *Rhizobium meliloti*

Species*	Age	Coenzyme content per gm. of fresh tissue (mμmoles)
Medicago sativa	78 days	347
Glycine max	39 days	231
Glycine max	73 days	116
Pisum sativum	26 days	219
Pisum sativum	45 days	103
Trifolium pratense	81 days	214
Alnus oregona	2 years	174
Rhizobium meliloti	36 hours	290

* Alfalfa plants (age 78 days), soybean plants (age 39 days), peas (age 26 days) and red clover (age 81 days) exhibited initial evidence of flower bud formation. Soybean plants (age 73 days) and peas (age 45 days) showed fully developed flowers. The alder plant, estimated to be 2 years old, was obtained near Corvallis, Oregon, in November 1961 by Mr. Michael Newton.

It is apparent from the results in Table 1 that the concentration of coenzymes in nodules of soybeans and peas during the early flower-bud stage of growth was nearly twice that of nodules from plants that had fully flowered. Nodules of alfalfa contained the highest concentration of B_{12} coenzyme. These results are consistent with Burton and Lochhead's[9] observation that the vitamin B_{12} content of *R. meliloti* was appreciably greater than that of other species of *Rhizobium*. The identification of B_{12} coenzyme in the nodules of alder is of particular interest since it suggests a role of cobalt in a non-leguminous species growing in symbiosis with an actinomycete.

Further investigations were conducted with pure cultures of *Rhizobium meliloti* to determine the identity of the cobamide coenzyme in this organism. The purification and isolation procedures of Barker *et al.*[5,6] were used. The absorption spectrum of a purified preparation isolated from 300 gm. of fresh cells showed peaks at 522–525 mμ, at 375 mμ and an inflexion at 288 mμ. Samples of the coenzyme isolated from *R. meliloti* were chromatographed using secondary butanol, acetic acid and water as the solvents[6]. The R_F values obtained were identical with those of an authentic sample of 5,6-dimethylbenzimidazolylcobamide coenzyme kindly supplied by Dr. H. A. Barker. From the spectral and chromatographic characteristics and from the micromolar activity of the coenzyme in relation to that of authentic cobamide coenzymes in the glutamate isomerase reaction[6] it is concluded that the compound isolated from *R. meliloti* is the 5,6-dimethylbenzimidazolylcobamide derivative. Further experiments are in progress designed to isolate the B_{12} coenzymes from various species of *Rhizobium* and from nodules of symbiotic organisms. Other investigations in progress in our laboratory (De Hertogh, A., personal communication) have shown the presence of methylmalonyl coenzyme A isomerase in extracts of *R. meliloti*.

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FORESTRY

Control of *Keithia thujina* Durand by Cycloheximide and Derivatives

Infection of seedlings and transplants of Western red cedar (*Thuja plicata* Lamb.) by *Keithia thujina* Durand frequently causes heavy losses in forest nurseries in Britain. In extensive field control trials carried out over the past ten years, the fungus has displayed a high resistance to a wide range of fungicides. Only copper fungicides showed any effectiveness, but the low level of control did not warrant their general field use. At the present time, *Thuja* is raised by the Forestry Commission by rotation sowing in a small number of nurseries which are isolated from possible sources of the disease. However, effective chemical control is still highly desirable, as avoidance of the disease by rotation sowing requires several economic forest nursery units which are not available to many private woodland owners.

Recent experiments in two Forestry Commission research nurseries, at Alice Holt, Surrey, and Wareham, Dorset, have demonstrated a high level of control of *Keithia* infection on *Thuja* transplants by cycloheximide and three derivatives. The cycloheximide used was in the form of commercial 'Actidione *BR*' concentrate, but the oxime, semicarbazone and acetate derivatives were in crystalline form. 'Actidione' was applied at concentrations of 150 p.p.m. and 50 p.p.m. in a 5 per cent white oil emulsion. The crystalline derivatives were dissolved in a small quantity of dimethylacetamide and made up to concentrations of 150 p.p.m. and 50 p.p.m. in water. 0·02 per cent 'Ethylan C.P.' wetting agent was added to all spray solutions.

In November 1960 at Wareham in a replicated layout of 2 sq. yd. treatment plots of badly infected two-year old transplants, plots were completely sprayed with each material at both concentrations. In March 1961, the same plots were sub-divided, one square yard receiving a further spray treatment, and the other left unsprayed. All solutions were applied at a rate of 300 ml./sq. yd. The experiment was assessed in May 1961, when plants in the unsprayed control plots were showing the late pustule stage of apothecial development on the leaves. Owing to the debilitated condition of the plants, an accurate numerical assessment was not possible, but it was clear that in the half-plots which had received only one spray treatment in November, the level of fresh infection was similar to that in the untreated controls. In the half-plots treated again in March, a high level of control was achieved by all materials at both concentrations. None of the derivatives produced symptoms of phytotoxicity on the transplants, but 'Actidione' at 150 p.p.m. and 50 p.p.m. produced pronounced shoot tip burn symptoms, and at 150 p.p.m. stunting and deformation of unburned parts of shoots were regularly observed.

The absence of control resulting from the November treatment suggests very low, if any, systemic activity of cycloheximide and the derivatives, but the success of the March application indicates excellent protective action against infection produced by the germination, in late March and early April, of ascospores which had overwintered on the surface of the foliage[1].

These experiments are being followed by nursery trials with a range of commercial cycloheximide fungicides at various concentrations, applied at different times.

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CYTOLOGY

Colchicine-Type Metaphases in HeLa Carcinoma Cells

Colchicine-like metaphases have been reported recently[1] in human leukæmic leucocytes treated with the dipotassium salt of (ethylene dinitrilo) tetracetic acid and sodium citrate. During a study of the effects of chelating agents on cultured HeLa cells similar metaphases were observed in cultures treated with phosphate-citrate buffer.

Actively growing cells cultured on coverslips in roller-tubes were treated with a preparation of McIlvaine's buffer in isotonic glucose solution. The buffer solution was used at *p*H 7·4. The final concentrations of disodium hydrogen phosphate and citric acid were 2·841 and 0·210 mgm./ml., respectively, in 5·25 per cent aqueous glucose solution. After treatment the cells were thoroughly washed with buffered saline solution and supplied with normal growth medium. This medium was essentially that described by Pereira and Kelly[2].

An abundance of colchicine-like metaphases was found in the cultures 48 hr. after treatment. By this time the cultures had recovered from the acute effects of the experimental treatment. Cells in division at the time of treatment were killed by the buffer solution. Cells with colchicine-like metaphases were enlarged and flattened. They tended to have vacuolated peripheral cytoplasm. Colchicine itself causes bubbling at surfaces of cultured fibroblasts[3]. The chromosomes were usually seen grouped in homologous pairs, well spread and not radially orientated. They were longer and thinner than normal HeLa metaphase chromosomes, stained palely but were structurally intact. Mitotic spindles were not distinguishable in preparations stained with hæmatoxylin and eosin. Most of the metaphases were of the colchicine-type; but other types of mitotic aberrations, including chromosome stickiness and fusion into pyknotic masses, were present. A small percentage of mitoses were normal.

With continued cultivation of the treated cultures the incidence of colchicine-like metaphases decreased while that of other forms of degenerated mitoses increased. In these older cultures the colchicine-like metaphases were highly polyploid and the cells commonly died without entering anaphase. Viable giant interphase cells appeared concurrently with the highly polyploid metaphases and likewise later greatly decreased in number.

Kinlough and Robson[1] obtained colchicine-like metaphases immediately after treatment with the anticoagulants used. In the present experiments the effect was delayed and persisted until the affected cells were eliminated. However, a basic similarity in the mechanisms of the effects is probable. Heparin[4] and another anticoagulant, janus green *B* (ref. 5), have been reported to have a colchicine-like effect on dividing cells. Mazia and Dan[6] isolated the mitotic apparatus of colchicine-treated echinoderm eggs as an amorphous gel, and it was inferred[7] that colchicine affected secondary bonds between individual spindle fibres. These experimental resu'ts are in agreement with Heilbrunn's[4] suggestion that the formation of the mitotic apparatus is essentially dependent on the presence of calcium ions. The delayed effect of phosphate-citrate buffer appears to require a more complex explanation than that for an immediate response during treatment.

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Micropinocytosis and Rhopheocytosis

In a recent paper, Brandt and Pappas[1] raise a point of terminology the answer to which appears timely when one considers the present condition of ultrastructural cellular biology.

In his classical work, W. Lewis[2] described a microscopical process of cell absorption different from phagocytosis. As regards this last phenomenon, the first operating factor is known to be adhesion of the cell to the particle it is going to absorb. Once adhesion is achieved and the cell fixed to its prey, the cytoplasm flows, so to say, around it, closes on it and finally incorporates it.

In pinocytosis, the very essence of the mechanism is different. A cytoplasmic veil from the cell, and this, by falling back on the surface of the cell, encloses a droplet from the environment, incorporating it into the cytoplasm in the form of a vacuole (Fig. 1, bottom).

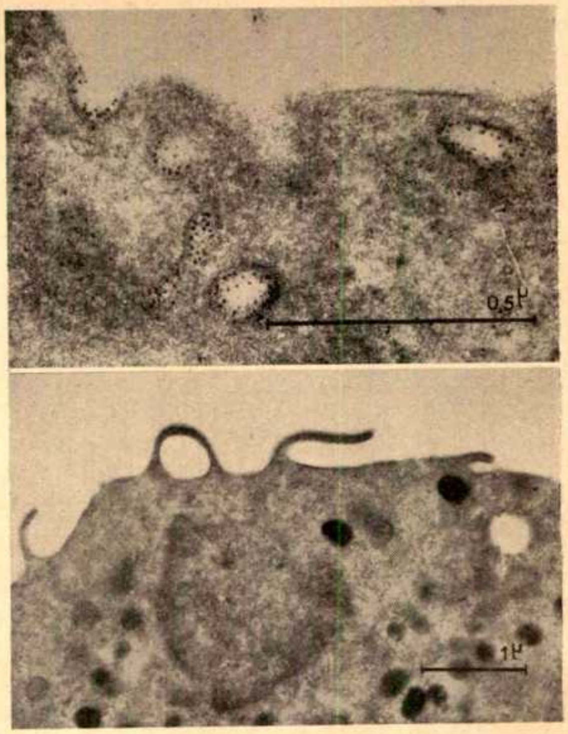

Fig. 1. Above: an example of rhopheocytosis (molecules of ferritin penetrate an erythroblast). Below: an example of micropinocytosis (a polymorphonuclear leucocyte)

Thus pinocytosis, the action of drinking (*pineo*, I drink), and phagocytosis (*phago*, I eat) are differentiated. These facts are well known. While investigating the erythroblasts with the electron microscope, we observed[3,4] the absorption of ferritin molecules fixed to the cellular membrane; we also found them in some of the intra-microscopical depressions of the membrane and again in intra-cytoplasmic vacuoles of the same size as the depressions (Fig. 1, top).

These findings obtained with electron microscopy were obviously of a static order. They were in agreement with those of Palade[5] concerning invaginations of the cellular surface. It therefore seemed reasonable to think of an infra-microscopical absorption mechanism of substances from the milieu. The cell membrane invaginates under the influence of these fixed substances.

By pinching of its edges this invagination becomes a vacuole which sinks into the cytoplasm and becomes part of it with the particles that have led to its formation. In this process, no folding back of a veil takes place and is therefore different from pinocytosis. The phenomenon is termed 'micropinocytosis' and is identical to pinocytosis but on a smaller scale, and only detectable by electron microscopy. For that phenomenon in which no folding back of veils is in action, but in which the cell seems to aspirate the surrounding milieu, we have proposed the name of 'rhopheocytosis' (*rhopheo*, I aspirate).

The phenomenon is of the type of that described by Bennett[6] as 'membrane flow'. As this term is not easy to translate, 'rhopheocytosis' might be preferable.

Akin to rhopheocytosis is the incorporation of hæmoglobin by intra-erythrocytic parasites recently described as phagothrophy[7].

Phagocytosis, pinocytosis, phagothrophy and rhopheocytosis are probably concerned with the same general mechanism. However, in detail, these phenomena are certainly different and we therefore consider it desirable to give them different names.

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GENETICS

Immunogenetic Confirmation of the Origin of a Presumed Natural Hybrid between Goldfinch and Greenfinch

A PRESUMED natural goldfinch × greenfinch (*Carduelis carduelis* × *C. chloris*[1]) hybrid was trapped near the town of Parma in September 1960. This bird is the second one captured in these surroundings, the first having been trapped in 1951[2]. The bird answers quite well the description of the goldfinch × greenfinch male hybrids reared in captivity[3]: it has the shape, the size and the heavy, whitish bill of the greenfinch, a small orange mask like the scarlet one of the goldfinch, a yellow wing-bar as in the goldfinch and yellow on the external vane of the primaries as in both of the supposed parental species.

Although, according to Gray[3], presumed natural hybrids have sometimes been reported, absolute certainty of their genetic origin has never been attained. With the aim of identifying beyond doubt the origin of the actual presumed hybrid, an immunological analysis of its red-cell antigens has been made. The inter-relationships of the red-cell antigens of goldfinch, greenfinch and of many other carduelines are well known[4]. Particularly, goldfinch and greenfinch have common antigens and specific ones. According to many researches on F_1 interspecies hybrids, these animals possess both common and specific antigens of the parental species[5].

The red cells of the presumed hybrid were tested by means of anti-goldfinch and anti-greenfinch sera absorbed reciprocally by the red cells of greenfinch and goldfinch, and by the red cells of the presumed hybrid. The immunological technique has already been described[6].

Table 1

Antiserum	Absorbing cells	Test cells		
		Goldfinch	Greenfinch	Hybrid
Goldfinch	Greenfinch	+	−	+
	Hybrid	−	−	−
Greenfinch	Goldfinch	−	+	+
	Hybrid	−	−	−

+, Agglutination; −, no agglutination.

As can be seen in Table 1, the presumed hybrid possesses in its red cells the specific antigens of the goldfinch (shown by anti-goldfinch serum absorbed by greenfinch red cells) and of the greenfinch (made evident by anti-greenfinch serum absorbed by goldfinch red cells). Besides, the red cells of the presumed hybrid contain the antigens common to goldfinch and greenfinch (clearly shown using anti-goldfinch and anti-greenfinch sera absorbed by the cells of the presumed hybrid).

Finally, the presence together of both specific and common antigens of the goldfinch and of the greenfinch in the red cells of this bird clearly indicates that it is a true F_1 hybrid between these two species, this being the unique genetic combination in which all these antigens can be found together.

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A New Eye Colour Mutation in *Calliphora erythrocephala* Meig

IN the summer of 1960 a variant eye colour appeared spontaneously in a culture of *Calliphora erythrocephala* which was being kept in the Zoological Institute of the Free University of Berlin. Dr. Peters, a member of the Institute, bred a pure strain from the few mutant individuals and gave us some of their progeny for research.

Until now only one mutation in the eye colour of *Calliphora erythrocephala* had been observed. It was described by Tate[1] and called 'white' (*w*). In the *w*-mutation the eyes of the females alone are a light yellow-orange. This character is both sex-linked and sex-limited and occurs only in double recessive females. The eyes of the new mutant are pure white and are thus very different from the mutation described by Tate, which is also being kept here at the Zoological Institute of Munich. The new character occurs equally in both sexes; it is recessive and independent of the gene *w*. Because of their pure white colour it is suggested here that the name 'chalky' be given to this mutation and that the corresponding gene should be designated by the symbol *c*.

In order to find the source of the white coloration the eyes of the wild-type, of mutant *w* and of mutant *c* females were examined by selective extraction and spectrophotometry for the presence of the main groups of insect eye pigments, the ommochromes and the pteridines.

The ommochromes were extracted quantitatively from the isolated eyes by means of a mixture of methanol and hydrochloric acid[2]. In the case of the wild-type the colour of the extract was red; whereas in both the mutant eyes it manifested almost no absorption above 300 mμ. The difference spectrum, calculated from the absorption curves of the red and the colourless extracts, was compared with the spectra of several different pigments and agreed almost completely with the absorption curve of dihydroxanthommatin. Xanthommatin, the only ommochrome to be found in the eyes of Diptera[3], occurs mainly in the red dihydro-form in living flies. From the difference spectrum it can therefore be concluded that there are no ommochromes in the eyes of the new mutant *c*, just as there was practically none in the mutant *w* under normal breeding conditions[1].

A second mixture, which was prepared from 40 per cent methanol, 1 per cent acetic acid and 59 per cent distilled water, removes pteridines totally and almost exclusively from the tissue. The extracts from the eyes of mutants *w* and *c* had different absorption curves. Between 400 and 430 mμ there was an absorption maximum for the *w*-eyes, whereas there was again no extinction to be found for *c*-eyes above 300 mμ. The difference spectrum for the absorption curves of mutant *w* and *c* shows the main characteristics of the pteridines and is very similar to that of 'sepiapterin'[4]. This light-sensitive derivative of pteridine-6-carboxylic acid is the main pteridine to be found in the eye of *Calliphora*. The pteridine content of mutant *w* does not differ from that of the normal form[5]. The mutant *c*, on the other hand, differs from the wild-type in respect to these substances as well. For this reason a comparative chromatographic and fluorometric test was made (Patat, U., unpublished results), with the result that no fluorescent pigments were to be found in the eyes of mutant *c*. Thus the compound eyes of mutant *c* adults contain none of the pigments characteristic of eyes of insects. The white colour is only of physical origin; most likely it comes from the reflexion of light against the air-filled tracheoles and the background of the eye.

The behaviour of mutant *c* is in complete agreement with the wild-type flies. Although mutant *w* has manifested a diminution of its vitality in several respects, in mutant *c* no such diminution could as yet be found. Through observation of its behaviour, especially in eating and mating, and of the presence of positive phototaxis, one can conclude that the visual sense of these flies is normally developed. The eyes yield the same retinograms as Autrum[6] found in the wild-type and in mutant *w*.

Thus this new mutation in eye colour offers new possibilities for analysis of the significance of pigments in the photoreceptive function of the compound eye. Such investigations are now being conducted[7].

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SOIL SCIENCE

Nutrient Enrichment of the Coconut Soils of the Humid Kerala Coast through Monsoon Precipitations

ASSESSMENTS of the agricultural value of the mineral nutrient contents of rain water have been made and recorded in several different geographical and climatic zones of the world[1-12]. Some regions appear to receive appreciable replenishments from the precipitations while in others the amounts so added are comparatively small and of doubtful significance for the nutrient economy of the arable soils of the region. In general, in the equatorial regions nitrogen, including both the inorganic and organic forms, is the element reported as received in appreciable quantities[1,11], although Jones[2] reported rather high figures for potassium (32·8 lb./acre) and sodium (53·8 lb./acre) as well, from Nigeria. The information so far available, however, pertains only to comparatively low and medium rainfall regions.

A preliminary investigation of the nutrient content of rain water was made at this Station, which is in the main coconut belt of the country. The nutritional aspects of the coconut palm, which is purely a rain-fed crop, and the soil fertility factors in relation to palm growth form the main subject of different investigations here. The average annual rainfall of this locality is about 3,750 mm., of which about 75 per cent is usually received during the south-west monsoon, which lasts from about the end of May to the beginning of September, and during the north-east monsoon, which begins during late September and continues up to January. Hence this investigation was confined to the maximum rainfall period. Nitrogen, phosphorus and sodium were determined in the rain water throughout the monsoon period of June–November. The estimate of the potassium content is not included in this communication as complete results are not available. The rain water was collected in the open, care being taken to avoid all possible contaminations by terrestrial dust containing plant or animal debris, atmospheric pollen, etc., during sampling. The analysis was carried out every day in a composite sample and when the rain was scarce it was done after sufficient quantity was collected, the sample under such conditions being preserved by the addition of a little toluene. So far as was practicable the rain water was quite fresh for analysis after collection. The different nutrients were estimated by the Association of Official Agricultural Chemists methods. The results are summarized in Table 1.

Table 1. AMOUNTS OF NUTRIENTS IN THE RAIN-WATER AT KASARAGOD. TOTAL QUANTITY OF RAINFALL RECEIVED DURING JUNE 1–NOVEMBER 30, 1961 = 2,919·7 MM.

Nutrients	p.p.m.	kgm./hectare
Nitrogen		
Ammoniacal	0·44	2·130
Nitrite	0·016	0·076
Nitrate	0·03	0·155
Organic	5·90	27·992
Total (Kjeldahl) nitrogen	6·20	29·779
Phosphorus (P)	0·98	4·748
Sodium (Na_2O)	1·70	8·291

These figures show that the rains do bring down some quantities of nitrogen (mostly in the organic form) and sodium to the soils; the phosphorus content is rather insignificant.

These nutrient materials are received through extremely heavy downpour and a portion of this is likely to be lost through leaching and also surface run off. It is, therefore, not possible to say to what extent these nutrients, particularly the nitrogen, are retained in the soil and utilized by the palms. Indeed, the occurrence of conditions like the foliar yellowing usually observed in the palms during the season actually indicate either nitrogen deficiency in the soil and palms or the impaired utilization of the necessary nutrients due to other conditions. However, a further detailed study of these aspects is being undertaken to obtain a definite idea of the part played by rain in the nutrient economy, particularly of nitrogen, of the coconut soils of rather heavy rainfall areas.

We thank Mr. M. M. Krishna Marar for his advice.

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VIROLOGY

Effect of X-Rays on Plaque-forming Ability of Vesicular Stomatitis Virus

The animal virus vesicular stomatitis can be easily propagated in tissue cultures and quantitatively assayed by the plaque-count technique using tissue cell monolayers[1,2]. In preliminary experiments dealing with X-ray inactivation of vesicular stomatitis virus, several morphological plaque-type variants were observed among the irradiated survivors. This observation suggested that vesicular stomatitis virus offers additional material for radiobiological and genetic studies on animal viruses and dictated a quantitative study of the plaque-forming ability of vesicular stomatitis virus when irradiated under a number of conditions suitable for the quantitative estimation and subsequent isolation of plaque-type variants.

The vesicular stomatitis virus strain used was the Ogden isolate, New Jersey antigenic type, kindly supplied by Dr. S. Madin of the Naval Biological Laboratory, University of California, Berkeley. Crude virus preparations were obtained from culture fluid of 24-hr.-old chick embryo cell monolayers[2] previously infected at a multiplicity of 0·2. The culture fluid was centrifuged at low speed to remove bulk host cell debris and then subjected to 38,000*g* at 4° C. for 100 min. The sediment, containing about 95 per cent of the virus, was washed twice in Dulbecco's phosphate-buffered saline[3] by successive centrifugations at 38,000*g*. The final pellet was suspended in phosphate-buffered saline to 10^{10} plaque-forming units/ml. and stored at $-15°$ C. Virus titres generally fell to about 1 per cent of the initial concentration over a six-week period.

The source of X-rays was a Westinghouse Duocondex deep-therapy machine operated at 200 kV., 20 m.amp., 1 mm. aluminium inherent filtration, with an average dose of 1,180 r./min. at the surface of the irradiated suspension. For irradiation, virus samples were thawed (37° C.) and diluted to about 10^6 plaque-forming units/ml. in phosphate-buffered saline, in 1, 10, 15 and 25 per cent 'Difco' nutrient broth or in inactivated 20 per cent lamb serum (final *p*H of each medium, 6·9–7·0). 3·5 ml. samples were irradiated in glass dishes, 2 cm. deep × 3 cm. diameter, centred in the path of the X-ray beam and mounted on a platform which rotated horizontally to the beam during exposure. Except where noted, control and irradiated samples were diluted in the same medium in which they were irradiated, and aliquots of appropriate dilutions seeded in triplicate on 24–30-hr.-old chick embryo cell monolayers grown in prescription bottles[4]. Following 1 hr. for virus adsorption[2], monolayers were overlaid with nutrient agar containing 0·001 per cent neutral red. Plaque counts were made after bottles had incubated at 37° C. for 36–40 hr.

Fig. 1 depicts the effects of X-rays on plaque-forming ability of virus (*a*) irradiated and diluted in phosphate-buffered saline (curve 1); (*b*) irradiated in phosphate-buffered saline and diluted in modified Hanks's solution[5], containing 0·5 per cent lactalbumin; (*c*) irradiated and diluted in 1 and 10 per cent nutrient broth (curves 3 and 4, respectively); (*d*) irradiated and diluted in 20 per cent lamb serum (curve 5). The inactivation of virus in buffer can be attributed to both direct and indirect radiation effects, the latter of which was demonstrated by the inactivation of non-irradiated virus incubated in X-irradiated buffer (47,200 r.) (Fig. 2). A comparison of curves 1 and 2 (Fig. 1) shows that virus irradiated in buffer and diluted in buffer is more sensitive than equivalent virus samples diluted in buffer containing lactalbumin. This post-radiation effect is qualitatively similar to that obtained with bacteriophage[6] and can be accounted for on the basis of long-lived indirect effects which are partially negated by the addition of lactalbumin to the buffer.

Lamb serum and 10 per cent nutrient broth exhibited a pronounced protective effect against X-ray inactivation of virus (curves 4 and 5). Nutrient broth at concentrations of 15 and 25 per cent, although affording protection comparable with that obtained with 10 per cent broth, inactivated control virus rapidly (50 per cent survival) after extended incubation (80 min.). Nutrient broth at a concentration of 1 per cent did not inactivate non-irradiated virus but exhibited only partial protection against X-ray inactivation (curve 3). Thus, either 10 per cent nutrient broth or 20 per cent lamb serum provides maximum protection for the study of direct effects,

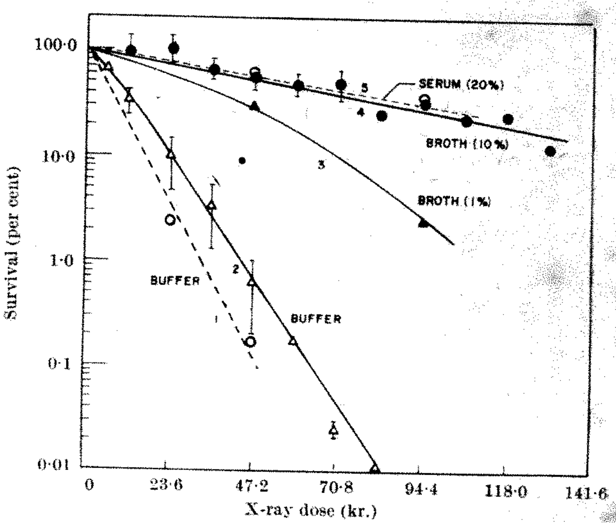

Fig. 1. Effect of X-rays on plaque-forming ability of vesicular stomatitis virus: (1) irradiated and diluted in buffer; (2) irradiated in buffer and diluted in modified Hanks's solution containing 0·5 per cent lactalbumin; (3) irradiated and diluted in 1 per cent nutrient broth; (4) irradiated and diluted in 10 per cent nutrient broth; (5) irradiated and diluted in 20 per cent lamb serum

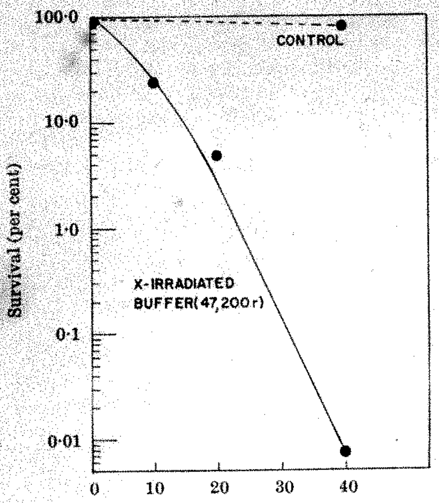

Fig. 2. Inactivation of plaque-forming ability of non-irradiated vesicular stomatitis virus suspended in X-irradiated phosphate-buffered saline

uncomplicated by inactivation of control virus samples. Utilizing the data obtained with 10 per cent nutrient broth, and assuming the inactivation rate of virus under this condition is exponential (Fig. 1, curve 4), the radiosensitive target volume of the virus was calculated using the relation $N/N_0 = \text{exp}^{-VD}$, where N/N_0 is the surviving fraction, V is the radiosensitive target volume (c.c.) and D is the dose in primary ionizations (c.c.) (ref. 7). The dose required to produce 37 per cent survival was 74,000 r. or $4{\cdot}54 \times 10^{16}$ primary ionizations (c.c.). Assuming a single ionization per radiosensitive target volume, $V = 2{\cdot}2 \times 10^{-17}$ c.c. This value is in close agreement with that calculated by Franklin[8] for a Missouri strain of vesicular stomatitis virus irradiated after adsorption. Consequently, the target volume by and large measures the radiosensitive site(s) for infectivity rather than site(s) governing adsorption. If the radiosensitive structure is considered to be a sphere, this volume corresponds to a target diameter of approximately 35 mμ or about 46 mμ by Lea's associated volume method[9], and represents about 4 per cent of the total virus volume assuming a rod of 65 mμ × 165 mμ[10]. The relation between radiosensitive target diameter and the physical virus diameter for vesicular stomatitis virus is similar to that for several large animal viruses[11] in that the former dimension is significantly smaller than the latter.

It should be noted that the plaque count for non-irradiated virus diluted in buffer is lower than that obtained when dilutions are made in broth. Furthermore, the titre of virus suspended in broth may rise on extended incubation (10–40 min.). Microscopic observation of virus samples in buffer and titres of virus samples following vigorous agitation indicate that a proportion of the virus particles forms aggregates in buffer. These aggregates disperse on standing in nutrient broth, thus accounting for the rise in titre of non-irradiated samples. Following low doses of radiation, an apparent increase in survival occurs for virus irradiated in broth if the survival ratio is calculated using the control titre D_0. This increase, however, disappears if the titre of control virus samples at each interval of radiation is used to calculate the survival ratio. If, as suggested by the available evidence, a proportion of the virus particles forms aggregates in buffer, we would predict the survival curve for virus irradiated in buffer to be initially exponential, followed by a 'tail portion' which is multi-hit in character. The failure to obtain this relation (curves 1 and 2) is interpreted to mean that virus aggregates do not serve as infectious units and that the curves depict basically inactivation of single particles.

Virus irradiated in broth was not only less sensitive to inactivation but also formed principally round plaques, while virus irradiated in buffer produced normal, 'ragged', and 'minute' plaques. These results show that irradiation in buffer may provide the most convenient means for the procurement of plaque-type variants.

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Purification and Separation of Tobacco Mosaic Virus and Southern Bean Mosaic Virus by Agar-gel Filtration

MOST procedures for the purification and concentration of viruses involve either dense packing of virus particles by sedimentation or by chemical or isoelectric precipitation. Although many viruses can be resuspended easily from pellets or precipitates, others are difficult to resuspend and to separate from contaminants following such treatment. In order to avoid this difficulty we have sought to develop virus purification and concentration procedures which: (1) are very gentle to the virus, allowing it to be retained in suspension throughout the procedures used; (2) require only limited amounts of inexpensive equipment; (3) are capable of handling large volumes; (4) are rapidly and easily accomplished.

Work which we have done on restricted diffusion through agar gels[1,2] led us to the conclusion that, if properly developed, this principle could be used for rapid purification of viruses. Polson[3] recently described the use of columns containing small agar pieces for the separation of proteins. We have examined the use of similar columns for virus purification. We have also explored the possibility of concentrating virus suspensions by ultrafiltration[4]. This communication describes: (1) the use of agar columns and ultrafiltration in the purification and concentration of tobacco mosaic virus and southern bean mosaic virus without chemical or physical 'packing' of the virus; (2) the use of agar columns for the separation of the same two viruses from an artificial mixture.

Suspensions of 'Ionagar No. 2' (Oxoid. This material is mentioned for specific identification only. No discrimination is intended and no guarantee of reliability implied), 4 and 8 per cent by weight in distilled water, are autoclaved for 30 min. at 15 lb. pressure, allowed to gel at 22° C., then chopped into small pieces and sorted by washing through sieves to obtain the fraction which will pass through a 40-mesh but not through a 60-mesh screen.

Glass cylinders are packed with a water suspension of agar chips of the pore size desired. Agar cores are allowed to pack to within 10 cm. from the top of each column and each core is covered with a filter paper disk to prevent disturbance of its surface when various solutions are applied. All columns are washed with sufficient 0·2 M sodium phosphate buffer of pH 7 to remove all traces of soluble material from the agar. The columns used in this experiment had bed dimensions of 2 cm. × 60 cm., 4·5 cm. × 60 cm., and 1·5 cm. × 100 cm.

Fresh tobacco leaves systemically infected with tobacco mosaic virus are dropped into an operating blender containing 100 ml. of buffer (0·2 M sodium dihydrogen phosphate–0·3 M sodium chloride) for each 100 gm. of tissue. Preliminary tobacco mosaic virus suspensions prepared with 0·2 M sodium dihydrogen phosphate had a brown pigment adsorbed to the virus. The pigment can be eliminated by increasing the molarity to 0·5 with sodium chloride. Juice is extracted from the pulp by pressing it through a cheesecloth bag and is then clarified by centrifuging for 30 min. at 500g.

Bean leaves systemically infected with southern bean mosaic virus are similarly ground in a blender with one volume of 0·2 M sodium phosphate–0·1 M ascorbic acid adjusted to pH 7 and one volume each of n-butanol and chloroform. The suspension obtained by squeezing the resulting emulsion through a cheesecloth bag is held overnight at 22° C. and only the upper aqueous phase is retained. Sodium chloride is added to this fraction to bring the total molarity to 0·5. The suspension is then clarified by centrifuging for 30 min. at 500g.

Clarified suspensions of each virus are layered on top of columns of 8 per cent chipped agar (150 ml. can be added to a column 4·5 cm. × 60 cm.) equilibrated with a buffer containing 0·2 M sodium dihydrogen phosphate and 0·3 M sodium chloride, adjusted to pH 6·8 by the addition of sodium hydroxide, and are washed through the columns with the same buffer. Ten ml. samples of effluent liquid are collected until the virus fraction (easily detected by its light-scattering properties) is eluted. The column is allowed to continue flowing until the coloured band of contaminants and all light-scattering material have moved out completely, when it is ready for a new sample.

As virus comes off the 8 per cent agar column it is contained in liquid of at least twice the volume of the crude juice present in the original infected tissue. We have chosen to use ultrafiltration[4] for concentration of these suspensions to between 1/10 and 1/100 of the original volume. The concentrate is clarified for 30 min. at 500g then filtered through a column of 4 per cent agar (2 cm. × 60 cm.) to eliminate a contaminant not separated from either virus by a single passage through 8 per cent agar. The final suspensions are clarified at 500g for 30 min.

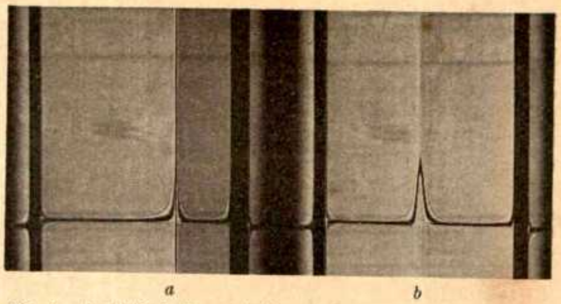

Fig. 1. Analytical ultracentrifuge pattern of virus suspensions purified with agar columns and concentrated by ultrafiltration. (*a*) Tobacco mosaic virus after 32 min. at 20,410 rev./min.; (*b*) southern bean mosaic virus after 40 min. at 20,410 rev./min.

Both tobacco mosaic virus and southern bean mosaic virus purified by the agar column procedure and concentrated by ultrafiltration were colourless and had ultra-violet spectra characteristic of the respective viruses. Each suspension revealed a single peak in the analytical ultracentrifuge (Fig. 1), and electron micrographs showed the expected particles without visible contaminants. Local lesion tests proved that the infectivity of these suspensions was equivalent to that of the same concentrations of these viruses purified by differential centrifugation.

In the development of this new purification procedure we have deliberately used two viruses which are stable and easily resuspended from precipitates or pellets. The method, however, should prove very useful for the purification of those viruses which have been more difficult to work with, and we are now examining this possibility.

Separation of tobacco mosaic virus and southern bean mosaic virus can be accomplished with 4 per cent agar columns in which the agar has a sufficiently large pore size that southern bean mosaic virus can move through, whereas the rod-shaped tobacco mosaic virus is excluded or nearly so. With these columns the tobacco mosaic virus comes off well ahead of the southern bean mosaic virus, and almost complete separation is attained on a 1·5 cm. × 100 cm. column equilibrated with the 0·2 M phosphate, 0·3 M sodium chloride buffers of pH 6·8 (Fig. 2). In this separation process the trailing of both viruses is considerable when 0·2 M phosphate is used.

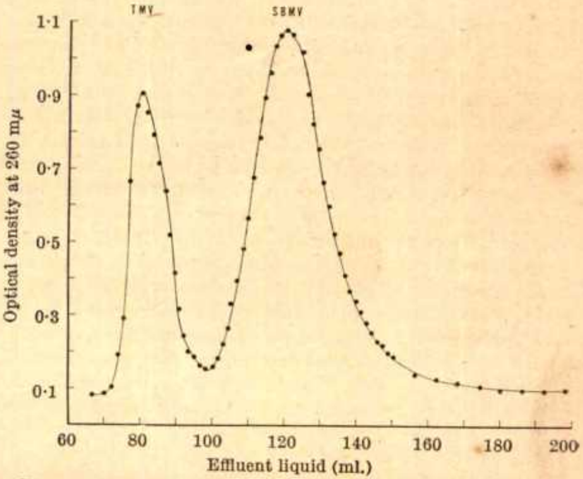

Fig. 2. 'Elution' diagram of a mixture of tobacco mosaic virus and southern bean mosaic virus from a granulated (40–60 mesh) 4 per cent agar column, 1·5 cm. × 100 cm. Ordinate: optical density at 260 mμ

Preliminary tests suggest that columns made up from bottom to top of layers of 8, 7, 6, 5, 4 and 3 per cent agar are superior to those containing only 4 or 8 per cent agar for the separation of particles of different sizes. Additional studies are under way which should give us more information regarding optimum conditions for purification and separation of viruses, cell particulates, nucleic acids, enzymes, and proteins.

We wish to thank Mr. Ralph M. Faust for his excellent assistance in the development of the purification procedure.

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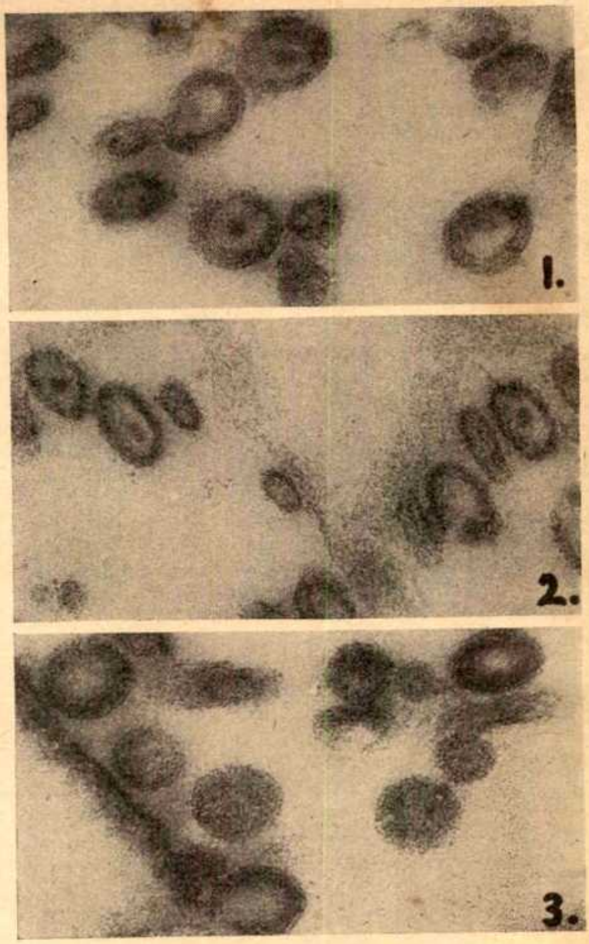
Nucleic Acid of Mature Herpes Simplex Virus: its Type and Location

THE nuclear inclusions of herpes infections have been shown, cytochemically, to contain deoxyribonucleic acid (DNA)[1], and it is further known that this material is synthesized in excess by cells soon after the herpes virus enters them[2]. However, no connexion has been established between the DNA and constituents of the agent, the nucleic acid type of which has not, as yet, been elucidated.

In order to investigate the nature of herpes virus nucleic acid, mature extracellular particles have been treated with specific nucleases, and the resulting changes caused within them have been observed in thin sections with the electron microscope, such methods having proved successful with the adeno and Rous viruses[3]. For the experiments, the *HFEM* strain of herpes was grown in HeLa cultures and the readily recognizable mature virus[4] was gathered along with the cells around which it accumulated. The cells were collected, fixed, dehydrated, embedded in *n*-butyl methacrylate, and sectioned for electron microscopy as already described[5], except that potassium permanganate was used as the fixative[6] since it permits subsequent digestion of nucleic acids by appropriate enzymes[7]. After fixation, samples of the material were placed in iced 30 per cent alcohol and brought to room temperature; the samples were then divided into three groups before continuing with dehydration and embedding. One group was incubated for 2 hr. at 37° C. in 30 per cent alcohol containing 1 mgm./ml. ribonuclease at *p*H 7, a second group was similarly incubated in the same medium containing 1 mgm./ml. deoxyribonuclease together with magnesium chloride (0·003 *M*), and a third group was incubated in the medium containing a similar concentration of this salt but no enzyme.

On examination, the control preparations which had been in the enzyme-free medium all contained numerous extra-cellular particles with well-marked nucleoids (Fig. 1), while the particles in material exposed to ribonuclease also retained these dense structures (Fig. 2). Particles which had been subjected to the action of deoxyribonuclease were quite unlike those of the other groups; their nucleoids were digested, leaving an empty space in the central area (Fig. 3).



Figs. 1–3. Electron micrographs of thin sections cut through permanganate-fixed mature herpes simplex virus particles lying between microvilli close to infected HeLa cells. The particles were treated in various ways before embedding in methacrylate. (× 80,000).

Fig. 1. Control preparation incubated in enzyme-free 30 per cent alcohol for 2 hr. at 37° C. The dense viral nucleoids are present.

Fig. 2. Preparation incubated as the foregoing but with ribonuclease (1 mgm./ml. at *p*H 7) in the alcohol; here, too, the nucleoids are intact, despite some compression of the particles as a result of sectioning

Fig. 3. Particles in a preparation which was incubated with deoxyribonuclease (1 mgm./ml.) and 0·003 *M* magnesium chloride in the alcoholic medium. The nucleoids have all been removed

This finding establishes that herpes virus contains nucleic acid of deoxyribose type and demonstrates that this material is localized, in mature particles, within the viral nucleoid. A full account of these experiments has been reported elsewhere[8].

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FORTHCOMING EVENTS

(*Meeting marked with an asterisk * is open to the public*)

Monday, April 9

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. T. Kendall: "The Impact of Epitaxial Technique of Semiconductor Devices".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Major E. J. E. Mills: "Two Journeys in Sawt Kohistan".

Monday, April 9—Friday, April 13

International Society for Fat Research (at the Imperial College of Science and Technology, London, S.W.7)—Sixth I.S.F. Congress.

Tuesday, April 10

Society of Chemical Industry, Industrial Water and Effluents Group (at 14 Belgrave Square, London, S.W.1), at 10 a.m.—Symposium on "Re-use of Water in Industry".

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Education or Technical Instruction ?"

Royal Society of Medicine, Psychiatry Section (at 1 Wimpole Street, London, W.1), at 8 p.m.—Prof. O. Ødegaard (Norway): "Psychiatric Epidemiology".

Tuesday, April 10—Thursday, April 12

Faraday Society (at the University, Cambridge)—General Discussion on "Inelastic Collisions of Atoms and Simple Molecules".

Wednesday, April 11

Laboratory Animals Centre (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 9.30 a.m.—Symposium on "The Environment of Laboratory Animals". Chairman: Dr. L. Harrison Matthews, F.R.S.

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. E. H. Bruce-Clayton and Mr. D. B. Clemow: "Long Range V.H.F. Air/Ground Communications".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Introduced Discussion on "Random Vibration Testing".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. F. M. Lea: "Development in Building Materials—Prospect for the Future".

Thursday, April 12

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. R. Couteaux: "The Differentiation of Synaptic Areas" (Special Review Lecture).

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. Th. Dobzhansky (Columbia University): "Species in 'Drosophila'" (Hooker Lecture).

Eugenics Society (at 69 Eccleston Square, London, S.W.1), at 5.30 p.m.—Prof. Lars Beckman (University of Uppsala): "Assortative Mating in Man".*

Institution of Electrical Engineers, Utilization Section (joint meeting with the London Joint Group on the Application of Electricity in Aircraft, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. W. Ford: "Brushless Generators for Aircraft—a Review of Current Developments".

Thursday, April 12—Friday, April 13

British Society of Rheology (at the Battersea College of Technology, Battersea Park Road, London, S.W.11)—Conference on "Temperature Effects in Newtonian and Near-Newtonian Materials".

Thursday, April 12—Saturday, April 14

The Anatomical Society of Great Britain and Ireland; The Society for the Study of Human Biology; and The Zoological Society of London (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10 a.m. daily—Symposium on "The Primates".

Friday, April 13

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Captain A. Webster: "The Arrangement of Navigational Equipment in a Modern Cargo Vessel".

Monday, April 16

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. L. G. C. Pugh and Mr. Barry Bishop: "Man at High Altitude".

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Eighth Annual General Meeting.

Monday, April 16—Wednesday, April 18

British Society of Soil Science (in the New Lecture Theatre, The London School of Economics and Political Science, Houghton Street, London, W.C.2)—Meetings on "Productivity and Soil Type" and "Soil Microbiology".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Leverhulme Student (with a postgraduate diploma in chemical engineering or equivalent qualifications) in Chemical Engineering—The Registrar (University of London), King's College, Strand, London, W.C.2 (April 11).

Lecturer or Assistant Lecturer in Philosophy—The Registrar, University of Singapore, Bukit Timah Road, Singapore 10 (April 14).

Lecturer or Assistant Lecturer in Theoretical Physics—The Registrar, The University, Manchester 13 (April 14).

Research Fellow in the Department of Pure Mathematics—The Deputy Registrar, The University, Edgbaston, Birmingham, 15 (April 15).

Assistant Lecturer or Lecturer in Pure Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (April 16).

Lecturer or Assistant Lecturer in the Department of Applied Mathematics—The Registrar, University College of Wales, Aberystwyth (April 18).

Sub-Librarian (Science) (honours graduate, with experience in a university or special library, and preferably with professional qualifications)—The Registrar, University College, Singleton Park, Swansea (April 18).

Senior Lecturer or Lecturer (preferably with a medical qualification) in the Department of Physiology—The Secretary, The University, Aberdeen (April 21).

Lecturer (Grade II) in Philosophy—The Registrar, The University, Bristol 8 (April 25).

Chair of Geography in Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 28).

Lecturer (with a good honours degree in mathematics and experience of teaching in schools) in the Department of Education—The Registrar, The University, Liverpool, quoting Ref. CV/40/T (April 28).

Horticulturalist or Botanist, to help in physiological studies on growing plants—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (April 30).

Lecturer in Civil Engineering—The Registrar, The University, Sheffield (April 30).

Plant Physiologist (graduate, or graduating this year, with a good honours degree in an appropriate science), initially for work on bulb crops—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (April 30).

Lecturer or Assistant Lecturer (with a special interest in the plastic deformation or fabrication of metals, and preferably research, teaching or industrial experience) in the Department of Metallurgy—The Registrar, University College, Singleton Park, Swansea (May 1).

Assistant Lecturer (with a good honours degree in mathematics, engineering or physics) in Engineering Mathematics—The Secretary, The Queen's University, Belfast (May 5).

Assistant Lecturer or Lecturer in Applied Mathematics; and an Assistant Lecturer (with qualifications and experience in the general field of physical chemistry, and preferably with a special knowledge of N. M. R. spectroscopy or reaction kinetics) in Chemistry—The Registrar, University College, Singleton Park, Swansea (May 5)

Lecturer (with an honours degree in geography, and preferably with special interests in one of the following fields: climatology, population and settlement studies or urban geography) in Geography at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, May 5).

Senior Lecturer or Lecturer (preferably with research experience in rock magnetism, ionospheric physics, or solid state physics) in Physics at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 5).

Lecturer or Assistant Lecturer in the Department of Psychology—The Registrar, The University, Leeds, 2 (May 7).

Lecturer (with a degree or diploma in agriculture, agricultural economics or a related subject and special training in farm management, together with some experience either in teaching or advisory work) in Farm Management—The Principal, Seale-Hayne Agricultural College, Newton Abbot, Devon (May 7).

University Students and Fellows for Experimental or Theoretical Research in Solid State Physics—The Professor of Natural Philosophy, The University, Glasgow, W.2 (May 12).

Esso Student (holding, or expecting to hold, a good university degree in chemical or other branch of engineering, or in chemistry) in Chemical Engineering—The Registrar, University College, Gower Street, London, W.C.1 (May 14).

Assistant Professor (preferably with a Ph.D. or equivalent degree, and experience in research and/or teaching) in Dairy and Food Microbiology—Head, Department of Dairy Science, University of Alberta, Edmonton, Alberta, Canada (May 15).

Lecturer or Assistant Lecturer in Botany and in Physics at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 15).

Lecturer in the Department of Biochemistry—The Deputy Registrar, The University, Edgbaston, Birmingham, 15 (May 20).

Baxter Chair of Mathematics—The Secretary, The University, College Gate, St. Andrews, Fife (May 21).

Chair of Biochemistry in the University of Adelaide—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 Australia, May 31).

Denman Baynes Research Student (preferably graduate of the University of Cambridge), to do research in mathematics, physics, chemistry or in an approved related subject—The Master, Clare College, Cambridge (July 1).

Lecturer (between 30 and 50, with a major interest in structural geology and palæontology, and capable of supervising geological field mapping) in Geology at Mandalay University College, Union of Burma—The Director of Recruitment, Department of Technical Cooperation, Carlton House Terrace, London, S.W.1, quoting Ref. RC.203/21/01.

Laurence Reader in Classical Archæology—Secretary of the Appointments Committee, Faculty of Classics, St. Catharine's College, Cambridge.

Master to teach Chemistry to all levels—The Headmaster, Berkhamsted School, Herts.

Research Fellow (with a good degree in physics and some research experience in high energy nuclear physics) for Bubble Chamber Track Analysis—The Deputy Registrar, The University, Edgbaston, Birmingham 15.

Senior Technical Officer (with a degree in agriculture or agricultural botany, or a National Diploma) in the Seed Production Branch—The Secretary, National Institute of Agricultural Botany. Huntingdon Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletins of Marine Ecology, formerly *Hull Bulletins of Marine Ecology*. Edited by Sir Alister Hardy, Dr. C. E. Lucas and R. S. Glover. No. 46, Vol. 5 : Continuous Plankton Records—The Distribution of Young *Sebastes marinus* (L.). By Dr G. T. D. Henderson. Pp. 173–193. 7*s*. Nos. 47 and 48, Vol. 5 : An Ecological Survey of a Scottish Herring Fishery. Part 3. Geographical and Ecological Groups in the Plankton. By R. S. Glover, G. A. Cooper and D. C. T. Forsyth. Part 4 : Changes in the Plankton during the Period 1949–1959. By Dr. M. H. Williamson. Appendix : A Method for studying the Relation of Plankton Variations to Hydrography. By Dr. M. H. Williamson. Pp. 195–229 9*s*. (Edinburgh : The Scottish Marine Biological Association, 1961.) [11

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Commonwealth Relations Office : Colonial Office. Second Annual Report of the Commonwealth Scholarship Commission in the United Kingdom for the year ending 30th September 1961. Pp. 36. (London : H.M. Stationery Office, 1962.) 2*s*. 3*d*. net. [91

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United States National Museum, Smithsonian Institution. Bulletin 228 : Contributions from the Museum of History and Technology. Paper 25, pages 133–154 : The Migrations of an American Boat Type. By Howard I. Chapelle. (Washington, D.C. : Government Printing Office, 1961.) [2712

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United States Department of the Interior : Fish and Wildlife Service. Bureau of Commercial Fisheries Fishery Bulletin No. 193 : Blood Properties of Prespawning and Postspawning Anadromous Alewives (*Alosa pseudoharengus*). By Carl J. Sindermann and Donald F. Mairs. Pp. iv+145–152. 15 cents Fishery Bulletin No. 184 : Effects of Copper Ore on the Ecology of a Lagoon. By Kenneth T. Marvin, L. M. Lansford and Ray S. Wheeler. Pp. iv+153–160. 15 cents. (Washington, D.C. : Government Printing Office, 1961.) [81

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RESEARCH IN INDUSTRY

IN speaking in the House of Lords on November 15, 1961, during a debate on technical and scientific manpower and research, the Minister for Science, Lord Hailsham, while agreeing that the co-operative research associations represented a field in which the Government could possibly do more to foster research in industry, said that he had asked the new chairman of the Council for Scientific and Industrial Research, Sir Harold Roxbee Cox, to make it his special concern to secure a "shake-up" in the research association field. Lord Hailsham himself believed that we had been right to abandon the idea that the research associations could become self-supporting, though he does not agree that there should necessarily be a straight increase in the Government grant. Over the whole field of industry an approach which relied primarily on tax-payers' money as a source of income was doomed to failure, and the need for research was much wider and more universal than could be met in this way.

Lord Hailsham thought that there were several ways in which we could encourage activity in the research associations; but neither the last report of the Department of Scientific and Industrial Research nor that of its Industrial Grants Committee on Research for Industry, 1960, appears to foreshadow the inquiry to which he referred in the House of Lords. Later, on December 5, the development and financing of research associations were discussed in addresses to the Parliamentary and Scientific Committee by Dr. D. W. Hill and Sir Charles Goodeve, while in the report *Industrial Research in Manufacturing Industry, 1959–60*, issued by the Federation of British Industries* also in December, some comments are made by the Federation's Industrial Research Committee on the work of the research associations. Prof. Bruce Williams, in a commentary on behalf of the National Institute of Economic and Social Research, does not discuss the distinctive contribution of the research associations, but the Institute's statistical report considers specifically the use made by industry of the research associations, as well as of the laboratories of the Department of Scientific and Industrial Research. Nevertheless, the Federation's Industrial Research Committee expresses the opinion that the contribution of the research associations is rather unequal and that the situation is unhealthy and merits further investigation from the point of view of industry itself. With this examination of the functions of research associations they would link a re-examination of the research and development problems of small, and perhaps also medium-sized, firms, with the view of stimulating the application of new scientific and technological knowledge.

Dr. D. W. Hill, who dealt with the development of the research associations, their achievements and their importance to the economy, began his address by referring to this instruction of Lord Hailsham to Sir Harold Roxbee Cox to consider afresh and as a matter of urgency the whole question of the development of the associations. The present fifty-two active associations cover some 60 per cent of the productive industry of Britain, and having regard to the very large direct Government expenditure on research and development in such industries as aircraft, electronics, building, civil and mechanical engineering and to the high level of industrial expenditure on research in such industries as chemicals and pharmaceuticals, Dr. Hill does not see much scope for further expansion in numbers. Indeed, it might be observed in passing that present Government policy seems designed to discourage pharmaceutical research. In Dr. Hill's view, the Department's policy should be concentrated on building up and strengthening the existing associations and that it would be unwise to encourage the formation of any new research association without a guaranteed minimum income of £100,000 a year.

Reviewing the achievements of the research associations, Dr. Hill put first, though not necessarily the greatest contribution, the scientific and technical advances produced representing many millions of pounds annually. He gave an estimate of more than £100 million a year in direct benefit to industry to-day from an annual expenditure now running at some £8 million. It is, of course, a matter of argument whether such a return or even a greater return might have attended similar expenditure by industry in its own establishments, for opinion is freely held that, with certain exceptions, the selection of research projects, and often their execution, is most effective within industry itself provided the research establishment has sufficient resources at its disposal.

That reservation could apply in lesser degree to the first of ten other achievements which Dr. Hill lists as immeasurably more valuable though incalculable, but scarcely to the remaining nine. The devising of new and revolutionary techniques that have permitted great advances on a wide front—Dr. Hill instances partition chromatography devised at Torridon, the industrial development of operational research by Tippett at the Shirley Institute and Sir Charles Goodeve with the British Iron and Steel Research Association—is not necessarily a product of co-operative research, but the latter is unquestionably better placed to foster the introduction of a scientific approach into the traditional industries, such as steel, textiles, leather and food, which are still the backbone of British economy, as well as to train personnel in scientific and industrial fields for industry. The educational function of the research associations has yet to receive adequate recognition and it is important that it should not escape the attention of the Robbins Committee. Related to this training for industry is the significant contribution of the many research associations to formal education in collaboration with technical colleges and universities, and Dr. Hill lists with the actual supply of teachers, examiners and senior staff, but separate in its importance, the fostering of the capacity to take broad and industry-wide views. Further, he claims that the research associations have carried out much fundamental research beyond the scope of individual companies and even beyond the interest or ability of universities, and in support cites the publication last year of some 500 papers in scientific periodicals of the highest standards of originality.

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* Federation of British Industries. *Industrial Research in Manufacturing Industry, 1959–60*: Results of a Survey, including Commentaries and a Statistical Report. Pp. 129. (London: Federation of British Industries, 1961.) 40*s*. (See also *Nature*, 193, 1246; 1962.)

That is undoubtedly a claim to be taken seriously but one that calls for an appraisal of the research associations as a part of Britain's organization for scientific research generally as well as in relation to the structure of higher education. What needs to be examined is the situation, industry by industry, and taking into account the facilities for co-operation in such a research association and in a university or college of technique, particularly where the installation of costly equipment is involved. The point was well made in the recent Zuckerman Report on the management and control of research, and at this point the Industrial Grants Committee obviously must be prepared to concert common policy with the Department's Research Grants Committee.

The last four contributions stressed by Dr. Hill are that the associations have provided unrivalled trouble-shooting service for industry, for large as well as small firms, which has greatly increased efficiency. They have assisted materially in fostering export trade, and here he instanced the contribution of research data supplied by the Spring Manufacturers' Research Association to the design of satisfactory high duty springs in machines for America. The reputations of the associations stood so high abroad that in a dispute between a United Kingdom supplier and a foreign customer their opinion was often accepted without question. The associations had also, as was specially emphasized in *Research for Industry*, 1960, contributed much to health and safety in industry and the community, and finally they were an important source of disinterested and expert advice to many Government Departments.

Dr. Hill's address gave a convincing account of the value of the research associations without necessarily answering the question whether this return could have been obtained with advantage in any other way. He is undoubtedly right as to the comparative neglect of publicity by the research associations, but what is required at this stage is some impartial appraisal not simply of the achievements of the research associations but of the possibilities of achieving a like return by alternative means, or if this is impracticable of increasing the effectiveness of their work. Such an appraisal should precede and not follow the increased support for which both Dr. Hill and Sir Charles Goodeve had pleaded.

Dr. Hill had himself pointed out that between 1920 and 1960 the annual industrial contributions to the research associations had risen from £100,000 to £6·1 million and the Government contribution from £70,000 to £1·8 million; but whereas the industrial contribution had risen by nearly £2·5 million in the past five years, the grant from the Department of Scientific and Industrial Research had only risen by £400,000. Sir Charles Goodeve, who was concerned more especially with the financial aspects, pointed out that two years ago, when the Federation of British Industries made its research survey, Britain was spending on research nearly £500 million, of which two-thirds was Government expenditure, and that of this, half went back to industry for research, mainly in aviation, light electrical engineering and nuclear power. The expenditure of the research associations represented only 1·5 per cent of the total research effort.

Sir Charles included in his paper an analysis of the income of the individual research associations, but when pointing out that in 1960 the £1·8 million grant from the Department of Scientific and Industrial Research represented 23 per cent of the total income compared with 37 per cent in 1950, he based his plea for more generous support on similar arguments to those advanced by Dr. Hill. More particularly, he stressed the value of research associations in fostering the practice of co-operation as well as saving manpower. Drawing on his own personal experience, he thought the structure of the research associations was an important factor in attracting staff of high quality and character who might have been indisposed to enter private industry.

Here Sir Charles enters a field covered by the Zuckerman Report, and his argument must be accompanied by the reservation that to attract staff of such quality and character, it is not sufficient that the conditions of service and the research facilities and equipment should be appropriate. The selection of research projects must also be manifestly wise and worth while, and here again if with Sir Charles we seek to achieve a balanced progress in industry, the problems must be examined comprehensively and objectively, not piecemeal. Neither Dr. Hill nor Sir Charles suggested there were no weaknesses in the research associations, but it is insufficient to identify these and consider them in isolation. The research associations must be considered in relation to our research organization as a whole, and it is in this connexion that it is to be hoped that Sir Charles Goodeve's sensible suggestion that care should be taken to see that the Councils which advise the Government on the distribution of its funds for research include some persons able to speak with direct knowledge of the problems of co-operative research in typical industries served by a research association.

The Federation of British Industries Report gives the expenditure of its members on research and development in 1959–60 as £250 million, excluding capital, and employing 120,000 persons, of whom 28,500 were qualified scientists and engineers, and this expenditure is believed to account for 90–95 per cent of the expenditure on research and development of all manufacturing industry. This applies to research expenditure within the firms: in some industries the picture is changed by the existence of the research associations themselves; for example, only about 5 per cent of the members of the Cotton Industry Research Association and some 20 per cent of the members of the Wool Industry Research Association were members of the Federation of British Industries and probably most of the 21,000 members of the research associations are not direct members of the Federation. Nevertheless, the greater part of the industrial income of the research associations and almost all their sponsored work comes from the larger firms who are members of the Federation. In five industries a substantial income from medium and small firms is derived from a compulsory levy, while in thirteen others some funds are also derived by allocation to research from Trade Associations supported by general subscriptions.

On the industrial use made of research associations, replies to the Federation's questionnaire suggest that perhaps double the proportion of large firms make use of the research associations' facilities compared with medium or small firms, but estimates of the value of the work done for particular firms varied widely. More than 90 per cent of large firms were members of one or more research associations, while among members of the Federation two-thirds of the small firms and more than a third of the medium firms were not members of any research association. Since large firms might be members of up to a score of research associations, comparisons between industries must be accepted with caution, but whereas in chemicals and electrical engineering, which have a high research ratio and very large internal research expenditure, the research associations account for only about 1 per cent of the combined total expenditure on research and development, in some of the traditional industries they may account for more than 40 per cent.

Comments on the work of the research associations frequently referred to the value of information services and informal background discussion, but while a large engineering firm expressed the opinion that practical application of research was more likely to be the direct outcome of their own work rather than that of research done elsewhere, other comment recognized that the research associations could no longer be criticized as too highbrow in their approach to the practical problems of industry, and only in a few industries did critical comments exceed the favourable ones. The former included reference to the need for greater resources to be devoted to the development of new machinery. Comments from the medium-sized or small firms did not support the view that the research associations are mainly of value to the smaller firms nor was there any clear consensus of opinion as to whether there was a substantial number of research projects which are too long-term or too expensive for a single firm which ought to be undertaken by the industry as a whole.

If the statistical report compiled by the National Institute for Economic and Social Research gives little guidance as to the functions or modification of the research associations, comments made by the Federation's Industrial Research Committee are more specific. While the contribution made by the research associations to the overall volume of research in Britain is not large, it includes many examples of first-class work, but a large part of the time of some research associations is devoted to information services and minor *ad hoc* investigations. Useful as these activities are, they cannot replace fundamental investigation, and it might be asked whether such activities could not be organized effectively in some other way.

The unequal level of the contribution made by the research associations revealed by the survey of itself points to the need for some further inquiry, particularly, as the Industrial Research Committee remarks, because the situation is a criticism of industry as of the research associations, which are directed by industrial committees, who may impose limits on the scope of the research. On this point again, the Zuckerman Committee made some comment. Further, the survey, while indicating a demand for improvements in external research facilities, revealed no demand for an increase in the number of research associations or of sponsored research institutes, while it appears also to be true that insufficient use is made in several industries of the research associations and other outside channels for the solution of specific problems. This is mainly true of the traditional industries, though there are others also in which research expenditure is low and there appears to be room for expansion of scientific and technical activity in small and perhaps also medium-sized firms.

On the research associations themselves, Prof. B. Williams's commentary adds comparatively little. He points out that it is not accidental that most of the research associations are in industries that derive little direct benefit from university research, and that while the associations are not simply substitutes for a university, many of them serve industries where both research and development problems are beyond the capacity of the individual firm and that their work must often range from fundamental research to the invention of technical gadgets. He thinks that there are probably certain engineering firms that would benefit from some form of organized contact with a centre of research and development in the appropriate branch of their industry and suggests that the new Machine Tool Industry Research Association, for example, may help to fill this gap in a small, but very important, sector of this industry.

Prof. Williams notes the need in many fields for more systematic dissemination and application of the existing body of knowledge, and his suggestion that more interchange of personnel, seconding of staff, joint work on specific projects and other forms of practical co-operation is in line with observations on these points in the Zuckerman Report on the management and control of research and development. The answers to the questionnaire, he observes, do not, moreover, help us to form a sound judgment as to whether the research associations are undertaking projects on a sufficiently large scale but they do suggest the need for a more co-operative approach to the problem of priorities. The wise selection of targets is of the utmost importance, and if the research associations are to succeed they must be managed by those who possess the necessary experience and sound judgment for taking wise decisions in that matter.

Possibly, as Prof. Williams suggests, the research associations could co-operate more in defining common problems the solutions which would add greatly to the productivity of their work. Apart from this, Prof Williams brings out the extent to which the functioning of the research associations is linked with the supply of qualified scientists and engineers. Any existing shortcomings of the research associations have to be considered in that light, and apart from suggesting that we need more research into the selection of research problems and the question of priorities, Prof. Williams indicates a number of related questions calling for inquiry parallel with those initiated by Lord Hailsham and recommended by the Industrial Research Committee of the Federation of British Industries.

Decisions as to the functions of a research association in any specific context must clearly depend in part on judgments as to the ability of its management to select appropriate targets of research as well as the efficiency with which it uses its qualified manpower. Further consideration of the use of qualified scientific and engineering manpower is needed in industry generally, as Prof. Williams suggests, and not only in the research associations. We need, too, further inquiry into the type of procedures useful in selecting projects in basic research, in applied research and in development, and into the variation of appropriate procedures with the type of activity and between industries. Moreover, the broad question of the function of the research associations and the magnitude of the public support they should receive is a particular facet of the organization of our scientific research effort in general which is at present under consideration by the Advisory Council for Scientific Policy as well as of the establishment of co-operative research institutes generally discussed in the Council's last report. It is to be hoped that the Minister for Science will apply the same mandate of urgency to that inquiry as he has already addressed to that into the research associations by the Council for Scientific and Industrial Research, and it is also to be hoped that the latter Council will be instructed no less firmly to give adequate publicity to the results of that inquiry. If the research associations are to function effectively within whatever limits are determined and to receive adequate public support, both the scientific and industrial world and the general public must be given much more information than has been contained in recent reports either of the Council itself or its Industrial Grants Committee.

METALLURGY OF PLUTONIUM

Plutonium 1960

The Proceedings of the Second International Conference on Plutonium Metallurgy, Grenoble, France, 19–22 April, 1960. Editorial Committee: Emmanuel Grison, William B. H. Lord and Robert D. Fowler. (Organized by Société Francaise de Metallurgie et le Commissariat a l'Energie Atomique.) Pp. xvii + 804. (London: Cleaver-Hume Press, Ltd., 1961.) 189*s*.

INFORMATION on the metallurgy of plutonium has been spasmodic since the Geneva Conference on Atomic Energy in 1958. The Conference, the proceedings of which are reported in this volume, could be described as revealing more information and offering a wider scope than any previous one, and, as such, presented a balanced picture of the subject up to 1960, as claimed by the editors in their preface.

Papers were presented in four sessions, dealing with physical properties, alloys, ceramics and nuclear fuels. The discussions included mention recent developments that have arisen since the writing of the papers, and are fully recorded in the book.

Through the whole proceedings, the question of impurity loomed large. Work on allotropic transformations and physical properties is obviously affected by purity, and one author admitted that the precise position is obscure as a result. The fascinating problem of negative thermal expansion in the delta phase of plutonium has moved a stage nearer to a solution with studies of properties illustrating the density of states at the Fermi surface. Ferromagnetic impurity elements seriously affect low-temperature specific heat, electrical resistivity and thermal expansion and the transformations.

A large number of alloys of plutonium has been investigated in recent years, with a concentration on the theoretical implications. Certain elements have been found to increase the rate of negative expansion of the delta phase in dilute alloys with a reversal of the effect with increasing alloy content. The point at which zero expansion occurs appears to be dependent on electron concentration. The smaller the size of solute metal (within certain limits) the less becomes the minimum amount of solute required to retain the delta phase. Although one might expect that the requirements of nuclear energy would strongly influence a more fundamental approach to alloy theory with respect to such rare metals as plutonium, it is encouraging to read how far this approach is being taken. The effect on metallurgical theory in general is bound to be widespread and welcome.

The chemical metallurgy of plutonium and its alloys is not forgotten, in spite of the extensive effort on the physical problems. Preparation of the metal and atmospheric oxidation are covered in this volume. Production of nuclear fuels, including mixed oxides of uranium and plutonium, and fabrication of fuel elements (chiefly based on alloys with aluminium) appears towards the end. Three papers discuss mechanical properties, two of these including fabrication methods on unalloyed plutonium. Considering the effect of allotropic transformations on this most peculiar metal, it is no small wonder that the measurements of mechanical properties at elevated temperatures give rise to difficulty in interpretation. The energy expended in deformation is bound to cause local changes in temperature, with inevitable repercussions on transformations. The speed of testing is, from these papers, a very pertinent variable.

Two review papers on the use of plutonium in fast fission reactors present the nuclear power facet behind the large experimental and theoretical effort covered in the Conference. Some idea of the tremendous cost involved can be inferred from the statement by one author, discussing fuel element development for one power reactor alone, that 5 million dollars will be necessary. If one multiplies this figure by the number of countries pursuing the same problem, and makes an allowance for variations in method and approach, then roughly 5 million pounds sterling may be a reasonable estimate for the whole world. This should emphasize the importance which the four countries producing significant quantities of plutonium attach to its future use as a nuclear fuel. The present stage of knowledge was very concisely summarized at the Conference, and a measure of the considerable ignorance in which nuclear scientists and engineers are still working. As a direct consequence, the book scarcely requires the recommendation which it deserves.

C. R. TOTTLE

REACTIONS IN ORGANIC CHEMISTRY

Name Index of Organic Reactions

By Dr. J. E. Gowan and T. S. Wheeler. Second Edition. Pp. v+293. (London: Longmans, Green and Co., Ltd., 1960.) 50*s*. net.

ORGANIC chemical processes are often named after their discoverer and this book lists alphabetically no fewer than 739 of such named reactions. A short description of each is given, usually with formulæ, and original papers or reviews are cited from which further information can be gained. There are plenty of cross-references, for example, to related processes, and, where more than one name is associated with a reaction, an entry is found under each. There are a general index based on compounds and also one based on types of reaction. The book undoubtedly satisfies a real demand, in that it provides a guide to the multitude of randomly named reactions that the organic chemist is for ever encountering and enables him to classify them chemically. It is an expanded, and much-improved, version of the author's earlier edition, which had the same title.

Though this book is most necessary, one can only yet again deplore that the need for it has arisen. Though few claim to be in favour of the practice of giving a reaction the originator's name in general chemical usage, very many of us perpetuate it in our writings and, worse, in lectures. The faults of the practice such as obscurity, lack of uniformity in naming, etc., are so well known that detailing here is unnecessary. In this book the name of Claisen is attached by itself to five different processes and, with another name, to seven others, while the name of Baeyer by itself is given to three reactions and, in association, to six others. No fewer than twenty reactions are linked with the name Fischer. With no disrespect to the names involved or to their work, do we really need the following terms in general usage: von Braun–Rudolph modification of the Sonn—Müller reaction, Gryskiewicz–Trochimowski–McCombie–Saunders preparation, Makarov–Zemylanskil–Prokin preparation, Padbury–Lindwall modification of the Grosheintz–Fischer–Reissert reaction ? Reaction No. 630 (the Stacey esterification) was not so called by any wish of its originators, and has never been thus named at Birmingham.

Is it too late to ask that organic chemists describe reactions in chemical terms and give credit to the the discoverers by citing their references ? More realistically, cannot editors of journals refuse to accept named reactions in their publications ? If a few very important processes are linked indissolubly with one name (for example, Grignard reagents), could there not be agreement on a list, as with nomenclature. In fairness to the authors of this book, it is clear from their prefaces that they do not like the practice of giving originator's names to reactions. They are dealing with the situation that already exists, and, as they say themselves, trying to combat the obscurity caused by this undesirable custom.

J. C. TATLOW

SUBSONIC PLANE FLOW

The Theory of Subsonic Plane Flow

By Dr. L. C. Woods. (Cambridge Aeronautical Series, Vol. 3.) Pp. xxii+594. (Cambridge: At the University Press, 1961.) 120*s*. net.

OF all the great scientific disciplines of the twentieth century fluid dynamics takes an honourable place, and if it is not quite such a household word as radioastronomy or nuclear physics, nevertheless its contributions to our modern technological society have been no less revolutionary: the supersonic aeroplane and the guided missile are among the supreme, if misapplied, achievements of man's skill and inventiveness.

The famous physicists of the past century, however, who laid down the immense structure of classical hydrodynamics, as we now call it, made few contributions of real value to twentieth-century technology; for they were perplexed (and knew it) by the effect of the viscosity of the fluids they studied. Certainly they had their successes: as with surface waves (where viscosity is often of negligible importance) and with flow in pipes (where it is overwhelmingly important). But an acceptable theory of flight was beyond their reach and indeed awaited the scientific break-through which eventually came through the genius of Ludwig Prandtl. His conception of the action of viscosity, explained by him in both physical and theoretical terms in 1908, opened the flood-gates of modern aerodynamic research. The boundary layer had arrived !

What, therefore, are we to make of a scholarly book on fluid dynamics which virtually leaves the boundary layer unmentioned and certainly gives it no entry in 6 pages of index ? Is this volume to be regarded as a Victorian phoenix or as a startling *avant-garde* approach ? The former we might well dismiss at once, and with the latter we might sit happily on the fence to see what the future brings. But neither shows the right attitude, for in fact we should most heartily welcome this first exhaustive account of a certain method of solving the two-dimensional Laplace equation under various types of boundary conditions.

For that is what this book is about. It is emphatically not fluid dynamics, nor is it *the* theory of subsonic plane flow as the title claims. There are other theories of compressible (but yet subsonic) flow, many other theories of plane (that is, two-dimensional) flow, even of inviscid, and even more of plane viscous flow. One has very serious doubts as to whether the methods advocated in this book for aiding the solution of real flow problems are, or even will be, in use to anything but a negligible extent.

Nevertheless, this is a fascinating book. The author has indefatigably applied and extended his central idea—namely, the determination, Cauchy-like, of the value of a function in an infinite strip given its real part on one boundary and its imaginary part on the other—to almost every conceivable aeronautical application. It requires considerable discernment to disentangle the wheat from the chaff, the mathematics from the pseudo-physics, which is why I would have preferred a frankly mathematical treatise from Dr. Woods; but the effort is always worth while, and I, for one, am more than grateful for this addition to my shelves.

B. THWAITES

AN INTRODUCTION TO THE ATOM

Modern Atomic and Nuclear Physics

By C. Sharp Cook. (University Physics Series.) Pp. x+296. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 58*s*. 6*d*.

THIS interesting book, the fifth in Van Nostrand's *University Physics Series*, is intended, we are told in the preface, "for the college or university student who has completed at least one year of classical physics and mathematics through calculus". It attempts to present in a relatively compact book a wide coverage of atomic and nuclear physics.

For the most part it succeeds in giving a clear and up-to-date account of the subject, beginning with gas-kinetic theory, properties of the electron and proceeding by the wave-particle dualism, the Bohr theory and elementary quantum theory to atomic structure and spectra. The coupling of angular momenta of atomic electrons is especially well described. There follows a brief but illuminating introduction to the theory of molecular structure. Solid-state physics and the emission of electrons from solids are described very briefly. The four final chapters deal with nuclear properties, nuclear reactions and elementary particle physics. It is refreshing to find an introductory book on this subject which leads up to the concepts of isotopic spin and strangeness. The only significant omissions are the methods of production and detection of nuclear particles.

However, the inevitable consequence of such coverage is that basic ideas are lost and the reader is forced to be content with much that is empirical. Relativistic mechanics is dealt with in less than eight pages in which we learn little more than the rules of the Lorentz transformation. The book aims to provide "at least a speaking acquaintance with the atom". It is difficult to see what value this has in a physics course in Britain, where emphasis is placed on basic understanding of the subject rather than superficial acquaintance with it. Perhaps it is best recommended as background reading for sixth formers.

The book is marred by a looseness of style and expression, with sometimes unnecessary repetition. Some details should be amended: the mathematical background assumed is sufficient to avoid the use of the Joule classification in the treatment of gas-kinetic theory; a well-known law is incorrectly attributed on page 27; the force on a moving charge is deduced from the force on a current-carrying wire—surely a tautology.

There are 23 pages of problems in the 280 pages of text, and these are probably of little value unless the book is studied in a class. The references given at the end of each chapter are excellent and the keen student will be wise to follow them up.

This is a stimulating and readable book, well illustrated and with clear typography. The subject is presented in an interesting manner, but it is a pity that the author has sacrificed the thorough development which is perhaps more essential in physics than in other sciences.

G. DEARNALEY

ECONOMIC MALACOLOGY

The Giant African Snail

A Problem in Economic Malacology. By Albert R. Mead. Pp. xvii+257. (Chicago and London: University of Chicago Press, 1961.) 7.50 dollars; 60*s*.

STARTING on what may well eventually prove to be a world-wide journey, the giant snail of East Africa, *Achatina fulica*, appears to have reached Madagascar and then Mauritius about the beginning of the last century. From the latter it was carried, presumably by accident, to the Seychelles, but quite deliberately into India by a conchologist, W. H. Benson, who released specimens near Calcutta in 1848. Separate entry from Mauritius took the snail to Ceylon at the beginning of this century and this proved a stepping stone to Malaya and Indonesia. Before the Second World War it had reached the Far East from south China to Japan and had been deliberately introduced into Hawaii and the islands of Micronesia. During the War the Japanese carried it still further.

This was no inconspicuous incomer, unrecognized until too numerous to combat, but a creature worthy of its name, the largest individuals reaching almost 1 ft. when fully extended. With the sole exception of Japan, where winter cold was possibly the deciding factor, attempts at extermination have everywhere failed before reproductive powers potentially capable of producing in three years eight billions (American) from a single gravid snail. In Java more than two million snails and half a million of their eggs were collected by hand in a few weeks.

Dr. Mead has produced a fascinating book of the widest biological interest. He has studied these snails in Africa, Asia and on many Pacific islands. He has also digested relevant information from nearly 900 listed references. He may fairly claim to have succeeded in his declared attempt to produce the first major work on economic malacology. His chapters cover dispersal, economic status, chemical, mechanical and biological control (with enemies ranging from predacious snails to beetle larvæ, giant toads and even hermit crabs) as well as control by human use and legislative action. The problem might be solved if the vast quantities of mucus-encumbered animal protein could be suitably utilized. Dr. Mead comes finally to an interesting consideration of population decline, most probably the result of disease. Taken at the right time, this appears to offer the best hope, if not of extinction, at least of permanent control.

C. M. YONGE

Theory of Elasticity

By V. V. Novozhilov. Translated from the Russian by J. K. Lusher. Pp. xii + 448. (London and New York: Pergamon Press, 1961.) 80s. net.

IN the first part of the book the author develops the theory of elasticity proceeding from the general concept of finite deformation and specializing to cases where (*a*) the strains are small and (*b*) the strains and rotations are small. In this way he examines the geometry of deformation, the conditions of equilibrium, and the strain energy and related concept of virtual work. The classical theory is then examined in detail. Topics considered include the Papkovich solution of the general equations, thermoelasticity, dynamic problems, and existence, uniqueness, minimum, and super-position theorems.

The theory is developed in rectangular Cartesian co-ordinates, but the field equations are also transformed to orthogonal curvilinear co-ordinates. This makes the initial development rather lengthy, but has the effect that many topics in non-linear elasticity become available to the newcomer without resort to tensor notation. The theory of finite deformation is referred to co-ordinates both in the deformed and in the un-deformed body. The careful transition by stages from the non-linear to the classical theory brings out a number of points that are sometimes overlooked in more sophisticated treatments. The book includes a brief mention of tensor notation.

The last two chapters are concerned with problems in classical elasticity. St. Venant's theory of torsion is developed and applied to the twisting of rods of circular, elliptical, triangular, square and other cross-sections. Bending theory is treated similarly. The theory of classical plane strain and stress is formulated in curvilinear co-ordinates and in complex forms. A number of problems is treated using the method of complex power series expansions, aided where necessary by conformal transformations. These include the plane deformation of an infinite plate containing circular, elliptical and (approximately) square holes. The half plane problem is also considered. The book does not deal in any detail with anisotropic bodies or with three-dimensional problems. The careful treatment of the basis of finite elasticity and the transition to the classical case, however, make this a useful book. J. E. Adkins

Proceedings of the International Conference on Nuclidic Masses

McMaster University, Hamilton, September 12–16, 1960. Edited by H. E. Duckworth. Pp. xi + 540. (Toronto: University of Toronto Press; London: Oxford University Press, 1960.) 80s. net.

THE complexities of nuclear structure having as yet defied all efforts at an overall interpretation, it is becoming increasingly recognized that more and more precise data on nuclear properties should be sought to provide clues which may lead us to the fundamental laws. An outstanding contribution to this cause is the present table of nuclidic masses published in 1960; it forms the hub about which revolve the discussions of the conference reported in this volume. For the mass compilation a vast array of data was drawn from mass-spectroscopic experiments as well as from all kinds of nuclear reaction measurements, and from β-decay studies. To users of these mass tables the papers in the first session of the conference afford an interesting opportunity of gaining some insight into the magnitude of the task which the compilers have undertaken. The second session directs attention to some of the physical properties of nuclei which can be derived from mass data, dealing particularly with the nuclear surface, total binding energies, and a shell-model interpretation. The experimentalists then take over with a series of lively discussions covering present-day problems and progress in energy standards and calibrations, the measurement of Q-values for nuclear reactions, including charged-particle, photonuclear, threshold and capture reactions, beta-decay energies and systematics, and finally mass-spectroscopic determinations of atomic masses, together with accounts of recently developed instruments, including a new type of radiofrequency mass spectrometer.

Considerable progress has been made in achieving consistency in the mass data, particularly between the reaction and mass-spectroscopic measurements. There remain, however, a number of discrepancies and gaps, which the contributors discuss, pointing the way to further desirable investigations. Another mass compilation and a similar conference are not anticipated for several years. Meantime this volume is likely to be of considerable use to low-energy nuclear physicists and mass spectroscopists. J. M. Freeman

Organic Chemistry Today

By F. W. Gibbs. (Pelican Book No. A461.) Pp. x + 294. (Harmondsworth, Mddx.: Penguin Books, Ltd., 1961.) 7s. 6d.

SINCE Woehler's synthesis of urea in 1828, staggering advances have been made in theoretical and applied organic chemistry. To-day chemical industries founded on oil, petroleum and other organic raw materials are still springing up in Britain. In his book *Organic Chemistry Today*, Dr. Gibbs attempts to describe some of the more important advances and underline their practical implications and significance.

He begins with a clear outline of the scope of organic chemistry and an introduction to molecular structure which should be familiar to every sixth-form chemistry student. Then, in the remaining eleven chapters, he deals with such topics as: oil and petroleum hydrocarbons, coal and coal chemicals, sugars and polysaccharides, proteins and living matter, food, drugs, dyes and pigments. Wherever possible, each topic is developed against a historical background of key-dates and the names of people most responsible for major advances. Schematic diagrams and charts, and structural formulæ even of the rather complex carotenes, porphyrins and vitamins, are most clearly and simply presented. The comprehensive subject index and the short bibliography with a comment on each book are most helpful.

The introductory chapters and the chapter on coal and coal chemicals should appeal to the layman, but the book as a whole is more likely to appeal to the reader who is able easily to interpret structural formulæ and is familiar with the technical terms of organic chemistry: the synonyms isobutene and isobutylene appear indiscriminately in the text without comment. This book is of considerable value to all students of chemistry at sixth-form level and beyond, and is cheap at the price. It also has something to offer those interested in the influence of chemistry on politics and economics, and vice versa. Michael C. Cox

USER RESEARCH FOR THE NATIONAL REFERENCE LIBRARY FOR SCIENCE AND INVENTION

By Sir FRANK FRANCIS, K.C.B.

Director and Principal Librarian, British Museum, London, W.C.1

AN editorial in *Nature* of July 1, 1961, p. 1, subjected the proposal for "The National Reference Library for Science and Technology" (as it was called) to a searching scrutiny, and voiced the anxiety of scientists that the services available in the new Library should be as complete as possible.

Both the British Museum and the Patent Office, the future and present parent organizations of the new Library respectively, are fully aware of the complexities and of the opportunities which the change-over offers. The librarian of the Patent Office, Miss Maysie Webb, who was referred to in the article, is most concerned of all, since she has the task of running the day-to-day machine while at the same time overhauling it and planning its conversion to new, expanded and, it is hoped, even more efficient uses. The Trustees of the British Museum, who have been charged with the control and the administration of the new Library, look on their task as a great opportunity to provide an exemplary kind of library service for special classes of users.

There are, however, some questions of general importance which should be answered so far as possible before more detailed planning takes place. These concern the nature of the service to be provided by the new Library, and it is felt that detailed research into the use which is made of the present Library is needed to provide the essential basis for this future planning. The free access which has always been permitted in the Patent Office Library has, by its nature, precluded any systematic study being made hitherto, and there are few relevant facts or figures to guide us. Briefly, we need to be told or to be able to deduce: (*a*) the degree of 'professionalism' or system shown by the readers in their use of the library and whether there is a pattern, or patterns, in their approach; (*b*) the tools they use in their approach to the literature; (*c*) whether the present acquisitions policy reflects the needs of present and future users; (*d*) the length of active life of the material in the Library.

Research into these questions has, so far as I am aware, never been undertaken on a large scale in this or in any other library. It has now been made possible for us, however, with funds generously placed at our disposal by the Goldsmiths Company, to put in hand such an organized study, and we intend, with the full agreement of the Patent Office, to employ for one year special staff to study the present usage of the Patent Office Library and to investigate so far as possible its likely future requirements, and thus to secure information on which the services of the new Library can be soundly planned.

It is intended to begin by making a broad survey of the categories into which readers fall and of their use of the library and to go on from there to make a much more detailed survey based on a valid statistical sample.

The programme we have outlined for this purpose is as follows:

(1) *Broad survey*
  Categories of readers
    Their profession (patent agents; information officers; research workers, translators; etc.)
    Their purpose (rigorous subject search; specific documents; inventive novelty; browsing; etc.)

(2) (Based on (1)) What is a valid statistical sample for deeper survey? How best to take it?

(3) *Deep survey*
  (*a*) Frequency of visits; time spent; distance travelled.
  (*b*) Foreign language usage
    Can they read foreign languages? }
    Do they? } which languages?
    Will they later? }
  (*c*) Readers coming to consult specific documents:
    How many did they inspect?
    Categories (that is, books, periodicals, etc.)
    What percentage was not examined and why?
  (*d*) Readers coming to carry out a subject search:
    Which sources did they use?
      Library catalogue
      Library indexes
      Abstracting journals
      Book references
      Journal indexes
      Other
    What did they examine (categories)?
    What percentage was useful?
    What percentage of references was not examined and why (not in library; language difficulty; doubtful subject value)?
  (*e*) Specific items examined (dates and titles)
  (*f*) Use of catalogues:

Author catalogue	How many items were sought? How many were found?
Classified catalogue	Did it lead to useful specific material? Difficulties?
Special indexes	Which was used? Did it lead to useful specific material? Difficulties?

Connected with this research into the use of the present Library and the likely use of the new Library, we propose to investigate the present scheme of classification and to see whether it should be replaced. To do this we expect that existing scientific literature classifications will be critically examined and evaluated in relation to the details we discover about the present use of the Library.

The Trustees of the British Museum have appointed Miss Barbara Kyle to carry out this work, and they plan to appoint, to work with her, a full-time science graduate and to call on the services of statisticians and other specialists as required. With the advice of these specialists it is hoped that questionnaires of the highest degree of practicality and significance can be designed and the results usefully assessed.

The editorial in *Nature*, to which I have already referred, stresses the importance of the co-operation of users in seeing that the needs of the new Library are clearly and continuously presented. We need the co-operation of present and potential users of the Library's services in our inquiry, and it is our earnest hope that they will be willing to respond generously to the calls we shall make on them and on their time.

We shall be glad to hear from any who are interested. Letters should be addressed to me, or to Miss Webb or Miss Kyle at the Patent Office Library, 25 Southampton Buildings, London, W.C.2. It is then hoped to make progress reports on our survey in *Nature* from time to time.

CHONDRULES AND THE ORIGIN OF THE TERRESTRIAL PLANETS

By Dr. JOHN A. WOOD

Smithsonian Astrophysical Observatory, Cambridge, Mass.

IN the past decade, hypotheses to explain the origin of the solar system have been advanced which are consistent with almost all that is known about the nature of the universe and about the behaviour of matter. Postulated *ad hoc* events have been minimized. Perhaps there is reason for believing that the science of solar system cosmogeny is converging on the truth.

The hypotheses (see especially Hoyle[1], Cameron[2]) involve, briefly, gravitational contraction of an interstellar cloud; increasing angular velocity of the contracting mass until it became rotationally unstable and spun off matter at its equator; and finally a magnetic coupling which transferred angular momentum from the remaining contracted mass (Sun) to the spun-off disk or nebula. The latter became, eventually, the planetary system.

Meteoritic studies are expected to help us understand the latter part of this history: the formation of terrestrial planets. I would like to point out an important structural characteristic of meteoritic material which I feel is especially closely related to the astrophysical process of solar system formation.

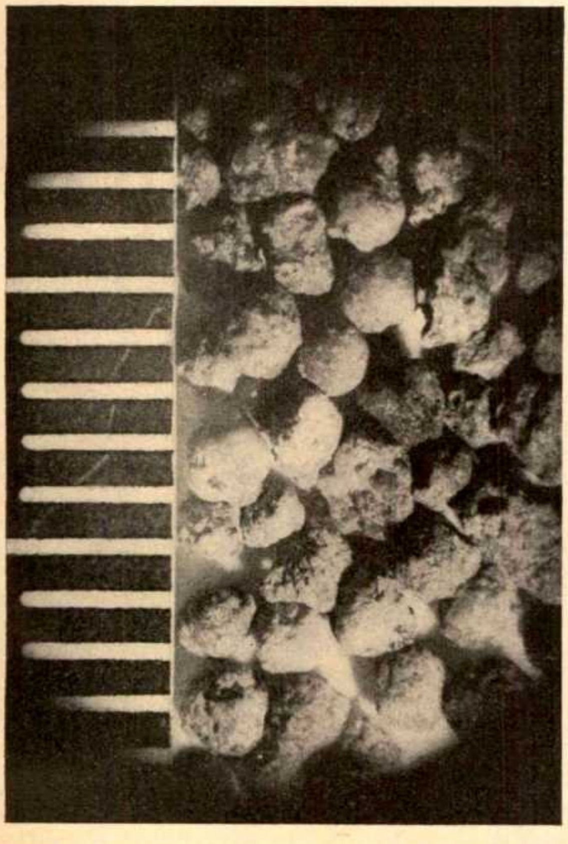

Fig. 1. One particular size fraction (− 10 + 20 sieve meshes) of chondrules separated from the chondrite Bjurböle, with millimetre scale

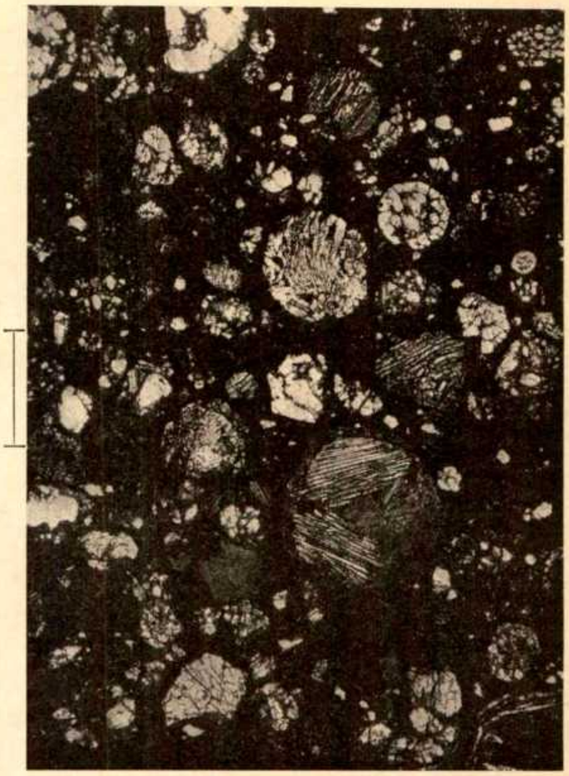

Fig. 2. Thin-section of the chondrite Tieschitz, viewed by transmitted non-polarized light. Length of scale bar, 1 mm. The chondrules (light areas) consist largely of olivine, $(Mg,Fe)_2SiO_4$, and orthopyroxene, $(Mg,Fe)SiO_3$ which are poor in iron. Scattered grains of metallic iron (black) are visible in the chondrules. The opaque (black) matrix in which the chondrules are embedded is a fine-grained ($< 1\mu$) mixture of olivine and Fe_3O_4, with minor amounts of troilite, orthopyroxene and iron metal. Photo. from *Geochimica et Cosmochimica Acta* (ref. 3)

The structures referred to are chondrules. These are solid spheres or spheroids of silicate and metallic minerals (usually mixed), ranging in diameter from 0·5 to 3 mm. (Fig. 1). No terrestrial geological process produces structures which resemble chondrules more than superficially.

Meteorites containing chondrules are called chondrites. Fig. 2 is a thin-section of the chondrite Tieschitz: chondrules make up roughly 50 per cent of its volume; the remaining opaque interstitial material is an extremely fine-grained ($< 1\mu$) mixture of silicates and iron oxide (Fe_3O_4).

Many chondrites contain fewer chondrules; but these have probably suffered re-crystallization which obliterated chondrules during a period of high temperature. Tieschitz and a few other chondrites appear to have been spared this thermal metamorphism[3]. If so, chondrules must earlier have been the dominant structural characteristic of meteoritic material, since 84 per cent of meteorites seen to fall are chondrites. Thus, an understanding of the origin of chondrules probably provides the key to the history of meteorites.

It seems clear that the chondrules were once dispersed molten droplets: their internal textures are igneous in character, and in some cases they contain glass. Not infrequently chondrules are seen which indented one another, apparently while one was still plastic.

I have previously proposed that these droplets condensed from cooling solar gases during the formation of the solar nebula[3,4]. Gases being spun off by a rotationally unstable protosun would have expanded and cooled, and there liquid chondrules might have condensed. Hans Suess[5] earlier suggested that chondrules might be liquid droplets which condensed in cooling gaseous protoplanets, after the hypothesis of Eucken[6].

The observation that chondrule minerals in the apparently unrecrystallized chondrites Tieschitz and Renazzo are unusually poor in iron silicates[3] lends support to this hypothesis: hot solar gases constitute an intensely reducing environment, and all iron condensing and freezing in such an environment must remain in the metallic state[7]. None can enter the silicate melt as iron oxide.

The compatibility of the liquid-droplets concept with the astrophysical framework of Hoyle or Cameron depends on two questions: is it probable that solar gas would cool through the liquid fields of silicates and iron during the contraction and shedding postulated ? If so, could we expect to find the condensation products (chondrules) as far away from the Sun as the asteroid belt (usually held to be the immediate source of the meteorites) ?

The first question requires knowledge of temperatures and pressures in the hypothetical contracting protosun. These cannot be known in detail, but the average temperature and average pressure in a stellar body, as defined by Aller[8], give some measure of what we can expect, and these can be calculated.

$$\bar{T} = \frac{1}{M}\int_0^R T \, \mathrm{d}M_r$$

$$\bar{P} = \frac{1}{M}\int_0^R P \, \mathrm{d}M_r$$

(R is total radius of sphere, M is total mass, M_r is mass beneath radius $= r$).

Aller shows that:

$$\bar{T} = \frac{G(\mu\beta)}{3M\mathcal{R}}\int_0^R \frac{M_r \, \mathrm{d}M_r}{r}$$

and:

$$\bar{P} = \frac{G}{4\pi M}\int_0^R \frac{M_r^2 \, \mathrm{d}M_r}{r^4}$$

independent of mass distribution in the star. The equation for $\bar{T}$ assumes that the ideal gas law holds. ($\mathcal{R}$ is gas constant, G is gravitational constant, μ is average molecular weight of gas, and β is 1 − (radiation pressure/total pressure).)

Assuming radiation pressure can be neglected, $\mu \sim 1$, and $M \sim$ present mass of the Sun, these reduce to:

$$\bar{T}\ (^\circ\mathrm{K.}) \sim 107{,}000\,\frac{d}{R}$$

and:

$$\bar{P}\ (\mathrm{atm.}) \sim 1{\cdot}68\,\frac{c}{R^4}$$

(R in astronomical units), where c and d are functions of mass distribution in the star. These coefficients were calculated for three particular mass distributions, and are presented in Table 1. As the hypothetical protosun contracts, $\bar{T}$ and $\bar{P}$ in the case of model 1 or 3 migrate along *ABCD* in Fig. 3. The temperature–pressure history of model 2 is not significantly different from that of model 1.

Table 1. COEFFICIENTS c AND d FOR THREE STELLAR DENSITY DISTRIBUTIONS

Model		c	d
1. Uniform density	$\rho = A$	0·15	0·20
2. Density varies linearly	$\rho = B\,(1 - r/R)$	0·34	0·25
3. Mass concentrated in centre	$\rho = C \quad (0 \leqslant r \leqslant 0{\cdot}1\,R)$, $\rho = 0 \quad (0{\cdot}1\,R < r \leqslant R)$	1,500	2

The liquid field of meteoritic silicates is approximated in Fig. 3, using the vapour–liquid curve of pure silica in a solar atmosphere (calculated from thermochemical data[7]), and the temperature at which basic igneous rocks begin to melt. The liquid field of iron has been determined experimentally[9]. $\bar{T}$ and $\bar{P}$ in the contracting protosun miss these liquid fields by a substantial margin.

However, Hoyle[1] estimates that the contracting protosun became rotationally unstable at $R \sim 0{\cdot}2$ A.U. and that mass shedding took place at this and lesser radii. In Fig. 3, B corresponds to $R = 0{\cdot}2$ A.U. for model 1 (uniform density) and C to $R = 0{\cdot}2$ A.U. for model 3 (mass concentrated in centre of protosun). The actual density distribution in the protosun probably lay between these extremes. Then, 'average' solar gas spun off at $R \sim 0{\cdot}2$ A.U. or less (that is, closer to D on *ABCD*) and allowed to expand and cool adiabatically or near-adiabatically, would have passed through the liquid field of meteoritic silicates and possibly through the liquid iron field as well. (The lines labelled a in Fig. 3 are adiabats.)

The reader will have mentally objected that matter shed from the surface of the spinning protosun would be at substantially lower temperatures and pressures than the $\bar{T}$ and $\bar{P}$ contemplated. This is so, but as equatorial surface gas was spun off, it must have been replaced by gas from greater depth, which was initially closer to $\bar{T}$ and $\bar{P}$. It is possible that the condensation of chondrules actually occurred within the body of the protosun, as hot gases moving up from depth expanded and cooled. Eventually expulsion of the gas-liquid mixture from the protosun would have accelerated expansion and cooling; this could account for the presence of basic glass in some chondrules, the preservation of which requires very rapid cooling (from 1,200° C. to perhaps 800° C. in a matter of minutes).

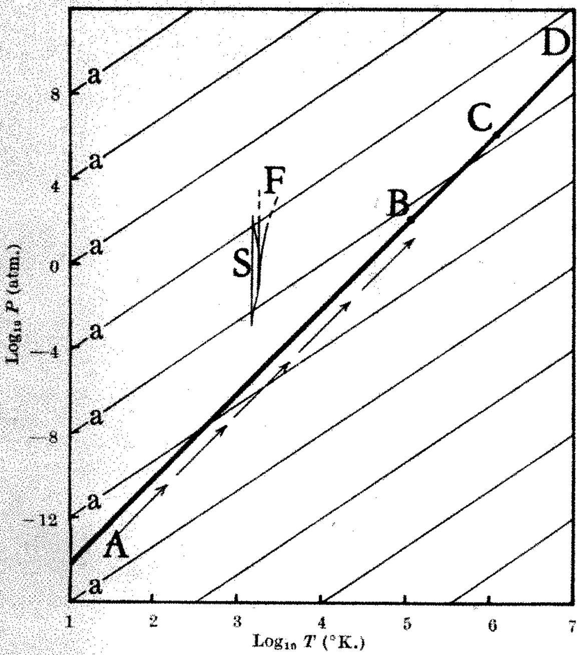

Fig. 3. A temperature-pressure plot, showing the evolutionary path (*ABCD*; arrows indicate direction) of $\bar{T}$ and $\bar{P}$ in a contracting protosun. *B* corresponds to radius = 0·2 A.U. for a protosun of constant density; *C* to the same radius for a protosun with mass concentrated near its centre. Parallel lines (*a*) are adiabats for an ideal monatomic gas, $\gamma = 5/3$. *F*, liquid field of iron; *S*, approximate liquid field of meteoritic silicates

The second pertinent question raised earlier now takes the form: if chondrules could have been manufactured near the protosun's surface when $R \sim 0{\cdot}2$ A.U., how could they have been afterwards transported out as far as the asteroid belt ($R \sim 3$ A.U.)? Hoyle's model provides the appropriate mechanism. Since, while it existed, the spun-off gaseous nebula was constantly being accelerated by the Sun's rotating magnetic field, the velocity of the gas was everywhere slightly in excess of that demanded by a circular Kepler orbit. Hoyle points out that this small excess of velocity would have acted to accelerate orbiting solid particles, moving them farther and farther from the Sun. He demonstrates that particles up to ~100 cm. in diameter would have been so accelerated.

The condensation of liquid silicate and iron droplets is also possible in the astrophysical model of Cameron[2]. This model assumes a much larger initial mass of contracting gas than does Hoyle's, and an earlier onset of rotational instability and mass shedding (at $R \sim 230$ A.U.). Cameron finds that temperatures and pressures which are adiabatically 'upstream' of the liquid fields in Fig. 3 would have prevailed in the interior of the gaseous nebula shed by the contracting protosun, as far out in the nebula as the present asteroid belt. Chondrules would have condensed in gases which convected between the hot interior and cooler surface of the nebula.

If chondrules are indeed surviving condensations dating back to the origin of the solar system, it is not unreasonable to suppose that the whole system of terrestrial planets accreted from 'planetesimals' of this type. Two consequences of such a process are worth developing: one is the opportunity it provides for chemical fractionation among the planets, the other is a possible mechanism of accretion.

It is not possible that all the non-volatile elements and compounds (silicates, iron) contained in the spun-off solar gas condensed into liquids. It is very probable that some or perhaps the great bulk of expanding, cooling solar gas missed the liquid fields delineated in Fig. 3. Certainly condensation continued at temperatures below the melting-range of silicates and the melting point of iron. Much silicate and iron must have condensed directly from vapour to solid, yielding particles of colloidal dimensions: a solid aerosol or smoke. Presumably the fine-grained material occurring between chondrules in Tieschitz was once just such a dispersed aerosol.

Urey[7] has pointed out that when the temperature of such a gas-solid mixture fell to ~ 330° C., the reaction:

$$\mathrm{Fe} + \mathrm{H_2S} \rightarrow \mathrm{FeS} + \mathrm{H_2}$$

would have proceeded until all accessible iron or else virtually all hydrogen sulphide in the system was used up. Any accessible iron remaining would completely react with water vapour when the temperature reached ~ 120° C.:

$$3\mathrm{Fe} + 4\mathrm{H_2O} \rightarrow \mathrm{Fe_3O_4} + 4\mathrm{H_2}$$

We must suppose that $\mathrm{S/Fe} < 1$ in the system, or the chondrites would contain more troilite (FeS) than they do, in place of their substantial content of iron oxide (9–15 per cent by weight FeO). (This supposition is contradicted by recent spectroscopic studies of the solar atmosphere[10], which find $\mathrm{S/Fe} \sim 5{\cdot}4$. However, this value is uncertain; the sulphur spectrum is especially difficult to assess. Also, the compositions of the present solar atmosphere and the primordial nebular gas are not necessarily identical.)

Clearly the colloidal iron 'smoke' particles would have responded immediately to the above reactions as temperature fell; but macroscopic iron grains embedded in the chondrules are shielded from reaction, and would have oxidized only if the chondrules remained in contact with an oxidizing atmosphere for an appropriately long time. (Note that iron grains in museum meteorite specimens, exposed to the Earth's atmosphere for a century or more, are only slightly oxidized.) Hence the use here of the term 'accessible iron'.

Thus, solid particles orbiting in the cooling nebula would have comprised two components: smoke or dust with all iron in the forms FeS and $\mathrm{Fe_3O_4}$, and chondrules containing varying amounts of metallic iron. Obviously the mechanism proposed by Hoyle to move particles outward in the solar system would operate more effectively on dust particles than on chondrules, and more effectively on iron-poor chondrules than on denser iron-rich chondrules. In Hoyle's model, and to a degree in Cameron's model, this fractionating effect could account for the apparent decrease in density of the terrestrial planets with increasing distance from the Sun. It might also explain the existence of two discrete groups of chondrites which differ in both total iron content and degree of oxidation[11].

Cameron's model offers a different fractionating mechanism. Internal pressures in the large gaseous nebula, he pictures, become smaller as greater distances from the centre (Sun) are considered. Note that in Fig. 3, gas cooling through the liquid fields at, say, 10 atm. would pass through liquid iron and liquid silicate fields of similar width; gas at 10^{-1} atm. would pass through a silicate field of substantial width, but liquid iron could condense only over a very narrow temperature-interval; and that from gas at 10^{-3} atm. liquid silicate could condense only over a narrow temperature interval and liquid iron not at all. Non-volatile compounds denied a liquid field, as in the latter instance, would instead condense into colloidal particles. Thus, qualitatively, the nature of the condensate would vary with distance from the Sun just as the planetary densities seem to require: the farther out from the Sun, the more colloidal matter and less chondrules; and the less iron in the chondrules.

For both Cameron's and Hoyle's models, differences in time elapsed before accretion could also have a fractionating effect on particles. Conceivably the Moon accreted at a late date from chondrules which had been exposed to an oxidizing atmosphere so long that they no longer contained metallic iron. Oxidation of all metallic iron in the 'average chondrite'[11] would depress its specific gravity from 3·58 to ~ 3·4, close to the Moon's density of 3·36.

Urey[7] has concluded that planetary accretion cannot have been the result of gravitational attraction alone, but that some "sticking agent" must have been involved. The model discussed in this article provides two possible sticking agents; one is magnetism, since both solid particle components contain ferromagnetic minerals (magnetite in the smoke fraction, iron metal in the chondrules). The other is electrostatic attraction; the enormous surface to volume ratio of the smoke particles would have maximized the effect of charge unbalance, and might have promoted a flocculation or clumping of particles (material between the chondrules in the chondrite Renazzo[3] has a grain size of ~ 200 Å.).

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A HYPOTHESIS OF VIRAL PATHOGENESIS

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Supposedly Genic Nature of Viruses

THE early history of virology was marked with much dispute concerning the question of whether the virus was an autonomous biological structure or merely a by-product of biological activity. D'Herelle[1] stood almost alone, in opposition to Northrup[2], Krueger[3], Bordet[4], and Bronfenbrenner[5], in his insistence that bacteriophage was a living organism.

In the past few decades the pendulum has swung to the opposite extreme, and many have conceived of viruses as the primitive organism which possesses all the basic characteristics of living matter. To many biologists, especially in the Western World, the gene is the fundamental biological unit and, in keeping with this view, viruses have been identified with genes. The virus has been looked upon as a gene, reproducing in the protoplast of its host by virtue of its genic nature. It is often said that the nucleic acid of the virus 'directs' its own replication and nucleic acids have been called 'living' molecules. Although these expressions are obviously objectionable because of their teleological implications, they are interesting since they suggest that the basic concept of viral pathogenesis involves the hypothesis that the virus is a gene operating in the cell by a mechanism indistinguishable from the mechanism of gene-action and diverting the metabolism of the host to its own uses by an active act of enzyme synthesis, rather than by passive growth.

This hypothesis of the 'genic' nature of viruses was the natural outcome of the current conventional view held by many indoctrinated Mendelists that the gene is the only living material and, hence, that only genes are 'self-duplicating'. This extreme view has been intensified rather than diminished by the remarkable and significant discovery that a virus contains a hereditary substance which comprises a molecule of nucleic acid[6]. It has been proposed: (1) that the nucleic acid in the virus is the homologue of a chromosome carrying genes; (2) that since the virus 'chromosome' contains only nucleic acid, genes are composed only of nucleic acid; and, hence, (3) that a knowledge of the structure of the nucleic acid in a virus would be knowledge of the 'structure' of the gene[7]. But, if viruses are non-genic particles, the study of the structure of their 'chromosomes' might not yield any information concerning the structure of the gene. It is the purpose of the present article to suggest: (1) that viruses are non-genic particles; (2) that viral pathogenesis is a process which results from the disruption of the normal mitotic cycle by the presence of the virus. Two basic assumptions of this hypothesis are: (1) that each successive phase of mitosis is initiated by a gene-controlled adaptive enzyme of the host; (2) that the presence of a virus of peculiar conformation may lead to the appropriation by the virus of nucleotides essential to these genes and, by inactivating them 'lock' the cell in a phase of nucleotide synthesis which produces those nucleotides essential to the virus and thus leads to the unrestrained growth of the virus. A subsidiary aspect of this concept is that alteration of other genes may lead to the synthesis of altered enzymes which would synthesize 'new' accessory substances producing protective or invasive envelopes for the viruses.

The hypothesis that the viruses contain genes does not consider the problem of genic balance. When one considers the demonstrated effects of genic imbalance it seems improbable *a priori* that viruses carry genes, since most of the viruses are so very well tolerated by their hosts—a circumstance which would be unexpected if viruses carried active genes.

Pattern-Dependent Heredity

Before considering the virus as a hereditary structure, it will be useful to review the earlier theories of preformation and epigenesis. The preformation theory held that the germ contained the preformed embryo. Early preformationists 'saw' the homunculus in the sperm and conceived of the sperm as a precise duplicate image of the man himself in smaller form. The atomic theory reduced the preformation theory to an absurdity and made the theory of epigenesis, which holds that germs are formed *de novo*, more acceptable to many. There are certain structures under the control of heredity that are obviously epigenetic. An example is supplied by the variety of birds' nests. Some birds make nests by laying twigs one on another, others by weaving threads into a basket and still others by lining dried grass with feathers. Each of the nests is the expression of inherited behaviour; but each nest is built without dependence on a pattern which either resembles the nest or is made of materials similar to, or like those present, in the nest. The possibility exists that many of the structures in the cell are epigenetic to the same degree that a bird's nest is an epigenetic structure.

One might distinguish epigenetic hereditary behaviour from pattern-dependent heredity (since preformation must be rejected *in toto*) by specifying whether the structure supplies the specific pattern for its reconstruction. The prime example of a hereditary structure which supplies the pattern for its own reconstruction on a molecular level is the double-stranded nucleic acid molecule which splits to provide a template capable of attracting the precise complementary components which reproduce the previously existent, double strand. If one describes nucleic acids as pattern-dependent molecules, one might suppose that the chromosome is a pattern-dependent organelle on the following hypothesis: Alexander and Bridges[8] pointed out that a crystal of alum can grow only on a pre-existent crystal which supplies the pattern capable of attracting specific components from the surrounding environment. Their theory is based on the fact that nearly every substance exerts certain residual unsatisfied attractive forces which could function to attract other molecules and, thus, explain how the patterns of a molecule of alum or of a gene may be perpetuated. Their proposal could apply to hereditary organelles which contain deoxyribonucleic acid (DNA) such as chromosomes, or to others such as chloroplasts, mitochondria, cell-walls, membranes, etc., which do not contain DNA. Bungenberg de Jong's[9] theory of coacervation shows by specific examples how the residual attractive forces of molecules can build structures comparable to biological organelles. Bungenberg de Jong's theory (explaining how hydrophilic colloids may become hydrophobic structures) and Alexander's and Bridges's concept of the duplication of a pattern by specifically oriented residual attractive forces are united in the concept of pattern-dependent heredity. Pattern-dependent hereditary structures would supply the patterns for their reconstruction while epigenetic hereditary structures would not. One advantage of this formulation is that it avoids the teleological expression—'self-duplication'.

The *B*-Chromosome of Maize

There are many indications that the inference that the gene is composed only of nucleic acid is unacceptable; the most pertinent example is supplied by the *B*-chromosome of maize[10]. The *B*-chromosome is conventional in appearance and contains nucleic acid distributed like that in normal chromosomes but carries no genes either in the euchromatinic or in the heterochromatinic DNA. It is probably not a maize chromosome but one which drifted into the cell by an illegitimate pollination. Once established in the cell, it continued to duplicate as a chromosome because the cell supplied all the necessary components for its reconstruction as well as the enzymes which reassemble them into the chromosome. The *B*-chromosome, like all chromosomes of higher organisms, is more than a strand of nucleic acid; it contains a great variety of other materials, including at least two kinds of proteins and at least three kinds of nucleic acids—one kind of deoxyribonucleic acid comprising the heterochromatin and another kind making up the euchromatin, as well as ribonucleic acid. The chromosome also contains other conventional localized structures such as the kinetochore and chromomeres. The euchromatinic and heterochromatinic regions of the chromonema of the *B*-chromosome divide synchronously at meiosis and mitosis, but the synchrony of the kinetochore is altered in the conventional way at meiosis. The synaptic behaviour of triploid *B*-chromosomes[11] is identical to the synaptic behaviour of conventional triploid chromosomes and suggests that the chromonema of the *B*-chromosome (and other chromosomes) is asymmetrical in cross-section, as well as along the long axis, since only two chromonemata synapse at any given point while the third chromonema is excluded at that point.

There is a prevailing conviction that the 'fine structure of the gene' may be revealed by genetical and biochemical analysis of the viral nucleic acid, but a complete analysis of the organization of the nucleotides in the euchromatin of the *B*-chromosome could not yield any information concerning the structure of genes.

Genes and Enzymes

Mendel[12] specified that the hereditary particle produced a 'factor' which controlled a 'character', and even Sturtevant[13], who insists that the gene be defined in terms of its location on the chromosome, speaks of 'gene-products' as if the gene were active in the control of a hereditary characteristic. It has been proposed that each gene is associated with a specific enzyme. Hence, the concept of the gene has always embodied the idea of a region of a chromosome active in the production of a 'factor', now generally assumed to be an enzyme.

Replication of Gene-Free Hereditary Structures

Since one must assume that the *B*-chromosome carried genes in the organism from which it was derived, the absence of genes in maize suggests that the active manifestation of a gene requires more than euchromatinic chromosomal DNA to make it operative. It is proposed that the *B*-chromosome, with its gene-less DNA, together with all the other complex components in their characteristic spatial dispositions, is perpetuated passively as a pattern-dependent organelle, the total length being limited by the DNA core. The molecular pattern of the nucleic acid and the organelle pattern of the chromosome together determine the spatial arrangement of the variety of proteins, and other chromosomal constituents in the reconstruction of the chromosome.

The propagation of the *B*-chromosome synchronously with the standard maize chromosomes, suggests the possibility that the prophage[14] may be homologous to the passive *B*-chromosome (rather than to an active chromosome) and that the pattern-dependent prophage may be replicated according to the Watson–Crick hypothesis with the materials and enzymes already available and present in the bacterial cytoplasm as a gene-less fragment of DNA which does not determine directly the composition of any of the bacterial enzymes. On this view, the prophage, the tobacco-mosaic virus[15], the *B*-chromosome and the kappa[16] substance would be examples of genetic, but non-genic, hereditary, DNA-containing structures which are passive in terms of gene action.

This concept is not inconsistent with the idea that the bacteriophage may transmit genes from bacterium to another, if one supposes that the function of the nucleic acid in the chromosome is to provide the base on which genes are arranged[17], rather than being the only component of the gene.

Neither the *B*-chromosome nor the prophage of lysogenic bacteria affects their hosts disastrously because their divisions are synchronized with mitosis, and they draw on the supply of nucleotides only during the phase of mitosis when these materials are being synthesized and are presumably in excess.

Evolution of Mature Infectious Bacteriophage from the Prophage

If prophage is analogous to the *B*-chromosome of maize, one might suppose that the original strands of prophage DNA were incorporated accidentally into bacterial cells and propagated for an indefinite period as relatively harmless parasites. The original accident may have occurred when several different kinds of bacteria were converted to protoplasts together. During the early stages of the evolution of bacteriophage, the newly incorporated DNA segment, the prophage, divided synchronously with the bacterial genome, using the nucleotides and enzymes available during mitosis, and remained without change in the cell during interphase. Unless some direct visual observations were possible this condition would be indistinguishable from direct attachment to the bacterial chromosome. The latter interpretation is at present exploited to explain the phenomenon of one DNA prophage fragment per bacterial nucleus; but it may have less relation to reality than the concept of synchrony.

The first important change in the evolution of the virus occurred when the prophage was altered in a manner which made it possible to multiply out of synchrony with the bacterial genome (vegetative phage). The fact that mature infectious bacteriophage readily reverts to the prophage shows that the restoration of synchrony can be achieved and implies the existence of a mechanism in the cell which makes the chromosomal nucleotides and enzymes available at a specific stage of mitosis. The phenomenon also implies that the cellular mechanism controlling synchrony is stable and difficult to disturb. When prophage first achieved the capacity for unrestricted multiplication, by whatever means, its growth in the bacterial cell led to the destruction of the cell and the release of strands of DNA, but these were not readily infectious, at least for the intact cell. Mutations of the *B*-chromosome which interfered with synchrony would be lethal and escape detection because the *B*-chromosome is transmitted only by cell-division.

The theory that cell division is controlled by the nucleo-cytoplasmic[18] ratio has been invalidated[19] by experimental analysis and a new hypothesis is required. A notable example of nuclear behaviour which is under hereditary (possibly genic) control involves the orientation of the spindles in *Neurospora*. In *N. tetrasperma* the spindles of meiosis are oriented diagonally while in *N. crassa* they are oriented parallel to the long axis of the ascus[20]. In all first generation hybrids the spindles are oriented parallel to the long axis of the ascus, but the genetical analysis of the phenomenon has not been pursued by inbreeding the hybrids. In *Saccharomycodes ludwigii* both types of orientation occur[21,22]. Although no experiments have been made to determine if the spindle orientation is gene-controlled, it seems to be a reasonable hypothesis. It is proposed that the different stages of cell division are under the control of genes which produce adaptive enzymes that successively initiate and terminate the different phases. If this were true, viruses could interfere with the sequence of the phases by the prior appropriation of the nucleotides essential to the construction of the genes controlling the adaptive enzymes responsible for the changes from phase to phase. (This hypothesis accepts the view that DNA is an essential component of a gene; but rejects the view that DNA is the only component.)

The absence of the required nucleotides would destroy the capacity of a host to control mitosis and, hence, disturb the orderly time sequence. The destructive action on the host would have survival value for the virus if it stopped the process at a specific stage in the cycle which limited the metabolism of the bacterium to the construction of those component parts which were essential to the construction of more virus. In *Saccharomyces*[23] homozygous for adenine deficiency an excessively high frequency of apparently non-specific gene-conversion occurs among genes located near each other, as if the deficiency of adenine (an essential component of nucleic acid) led to the abnormal construction of the chromosome with a non-specific effect on all the genes in a given region. (Some genes became unexpectedly normal; others became unexpectedly defective.) In a similar way, the relatively passive action of prior pre-emption of nucleotides essential to a bacterial gene could produce an effect which might appear to be due instead to the active production of new synthetic enzymes by the virus. Since growth occurs in successive cytoplasmic and nuclear phases specific DNA components must be available during nuclear growth and specific RNA components must be available during cytoplasmic growth.

The capacity for massive re-infection through the cell walls did not evolve until a type of virus nucleic acid became available which provided a suitable foundation structure on which other materials in the cells could be coacervated according to a precise and regular pattern. When such a nucleic acid multiplied out of control of the synchrony of mitosis, an epigenetic protein envelope was eventually built on the parasitic nucleic acid which enabled the virus to invade an intact bacterial cell. At this time the cycle of infection and parasitism began and natural selection led to the development of 'improved' parasites in which the accessory substances, coacervated on the nucleic acid DNA base, effected more rapid entry into a wider variety of hosts.

The only hereditary characteristic studied in the bacteriophage is the capacity of the mature virus to penetrate the wall of a bacterium. The capacity for infection is achieved by the arrangement and the specificity of the proteins present in the tail. On this concept, the structure of the mature bacteriophage depends on the maintenance of the nucleotide sequence of the standard wild-type nucleic acid, and (as Benzer's work indicates) almost any alteration of this sequence will lead to the reassembly of a bacteriophage particle without the capacity to reconstitute a virus particle capable of making an infective contact with the wide variety of receptors present in the normal host. But the changes in the sequence of nucleotides in viral nucleic acid which lead to changes in host range specificity are not necessarily comparable with gene mutation, that is, are not necessarily events resulting in a change in the kind or the amount of an enzyme produced by a gene present in the virus itself. The theory suggests that the DNA of the prophage only acts as a base on which the other components of the virus are arranged and that changes in the nucleotide sequence of the virus lead to a non-infective virus because the altered DNA does not form a base on which an effective protein envelope can be assembled.

The proposals concerning bacteriophage apply equally to tobacco mosaic virus; but the latter is transmitted primarily by abrasion and secondarily through the plasmadesmi, and the survival value of the protein which envelops the RNA core arises from the protection which the protein envelope gives the RNA core from the hydrolytic enzymes in the cytoplasm, in contrast to the protein coat of the bacteriophage which acts to effect reinfection of the host.

Does the Bacteriophage Directly Control the Synthesis of Enzyme?

It has been suggested that an analysis of 'mutations' in the bacteriophage may be used as a means of studying the 'fine structure of the gene', and that the gene may be defined in terms of variations in a sequence of the nucleotides present in DNA. During the long sequence of evolution of the *B*-chromosome in maize, many variations in the sequence of the nucleotides in the euchromatin must have occurred. Such changes in the *B*-chromosome are not gene mutations in the conventional sense since they do not result in any change in the synthesis of enzymes, because the *B*-chromosome does not control the synthesis of enzymes. If one called these changes 'mutations' of the *B*-chromosome, they would obviously not be mutations of genes.

The profound disturbances of the metabolism produced by the synthesis of mature infectious bacteriophage within the bacterium may not be the result of enzyme syntheses controlled by bacteriophage DNA but rather the result of some change in the nucleotide sequence of the bacteriophage DNA which leads to the disruption of synchrony due to the development of a greater affinity of the bacteriophage DNA for the available nucleotides in competition with the DNA of the bacterial genome. The mechanism which leads to the reconstitution of a mature bacteriophage particle may result from the selection of a specific bacteriophage DNA with the capacity to cause the disturbances which lead, first, to an alteration in synchrony and, secondly, to the synthesis of components specially adapted to coacervate on the nucleic acid base in such a way that virus particles with the widest possible host range specificity are produced. If the nucleotide sequence of prophage could disrupt synchrony by the prior appropriation of the specific nucleotides essential to the bacterial genome for the control of synchrony, then all the other events of viral pathogenesis, together with a variety of 'new' enzymes[24], could occur in the bacterial cytoplasm due to this basic disturbance in bacterial metabolism, without the nucleotide sequence of the virus having direct control over the synthesis of a single enzyme. The 'new' protein and other components of the viral envelope could result from the distortion of the enzymes previously active in the metabolism of the host by a deficiency of nucleotides essential to the construction of the 'normal' genes. The synthesis of the envelope materials provides an example of epigenetic heredity since the accessory substances do not supply the pattern for their reconstruction.

In present-day biochemical concepts of gene-controlled DNA specific enzyme (or protein) synthesis, it is not assumed that the 'messenger' RNA is derived directly from the chromosomal DNA. Some intermediary mechanism must exist, and I have proposed that this mechanism exists on the chromosome and comprises the gene[25]. The evidence that the prophage is nearly pure DNA would exclude the possibility that the prophage alone possesses the capacity to synthesize the messenger RNA but would not exclude the possibility that the prophage DNA could be replicated through pattern-determined heredity.

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KING'S COLLEGE, NEWCASTLE UPON TYNE (UNIVERSITY OF DURHAM)

NEW BUILDING FOR THE PHYSICS DEPARTMENT

By DR. R. ROSCOE

ON March 16 the new building for the Department of Physics of King's College, Newcastle upon Tyne, was opened by Prof. P. M. S. Blackett. A series of lectures were given by other distinguished physicists in order to mark this event, which represents an important stage in the development of teaching and research at Newcastle.

In this Department of Physics, as in many others, work has been carried on for years in an old building, largely designed for other purposes and only partially adaptable to modern requirements. Laboratory work has involved a constant struggle against dirt, bad illumination, poor ventilation, temperature instability, inaccessible servicing and cramped working conditions. In modern laboratories the dust of city air cannot be tolerated: it forms a conducting film on the surfaces of electrical apparatus, it fouls the surfaces of mirrors and the lenses of optical equipment, it makes engraved scales unreadable and plays havoc with the development of photographic plates. A high level of general illumination is necessary, for the inexperienced student is generally unaware that insufficient light is a major cause of inaccuracy in observation and fails to make use of sufficient local illumination. Temperature control is necessary not only for comfort in working but also for the correct functioning of sensitive instruments. But above all other requirements is a degree of flexibility in the arrangement of each laboratory so that it can be adapted to new techniques without recourse to structural alterations or makeshift devices in the years to come. For this reason, fitted furniture has to be reduced to a minimum and services have to be planned so that supplies are available at any reasonable work-place in a re-arranged laboratory. Electrical wiring festooned beneath the ceiling and power points sprouting from the floor are both unsightly and dangerous.

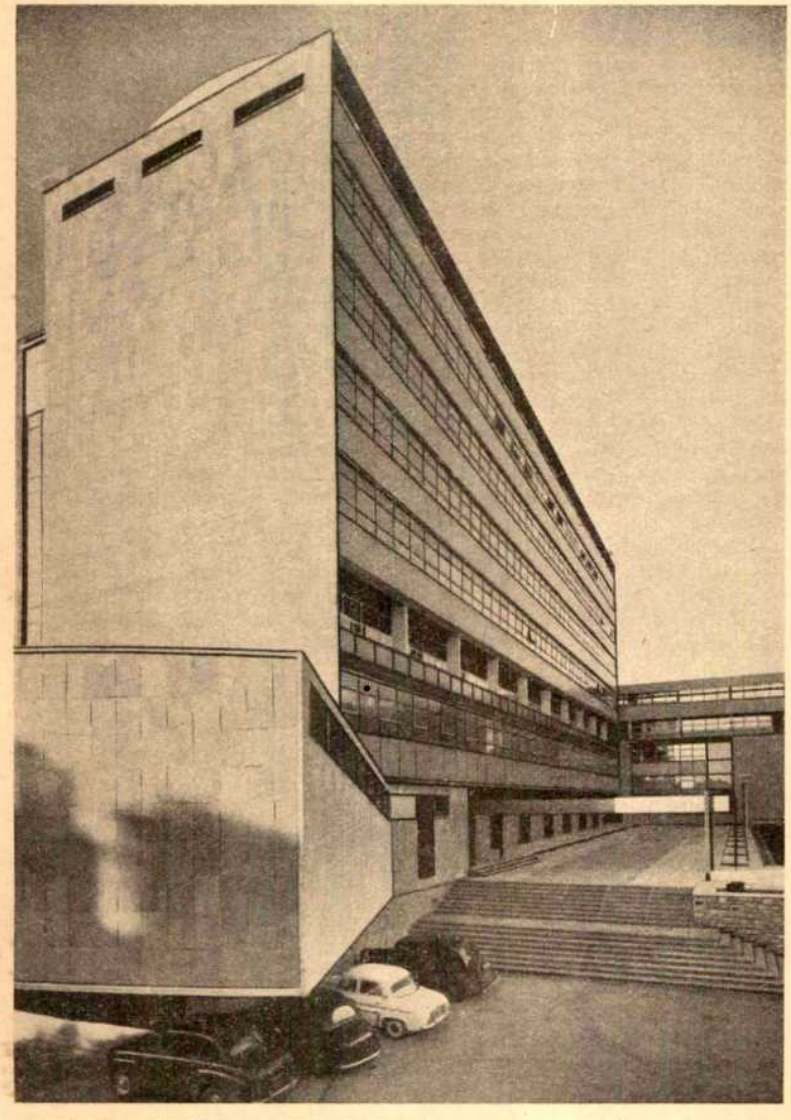
Fig. 1. View of the Teaching Block of the Physics Department, with the Research Block in the background

The architects, Messrs. Basil Spence and Partners, have collaborated closely with Prof. S. K. Runcorn, director of the Department, and his staff in designing the layout and servicing of the new laboratories. Use has been made of the simple principle that if adequate services are available at all points around the walls and the furniture consists of 8-ft. movable benches, placed each with one end abutting on a wall, a large laboratory can be filled with serviced work-places and the necessary circulation space is reduced to a minimum. Electrical supplies are carried in a metal channel with removable side-panels running just above bench-level around each laboratory: a system which has been installed by the architects with satisfactory results in other buildings. Although the main supplies to each room are controlled by master switches at the door, the trunking channel runs through from room to room so that special circuits can be arranged to connect up apparatus in different laboratories without knocking holes in walls or breaking up concrete floors. At present each laboratory is provided with a 240-V. single-phase ring-main with a lavish supply of 13-amp. points. Three-phase points are also fitted, and stabilized supplies of direct and alternating current are available in the Research Block.

The arrangements for supply of water, gas and compressed air also follow a scheme previously used by the architects. The pipe-work is enclosed by removable timber panelling, topped by a shelf 1 ft. wide running along the wall and level with the bench surfaces. The taps are mounted on this shelf, which also provides a useful extension to bench space. It has been found that a single sink is quite sufficient in a large laboratory, but taps and cone wastes must be fitted at regular intervals to provide water for cooling purposes.

The air for the building is filtered and heated before circulation. The main heating, which is thermostatically controlled, is provided by hot-water pipes running beneath the removable panels which form the ceiling. Radiators, which are unsightly and occupy valuable wall space, have been generally eliminated. The problem of providing space for the large horizontal ducts for the distribution of the heating, ventilation and other services over each floor of the teaching block has been overcome in an original manner. These ducts run along the centre-line of the building in the ceilings, the latter being sloped upwards towards the outer walls so as to allow maximum window height. The large area of window space, which contributes much to the interior appearance of the building, is double-glazed in order to reduce heat losses and secure uniformity of temperature. The high level of natural illumination is supplemented in the teaching block by fluorescent lighting. This produces a certain amount of electrical interference which has been reduced to a minimum by the use of tungsten ballast lamps. In the research block, where no interference is allowable, tungsten lighting alone is used.

The research block has presented particular problems of design because of the difficulty in foreseeing future trends of research. A standard laboratory size of 40 ft. × 20 ft. has been adopted, but variety has been achieved by dividing a number of these rooms unequally so as to give laboratories 25 ft. × 20 ft. and 15 ft. × 20 ft. These are all grouped round a central hall, two storeys in height, which can accommodate very large apparatus. Extremely heavy floor loading is allowable here, and a special hard concrete surface is provided which does not produce dust. Floor-ducting of services is necessary in this case as also in the workshops. A helium return system, consisting of a pipe network for the collection of the gas given off by helium cryostats, extends to all laboratories in the research block. A number of rooms has been set aside for special purposes: an X-ray laboratory, a glass-blower's room, a rock-cutting room, an anechoic chamber, a store for radioactive materials and several photographic dark rooms. In order to ensure maximum flexibility, however, an effort has been made to avoid specialization in laboratory design so far as possible.

The building forms a striking addition to the architecture of the University and the City. It demonstrates the possibility of satisfying both practical and æsthetic requirements within the stringent conditions imposed by present-day university finance.

OBITUARY

Dr. A. Muir

Soil science suffered a great loss by the death on February 1 at Harpenden, Hertfordshire, of Alexander Muir, deputy director of Rothamsted Experimental Station and head of the Pedology Department and of the Soil Survey of England and Wales.

Born on September 12, 1906, he was educated at Leith Academy and the University of Edinburgh, where he graduated with first-class honours in chemistry in 1928. As a research scholar he was for a time at the Edinburgh and East of Scotland College of Agriculture before going to the U.S.S.R., where he spent nearly two years at the Dokuchaev Institute of the Academy of Sciences, working with K. K. Gedroiz and B. B. Polynov, two of the most eminent Russian soil scientists. The Russians were pioneers in the infant science of pedology, and Muir was able to study at first hand their work on the soil-absorbing complex, their theories on soil genesis and their genetic system of soil classification which is based on the influence of climate, vegetation, parent material and other factors on the soil 'profile'. He also took part in a soil survey in the Crimea, thereby obtaining experience in the Russian methods of mapping. He made many lasting friendships, and the regard in which he was held by his Russian colleagues is evidenced by the fact that, at their invitation, he revisited the U.S.S.R. on three occasions since the War and was about to go again.

Muir returned home with his valuable Russian experience at a most opportune time. The Macaulay Institute for Soil Research had just been founded, and Muir was one of the small group of able young men who built up the reputation of the new Institute. The Department of Pedology, which he started, was the first in Great Britain, and his early field work developed into the Soil Survey of Scotland.

In 1945, Muir was invited to join the staff at Rothamsted to establish a Pedology Department and the following year he succeeded G. W. Robinson as head of the Soil Survey of England and Wales, the headquarters of which was then transferred to Rothamsted. From small beginnings, he built up the Soil Survey into a very efficient organization which has already mapped 7,500 square miles of England and Wales. He himself devoted much attention to the difficult problems of classification and correlation. At the same time he took a very active part in his Pedology Department, which attracted students from many countries, and he published a number of important papers, the last of which "The Podzol and Podzolic Soils" appeared recently in *Advances in Agronomy*. He became deputy director of Rothamsted in 1956.

Muir was the acknowledged authority on pedology and soil surveying in Britain, and his international reputation led to his advice and assistance being sought in many parts of the world. He became very interested in African soils and personally carried out the soil survey of the Shire Valley project in Nyasaland, publishing papers on this and on soils in Tanganyika. Other activities included an expedition to the Middle East in 1948 to study soils in relation to the corrosion of oil pipe-lines.

His keen interest in geochemistry was probably aroused by translating Polynov's book *The Cycle of Weathering* in 1937. Later, at the Macaulay Institute and at Rothamsted, he became closely associated with V. M. Goldschmidt, who had escaped from the Nazis in Norway and was engaged in writing what is now a standard treatise on geochemistry. Goldschmidt died before it was completed, and it would probably never have been published had not Muir undertaken to complete and edit it. This, in fact, involved compiling the book from Goldschmidt's very incomplete notes.

Muir took a prominent part in re-starting the International Society of Soil Science after the War, and was for a time president of the Commission dealing with Soil Genesis, Classification and Cartography. He gave invaluable help to the Colonial Office, assisting them to establish their Pool of Soil Surveyors based on Rothamsted, and making personal visits to many territories where there were soil problems. For the U.N. Food and Agriculture Organization he undertook many duties and acted as chairman of several of their committees. He renewed his interest in the Middle East as a member of a working party on Land Classification and Soil Surveys and during the past three years attended meetings in Turkey, Iraq and Iran held under the auspices of the Central Treaty Organization. Just before his death he visited India as a member of a Unesco Committee on the establishment of a Central Indian Institute for Pedology and Soil Mechanics.

Muir was a man of great vitality and vigour, equally at home in the field and the laboratory. Always ready to help others, he was held in affection and esteem by his colleagues and staff, and will be greatly missed, both as a scientist and as a man.

He married in 1934 Winifred, daughter of the late Prof. Drummond of the West of Scotland College of Agriculture, and the sympathy of their many friends will be extended to her and her two daughters.

W. G. OGG

NEWS and VIEWS

Henry Dale Research Professorship: Prof. J. L. Gowans

PROF. J. L. GOWANS, Fellow and medical tutor, St. Catherine's College, Oxford, and a member of the external staff of the Medical Research Council at the Sir William Dunn School of Pathology, Oxford, has been appointed by the Council of the Royal Society as its first holder of the Henry Dale Research professorship of the Royal Society. The professorship was established last year following a gift of £100,000 from the trustees of the late Sir Henry Wellcome to the Royal Society. Prof. Gowans will continue his researches in immunology and experimental pathology. He is well known for his solution of the controversial problem of the fate of lymphocytes and his demonstration that the lymphocyte is an immunologically competent cell.

Geology at Manchester: Prof. E. A. Vincent

DR. E. A. VINCENT has recently been appointed to the chair of geology in the University of Manchester in succession to Prof. W. A. Deer, who has transferred to Cambridge (*Nature*, 189, 793; 1961). Dr. Vincent is one of those rare and valuable scientists who belong to two disciplines: in his case, chemistry and geology. In 1936 he went to the University of Reading, intending to be a geologist, but after three years, having taken the general degree course, the War began and he was directed into the school of chemistry. For most of the War years he was an explosives chemist in charge of plant in the Royal Ordnance Factory, Bishopton. He was determined, however, to return to geology, and the acute shortage of geologists after the War gave him his chance. He joined the D'Arcy Exploration Co. as an oil exploration geologist and was concerned with logging the deep boreholes then being put down in the North of England. In 1946 he was appointed to a newly established lectureship in mineralogy and crystallography in the Durham Colleges (University of Durham), where he started a small chemical laboratory for silicate analysis and began his studies in chemical mineralogy, for which he is now so well known. In 1951 he moved to Oxford to continue similar work, first as a University demonstrator, and since 1956 as reader in mineralogy.

Dr. Vincent's research has been firmly based on his chemical analyses of rocks and separated minerals. He has developed modern methods as they became available, and in recent years has pioneered the use in geology of neutron activation and radioactive tracer methods of analysis. His earliest work was on Tertiary dykes, particularly the nature of the residual liquids produced by crystal fractionation, and he has collaborated from the chemical side in the investigation of several other aspects of Tertiary igneous rocks. His detailed studies of the iron–titanium minerals of the Skaergaard igneous complex, by analytical, optical and magnetic methods, have made him an authority on this group of minerals. Another of his interests is the distribution of trace elements, as determined by neutron activation; he has particularly worked on gold, silver, thallium and arsenic in differentiated igneous rocks. While Dr. Vincent's research has been in the field of chemical mineralogy and petrology, he has always retained a great interest in geology as a whole, and all will wish him well in his new sphere as a professor of geology.

U.S. Department of Commerce, National Bureau of Standards: Awards

DR. LEO A. WALL, of the U.S. National Bureau of Standards, has been awarded the Department of Commerce Gold Medal for Exceptional Service. A chemist in the Polymer Structure Section of the Bureau, Dr. Wall was honoured "in recognition of pioneering research in the synthesis of new fluorocarbon compounds for the preparation of organic polymers".

DR. H. W. KOCH, chief of the High Energy Radiation Section of the National Bureau of Standards, has been awarded the U.S. Department of Commerce

Gold Medal for Exceptional Service in recognition of his "outstanding scientific administration, research accomplishments in the field of scintillation spectrometry, high-energy bremsstrahlung spectra and attenuation of high energy radiation".

Silver Medals have been awarded by the U.S. Department of Commerce to the following members of the National Bureau of Standards: R. F. Ackermann, for his work in connexion with the development of high-precision techniques and equipment used for calibration of line standards of unusual length; W. W. Brown, for his part in the establishment of long-wave standard frequency broadcast stations including station *WWVL* at Sunset, Colorado; Dr. L. V. Spencer and C. M. Eisenhauer, who received a joint award for outstanding contributions to civil defence, through the development of radiation shielding engineering; Dr. D. Goodwin, for his work on measurements of the thermodynamic properties of fluid hydrogen; H. W. Lance, for outstanding performance in improvement of electronic standards, measurements and calibrations; C. E. Haven, for his contributions to dimensional metrology and standardization; G. Hefley, for his work in innovating and developing the "Loran-*C* Clock"; M. R. Meyerson, for his contributions to the field of physical metallurgy, particularly with reference to the development of gauge block steel; A. Mijares, for his contribution to the Bureau's technical programme over a long period of time; Dr. R. S. Roth, for contributions to the knowledge of inorganic phase relations in the field of non-metallic mineral products and for active and meritorious authorship; Dr. A. H. Scott, for his services in the field of precision measurements of the dielectric properties of matter; F. Sera, for his achievements in operation and improvement of the continuous broadcast of technical radio services; Dr. M. C. Thompson, for his part in the conducted research on the physics of the troposphere; Dr. W. L. Wiese, for outstanding research in the field of plasma physics.

Britain and the European Markets

UNDER the title *Producing for European Markets*, the text of five papers given at the Joint British Productivity Council–Export Council for Europe conference at the 1961 Factory Equipment Exhibition in London has now been published by the British Productivity Council (Pp. 40. London: British Productivity Council, 1962. 2*s*. 6*d*.). Mr. Bertil Neuman, a marketing consultant of Stockholm, discussing Europe's consumer goods requirements, makes some criticism of British business men, more particularly of lack of sales policy and attention to details of consumers' requirements, and urges the need to look well ahead. Mr. J. Hanning, managing director of a Paris firm, dealing with Europe's capital goods requirements, also insists on the importance of marketing capital goods which represent some technical advance, but his paper is noteworthy for constructive suggestions about the qualifications of agents, specifications, the importance of punctuality and of avoiding the use of abbreviations and ambiguities, etc. The Hon. Brian Rootes, dealing with the way Britain can face the challenge of Europe, is confident that Britain has the technical capacity, but stresses the need for rationalization of products, standardization including the adoption of the metric system of weights and measures, attention to safety and like requirements, and the framing of common policies in testing methods and like matters. The remaining two papers, by Mr. A. S. Booth and Mr. O. F. Reichwald, present case studies of experience with consumer goods and with capital goods, respectively. The former lays stress on packaging, the utilization of spare capacity, increasing the efficiency of production, and the guaranteeing of quality and delivery, while the latter emphasizes the vital importance of attention to detail and of prompt servicing.

Royal Dutch Shell Group : Chemical Enzymology Unit

A CHEMICAL ENZYMOLOGY UNIT, with a new laboratory near Sittingbourne in Kent, is being set up by the Royal Dutch/Shell Group. Jointly in charge of this Unit will be Dr. J. W. Cornforth, for some years a member of the National Institute of Medical Research, and Dr. G. J. Popják, at present director of the Medical Research Council's Experimental Radiopathology Research Unit. They will be assisted by Dr. Rita Cornforth, wife of Dr. John Cornforth. The Unit's work will embrace both basic research and research on topics of shorter-range interest—mainly, but not exclusively, in the sphere of 'biological chemistry', on which science Shell relies very largely in its development of pesticides and herbicides.

The search for new chemicals and their testing for this purpose is already carried out at Woodstock Agricultural Research Centre, near Sittingbourne. Close to it is the Tunstall Toxicology Laboratory. The new Enzymological Laboratory brings into this same area a third focal point—that for basic chemical studies in relation to life processes.

Dr. Popják will join the Unit on October 1 and Dr. and Mrs. Cornforth on November 1. Their laboratory is expected to be completed on April 1, 1963. In addition to the permanent staff, a limited number of scientists working in kindred fields in the universities will be welcome there for periods of research up to about three years in duration.

Register of Research in the Human Sciences, 1960–61

UNDER the heading *Register of Research in the Human Sciences, 1960–61*, the Warren Spring Laboratory of the Department of Scientific and Industrial Research has issued a most useful compendium concerning research bodies within the United Kingdom dealing with the human sciences (Pp. xi+44 (11 plates). London: H.M.S.O., 1962. 10*s*. net). Included within this title of 'human sciences' are such subjects as anatomy, cultural anthropology, industrial relations, management, medical sociology, physiological psychology, physiology, political science, psychology, social disorganization (criminology), social economics, economic history, social psychology, sociology and demography, and various marginal entries. The major section details the various fields of research, giving the address, telephone number and director of the research institutes, councils, laboratories, etc., concerned. Under these are listed the projects which have been or are being undertaken, including a brief description, the name of the person (or research committee) responsible for the research, research workers, approximate dates of starting and completion of interim and final reports, bodies concerned with the inquiry, etc. An index of institutes and their entries, dealing with universities, technical colleges, other institutes and private research workers, is supplied, and also a subject-index. The register is a most useful publication and an essential reference work to those who have to, or wish to, keep track of research in the human sciences, to those who wish to know where the effect of lighting installations and type of street surface visibility is being investigated, where retirement policies for industry are considered, what goes on in the Centre for Urban Studies, or what are the publications on smoking from the Department of Public Health and Social Medicine, Edinburgh, etc.

Films on Natural History Subjects

The Intelligence Unit of the Council for Nature has recently issued a list of 16-mm. films on natural history subjects, Part 1: *The British Isles and Europe*. The list has been prepared for the benefit of natural history societies, teachers and others wishing to prepare programmes and educational courses with a natural history content. Films aimed at very young children are omitted and those for children aged between 9 and 15 are indicated. Films are listed in the general headings of: world of life series, secrets of life series, secrets of nature series; ecological films; films on British regions; Europe; birds; fish, reptiles and amphibians; invertebrates and plants. Part 2 will be prepared during 1962 and will cover the rest of the world. Details are supplied of the distributors, whether the films are sound or silent, or black-and-white or colour, running times and whether or not teachers' notes are supplied. The cost of hiring these films is indicated when necessary. Copies of the list are available from the Council for Nature, 41 Queensgate, London, S.W.7.

County Naturalists' Trusts

The Council of Nature reports that, during 1961, naturalists' trusts have been founded in Glamorgan, Sussex, Hampshire and the Isle of Wight, Devon, Dorset, Suffolk, Gloucestershire and Derbyshire (*News for Naturalists*, 3, No. 2; January 1962). In addition, the West Wales Field Society has decided to turn itself into a naturalists' trust. Plans for trusts are also going forward in Northumberland, Durham and the Lake District, and discussions are taking place in several other countries. All counties south and east of a line drawn along the northern and western borders of Yorkshire, Derbyshire, Staffordshire, Worcestershire, Gloucestershire, Somerset and Devon are now covered by County trusts, except for Northamptonshire, Nottinghamshire, Somerset and Wiltshire.

Project Mohole Exhibition at the Geological Museum

The Project Mohole is featured in a special display at the Geological Museum, Exhibition Road, South Kensington, London, S.W.7. The display provides background information on the American plan to drill through the Earth's crust underlying the ocean to the Mohorovičić discontinuity and to the mantle beneath. The 'Moho' is the level, some 20,000 ft. below the deep ocean floor, at which there is a marked change in the velocity of earthquake waves, and is taken to be the base of the Earth's crust. The project is being carried out for the U.S. National Academy of Sciences National Research Council by the American Miscellaneous Society (see *Nature*, 184, 140; 1959). The central feature of the exhibit is a vertical 25-ft. scale section of the Earth's crust beneath the ocean with a model of the drilling barge and drill-string to the same scale. A cut-away globe summarizes the present state of knowledge of the Earth's interior, and a large coloured diagram shows the constitution of the continental and oceanic areas of the Earth's crust. Details are also given of the methods used in the successful experimental drilling carried out during 1961 in the Pacific Ocean in 11,700 ft. of water. A full-sized model of the diamond-armed drilling bit used in the experimental drilling is accompanied by specimens of the industrial diamonds used in this type of bit. The display will remain on show until the completion of the project, and it is intended to incorporate results as they become available.

The South African Museum

In the annual report of the South African Museum for the period April 1, 1960–March 31, 1961, reference is made to forthcoming building operations which will give additions to the main building and a new administrative block (Pp. vi + 34. Cape Town: South African Museum, 1961). In view of the restoration of the old Supreme Court to house the Cultural History Museum, it is suggested that it is linked with Koopmans de Wet House to form a separate entity with its own Board of Trustees and Director. With the aid of a trawler, the marine biologists at the Museum were able to obtain some valuable results in their research on tunny biology. Other research projects include serial sectioning of the skulls of many Deinocephalia and the Middle Triassic dinosaurs and advanced mammal-like reptiles. A large diorama depicting the bird life of Malgas Island, and exhibition on Bushman music and "Cape Town 1910–1960" have been completed during the year. As with so many recent reports on museum work, the format of cover, printing and illustrations are modern and attractive.

Computer Methods, Machines and Services

The proceedings of the sixth congress on theoretical and applied mechanics, held at Delhi in December 1960, have just appeared in print (Pp. xi + 316. Kharagpur: Indian Society of Theoretical and Applied Mechanics, Indian Institute of Technology). The papers range from mathematical analyses of magneto-hydrodynamic shock-waves to practical studies of flange connexion design for pressure vessels. An addendum contains material on a symposium entitled "High-speed Computation Methods and Machines" that was included in the programme of the meeting; it dealt with both analogue and digital computation, and gives a picture of some of the computing resources available in India. The January issue (Nos. 15–16, October 1961–January 1962) of the *Bulletin of the Provisional International Computation Centre*, Rome, contains interesting papers on machine translation and on learning machines. The *Bulletin*, of which this is the fifteenth issue, performs a useful service in documenting European computer activities and in providing detailed information on the facilities available in the various laboratories.

Age of Leads in Britain

Recent work on the abundance of lead isotopes in British galenas suggests the existence of at least six distinct periods of mineralization. These were: Caledonian, probably mainly of Middle and Upper Silurian age; Devonian; Lower or Middle Carboniferous; Hercynian, probably Lower Permian; Mesozoic, probably Upper Triassic; Upper Mesozoic, probably Upper Jurassic or Cretaceous. It is interesting to find that there is no isotopic evidence for Tertiary sulphide mineralization within the British

Isles. These conclusions follow from determinations of lead isotope abundances of 98 galena specimens from Great Britain and Ireland, the ages being calculated using the Holmes–Houtermans model (*Philosophical Transactions of the Royal Society of London.* Series A: Mathematical and Physical Sciences. *Lead Isotope Abundance Studies on Mineral Occurrences in the British Isles and their Geological Significance.* By S. Moorbath. Pp. 295–360. London: Royal Society. 21*s*.). These model ages show good agreement with published values of the absolute ages of genetically associated igneous rocks. Since most British galenas are vein-type deposits of epigenetic type which have traversed sedimentary and igneous rocks, this agreement suggests that the assumptions of the Holmes–Houtermans model do not contradict the hypothesis of a crustal rather than a mantle origin for lead ores. This view is in harmony with one of an early differentiation of the Earth's crust and repeated rejuvenation of ancient fold belts, rather than with one of continual continental accretion around marginal mobile belts.

Forest Research in Sweden

THE recent volume of reports from the Forest Research Institute of Sweden is devoted to a variety of subjects, and Scots pine is prominent among them (*Meddelanden från Statens Skogsforskningsinstitut,* Band 49, 1960–1961. *Reports of the Forest Research Institute of Sweden,* Vol. 49. Pp. iv+580. Stockholm: Statens Skogsforskningsinstitut, 1961). It has been found that mortality among pine seedlings caused by parasitic fungi can be prevented by pre-sowing treatment of the soil with methyl bromide. Storage of pine pollen is best done at very low temperatures (–18° C.) if the fertilization capability is to be preserved. Although there is nothing surprising in the conclusion reached that heavy low thinnings for Scots pine are economically and biologically superior to heavy crown thinnings, this is supported by a description and analysis of thinning experiments over a period of 54 years. A very good article on the sawfly, *Diprion* (*Microdiprion*) *pallipes* Fall., is well illustrated and includes an excellent colour plate. Another article on forest entomology is concerned with the larch shoot moth, *Argyresthia laevigatella* H.S. The reports are attractively presented and well supported with references. Four reports are in English, and of the remaining six there are English summaries for four and German summaries for two—those on forest entomology.

Indian Forest and Forest Products Terminology

PART 1 of a publication recently issued as an *Indian Forest Record,* entitled *Indian Forest and Forest Products Terminology,* deals with the general subject of forests (Vol. 10, No. 6. Pp. iv+125–215. Delhi: Manager of Publications, 1961. Rs. 6.00 nP., 9*s*. 6*d*.). This is a glossary of some 2,000 terms used in sylviculture, protection, mensuration, management, ecology, soil science and allied subjects. Most of them have been taken from the *British Commonwealth Forest Terminology,* while others have been gleaned from other well-known appropriate glossaries, and there are a few terms which are particular to India. The terms included in this glossary are comprehensive and correct, but 'assortment table' is perhaps too narrowly defined. It can be argued that 'crop' is used in an unfortunate sense at times, but this is a common failing nowadays when the growing of trees is too much likened to an agricultural crop. On the other hand, the correct 'to bark' is insisted on and improper 'to debark' is, according to the glossary, to be deprecated. Three appendixes provide classifications of the principal sylvicultural systems, trees by crown classes and thinning densities. This glossary is obviously intended for use in India and there it will be welcomed; it is a pity that a more convenient format and hard covers had not been provided. Part 2, dealing with forest products, will be published at a later date.

Gerontology and Geriatrics in the U.S.S.R.

SOME of the present-day developments in gerontology and geriatrics in Russia were discussed at a conference in Moscow during 1960. A brochure issued by the Division of General Medical Sciences, Center for Aging Research of the U.S. Department of Health, Education and Welfare, provides a translation of all the papers in summary form (Public Health Service Publication No. 884. Pp. viii+81. Washington, D.C.: Government Printing Office, 1961. 35 cents). These range from activities of the Republic Gerontological Society of the Kazakh S.S.R.; structural aspects of ageing; psychiatry of old age as an independent branch of psychiatry; organization and methods of conducting group physical culture activities among middle-aged and elderly persons; the effect of physical exercise on the cardiovascular system of elderly persons; the causes of death among elderly persons in one region of an industrial city of Zazakhstan; senile dementia; features of cataract surgery on persons 80–118 years of age; treatment of certain diseases of elderly persons with a 2 per cent solution of novocaine; the work capacity of persons with cerebral atherosclerosis; and premature senility and the treatment of cerebral artherosclerosis.

The Origin of Life

THE latest version of A. I. Oparin's hypothesis of the origin of life on the Earth is given by its author in a short article (*Priroda,* 7, 5; 1961). The story begins with an abiogenic generation of amino-acids in the primitive atmosphere, their gradual synthesis into proteins and the eventual formation of the 'primitive broth', composed of polymolecular 'concervate' drops, which eventually give rise to bacteria and blue-green algae. Future aeronauts may discover life existing on other planets, and carbon meteorites suggest the possibility of such an occurrence. Wider concepts of the probability of life in the cosmos, the progress of evolution and the future of mankind are discussed elsewhere by A. I. Baumstein (*Priroda,* 12, 76; 1961).

International Geological Congress:
Twenty-second Session

THE twenty-second session of the International Geological Congress will be held in New Delhi during December 14–22, 1964. The following subjects have been provisionally selected for discussion, each subject corresponding to a section of the Congress: (1) geology of petroleum; (2) geological results of applied geophysics; (3) Cretaceous–Tertiary boundary including volcanic activity; (4) rock deformation and tectonics; (5) genetic problems of ores; (6) minerals and genesis of pegmatites; (7) plateau basalts; (8) Tertiary mammals; (9) Gondwanas; (10) Archæan and Pre-Cambrian geology; (11) Himalayan and alpine orogeny; (12) isostasy; (13) charnockites; (14) laterite; (15) sedimentary geology and sedimentation; (16) other subjects. The programme will also include excursions. Copies of the first circular are obtainable from the Assistant Secretary, Royal Society, Burlington House, London, W.1, or direct from the Secretary General, Twenty-second Session, International Geological Congress, Geological Survey of India, 27 Chowringhee, Calcutta 13.

Carlsberg–Wellcome Travelling Research Fellowships, 1962–63

APPLICATIONS are invited for the Carlsberg–Wellcome travelling research fellowships, 1962–63, the object of which is to encourage co-operation, on an exchange basis, between Danish and British research workers in any branch of the natural sciences which has a bearing on human and animal medicine. One fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Denmark and one annually to a Danish candidate for a year's work in the United Kingdom. The stipend may range from £1,000 to £2,000 per annum (or the equivalent sums in Danish kroner), travelling and some incidental expenses being provided in addition. Further information can be obtained from the Assistant Scientific Secretary of the Wellcome Trust, 52 Queen Anne Street, London, W.1. Applications must be submitted before April 19.

Wellcome–Swedish Exchange Research Fellowships in Medical Science, 1962–63

APPLICATIONS are invited for the newly created Wellcome–Swedish travelling research fellowships, the object of which is to encourage co-operation, on an exchange basis, between Swedish and British research workers in any branch of the natural sciences which has a bearing on human and animal medicine. One fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Sweden and one annually to a Swedish candidate for a year's work in the United Kingdom. The stipend may range from £1,000 to £2,000 per annum (or the equivalent sums in Swedish kroner), travelling and some incidental expenses being provided in addition. Further information can be obtained from the Assistant Scientific Secretary of the Wellcome Trust, 52 Queen Anne Street, London, W.1. Applications must be submitted before April 19.

University News: London

TITLES have been conferred on the following in respect of their posts at Birkbeck College: professor of mathematics, on Dr. C. H. Dowker; professor of experimental physics, on Dr. W. Ehrenberg; professor of electrochemistry, on Dr. D. J. G. Ives; reader in mathematics, on Dr. Paul Vermes. The title of professor of anatomy has been conferred on Dr. A. D. Morgan, in respect of his post at Westminster Medical School.

California, Berkeley

THE building housing the new Department of Nutritional Sciences on the Berkeley campus of the University of California has been named the Agnes Fay Morgan Hall in honour of Prof. Agnes F. Morgan, emeritus professor of nutrition and chairman of the Department of Home Economics during 1918–54. Prof. Morgan, well known for her work in nutritional science, also holds the Garvan Medal in chemistry, the Borden Award in nutrition, and is a fellow of the American Institute of Nutrition.

Announcements

DR. CHARLES H. TOWNES, provost of the Massachusetts Institute of Technology, formerly professor of physics in Columbia University, New York, has been awarded the John J. Carty Medal of the U.S. National Academy of Sciences, in recognition of his pioneering work in the development of the maser (microwave amplification by stimulated emission of radiation).

DR. MONROE EDWARD SPAGHT, president of the Shell Oil Co. in the United States and a director of the American Petroleum Institute, has been appointed president of the Society of Chemical Industry (U.K.) for the year 1962–63. He has been a member of the Society since 1950 and was chairman of the American Section during 1956–57.

A SEMINAR on "Physics and Computers", arranged by the American Institute of Physics, will be held in New York City on May 22. Further information can be obtained from the American Institute of Physics, 335 East 45 Street, New York 17.

THE spring conference of the British Association for Commercial and Industrial Education on "Industrial Training—Whose Responsibility ?" will be held in London on May 10. Further information can be obtained from the Conference Secretary, British Association for Commercial and Industrial Education, 26A Buckingham Palace Road, London, S.W.1.

THE third general meeting of the International Mineralogical Association, sponsored by the Mineralogical Society of America, will be held in Washington, D.C., during April 17–20. In addition to business meetings and a session devoted to a general selection of papers, there will be two symposia on "Layered Intrusions" and on "The Mineralogy of Sulphides". Further information can be obtained from Alvin Van Valkinburg, National Bureau of Standards, Washington 25, D.C.

A CONFERENCE on "The Design of Talking and Writing Machines for the Rehabilitation of Communication Disabilities", organized by the Foundation for Medical Technology in conjunction with the Institute of Physical Medicine and Rehabilitation, will be held at the Institute of Physical Medicine and Rehabilitation, New York, on May 16. Further information can be obtained from Mr. C. Berkley, Foundation for Medical Technology, 2 East 63rd Street, New York, 21, N.Y.

AN international symposium on "Organic Chemistry applied to the Study of the Natural Products excluding Steroids and Polypeptides", to commemorate the seventy-fifth anniversary of the foundation of the Société Chimique de Belgique, organized by the Société under the auspices of the International Union of Pure and Applied Chemistry, will be held in Brussels during June 11–14. The symposium will be divided into five sections: structure of new natural products; methods used for the determination of chemical structures; syntheses and chemical reactions; biosynthetic theories; mode of action of naturally occuring compounds in biological processes. Further information can be obtained from the secretary of the symposium, Dr. F. Martin, 68 rue Berkendael, Brussels 6.

ERRATUM. In the heading of Table 1 of the communication "Gastric Esterases of Pigs", by R. A. Evans and D. A. Stansfield (*Nature*, 190, 1110; 1961), the words "of Triacetin" should be omitted.

AN INTERNATIONAL COMPARISON OF SCIENCE EDUCATION

MR. J. L. LEWIS, senior science master at Malvern College, has examined methods of teaching science in the United States, Western Germany and the U.S.S.R. In the first of two articles, he compares the relative merits of those methods with such as are used in English schools (*Esso Magazine*, Winter, 1961/1962).

Education in the U.S.S.R. is compulsory during the ages of 7–18 and the same course is followed by every child for the first eight years of his or her time at school. This basic course starts with the Russian language, Russian literature and mathematics. At the age of nine a foreign language is introduced, then history, geography, Leninism–Marxism theory and biology. A little later physics is begun and, a year later, chemistry. In other words, the Russian educational curriculum is fairly broad and the same standard, equivalent to the General Certificate of Education in England, is attained by every child. There is no streaming and no examination at 11-plus.

Mr. Lewis claims that pupils in Russian schools believe passionately in work. It is even a privilege, not a punishment, to be kept in after school. Education is the one way to open up the future for the Russian boy or girl, the one way to enable them to avoid spending their lives in factory or in field. That is an inducement. There is also the passion for the acquisition of knowledge which is too frequently lacking in English schools. Mr. Lewis includes in his picture of Russian life street corners abounding in bookstalls filled with books on physics, mathematics and astronomy. Throughout the day trestle tables are erected and crowds collect around their array of books. In the Moscow Metro and in the buses people are reading books on physics, mathematics and astronomy. Education in physics really matters. He rightly or wrongly questions whether it really does matter in Britain.

Western Germany has long had its tradition of good physics teaching. Physics is respected as a subject of major importance for the second half of the twentieth century and plays its part in the education of every boy and girl in the German *gymnasiums*. Could this be claimed for English grammar schools, where the educational system is still fundamentally classical in tradition? Latin, from the age of eight to sixteen, for several periods a week is said to provide "a broad liberal education". Two periods a week of science at the age of eleven have been called "gross specialization". It would seem that the English people are too complacent when others are believing passionately in the slogans "Work, work and work" and "Physics is life".

Other dangers, Lewis believes, also beset us. Teachers often tend to teach what they themselves were taught. This has doubtless contributed to the surprisingly few changes in the content of the school science courses despite advances which have been made. Physics has remained in its watertight compartments—heat, light and sound; magnetism and electricity. There seems to be an unawareness that there is any modern physics of relevance to the schoolboy or girl. There are, of course, still those who say that all modern physics should be left to the universities, but they forget how small is the proportion of those at school who will study physics at the university. In the second half of the twentieth century every boy and girl leaving school ought to have some knowledge of radioactivity and X-rays. The new syllabuses recently proposed by the Science Masters' Association have included some of this: however, the tragedy is that it has taken sixty years to begin to get topics included despite the fact that the U.S.S.R. and Germany have taught modern physics in schools for many years. Mr. Lewis rightly poses two questions which lead from such developments. What of equipment in English schools? What of laboratories? On the whole there is no great problem in England with regard to laboratories; however, with equipment, the situation is not satisfactory.

Throughout Western Germany there is an immense wealth of large-scale demonstration equipment for the teaching of science. University lectures are always accompanied by demonstrations which naturally stimulate and maintain the interest of the student. How often in England does the student in the university or the pupil at school have to be content with talk and chalk and occasionally the lantern slide. How easy it would be to capture the imagination of the boy with some of the excellent equipment available in Germany.

Good demonstration equipment is expensive. But in Germany, after the Second World War, it was decided that modern physics should be taught in German schools. To make this possible the Federal Ministry of Atomic Energy prepared a plan to make available 18 million D.-marks (just less than £2,000,000) and the funds were distributed at the rate of 12,000 D.-marks or more than £1,000 per school. This was given equally to State schools, municipal schools and to private schools, on condition that it was to be used on equipment for developing the teaching of modern physics in the last three years in the *gymnasium*. This special grant transformed the teaching, and it accounts for the expensive demonstration equipment used. Mr. Lewis warns that, despite the efforts now being made, England, and Britain as a whole, is in great danger of lagging behind.

Mr. Lewis now comes to the question of the teachers and whether or not, in England, the best use is made of the material available.

The English science teacher, in general, is given complete freedom to present his subject as he wishes. This will lead to the best results if the teacher is good and if he has sufficient time to think out his presentation. The trouble is that there are never enough good teachers and these seldom have sufficient time. The problem is also very real in the U.S.S.R., where it is conceivable that there will never be enough teachers, and consequently they have set about providing the material to enable indifferent teachers to teach well. The Russian science teachers are teaching as much science in less time than their British counterparts due to the fact that the teacher is given more guidance and far more aids to teaching. If the means of teaching science subjects more efficiently were available, the English schoolmaster might achieve much more in fewer periods and the shortage of teachers might not be so acute.

In the U.S.S.R. there are research institutes for devising school experiments and designing apparatus. In England this is usually done by the busy schoolmaster in any spare moments he may have, and, as Mr. Lewis states, "We cannot afford to continue in this amateurish way". Not only is the Russian teacher provided with a complete set of demonstration apparatus for his course, but he is well supplied with films on the subjects being taught. All this does much to help the teacher: it does not impede the good teacher from developing his own ideas, but it does enable the indifferent teacher to achieve a minimum standard, to teach with a minimum of efficiency.

Another serious problem is that, in England, the assumption is made that a young man merely has to take his science degree and can then teach for the next thirty years without ever returning for further instruction, refreshment or guidance. In the United States last year more than 17,000 high school science teachers attended summer institutes. In England, less than a hundred attended Ministry of Education courses for grammar school science teachers. The American summer institutes last from six to eight weeks; the courses in Britain usually last less than a fortnight.

Mr. Lewis has dealt with some of the problems confronting science education in England to-day. They are serious indeed if England, and Britain as a whole, is not to lag far behind what is being done in other countries.

EXPERIMENT IN EXAMINING FIRST-YEAR CHEMISTRY STUDENTS

In teaching chemistry, as with other scientific disciplines, there is a number of objectives. More often than not these objectives remain implicit in the minds of the lecturers. If they are made more explicit, it is usually in rather inexact and personal terms.

A general taxonomy of educational objectives has been developed by B. S. Bloom and others at the University of Chicago which contains six major classes: (1) knowledge; (2) comprehension; (3) application; (4) analysis; (5) synthesis; (6) evaluation. These six classes are subdivided, defined and illustrated by example. Several members of the University of Melbourne Chemistry Department explored the adequacy of this taxonomy for chemistry applying it to old examination papers, and the following simplification of Bloom's definitions of classes was produced:

(1) *Knowledge*, involves simply the recall of material directly taught.

(2) *Comprehension*, involves understanding of the material taught, to the extent that it is recognized when presented in any form.

(3) *Application*, involves the use of such material in a new situation.

(4) *Analysis*, where the breakdown into detail involves both the facts and their relation in a new situation.

(5) *Synthesis*, where previously presented material is assembled to create something new.

(6) *Evaluation*, involves judgments using undefined criteria about values in a chemical situation.

These categories, so defined, were found to cover most questions on a number of examination papers, although one question would often involve the components of several classes.

With this experience as a basis, a first-year paper was set covering all six classes. The questions were so designed that the percentage marks obtained for each class were easily extracted from the total, in the course of normal marking procedure. The examination was taken by 150 students, and as all the questions set were compulsory, the performances were directly comparable. The results have been described by P. J. Fensham of the University of Melbourne (*Vestes*, 4, No. 4; December 1961).

The customary way of assessing performance was in terms of the total percentage for the whole paper. This method gave the following results: honours (1st, 2nd and 3rd combined), 25; pass, 93; fail, 32. These three grades were then analysed by performance in the various classifications, particular attention being directed to the difference between honours and pass, and between pass and fail. It was clear that the major factor distinguishing between honours and pass was better performance of the honours group in classes 1, 2 and 3. This group averaged 78 per cent in the first three classes and 70 per cent for the last three, whereas pass students averaged 54 per cent in both sets of classes. However, twelve students who were graded as pass students achieved at least 66 per cent in classes 4, 5 and 6, whereas eight of the students who were awarded honours all scored less than this for those same classes.

A reasonable hypothesis for this type of examination might be that honours should be awarded to those who perform better at the more complex objectives of the last three classes, assuming a certain competence in the first three. If so, the experiment suggests that the traditional grading by overall percentages involves serious errors in distinguishing between honours and pass students. (A single paper of this type, however, appears unsuitable to make the distinction, for 70 per cent of it had to be devoted to questions in the first three classes, if the material of the course had to be reasonably covered. This meant there was not enough room left in the paper for a sufficient number of questions of classes 4, 5 and 6.)

So far as the distinction between pass and fail is concerned, it might be reasonable to award a pass for a minimum level of performance in the first three classes, ignoring performance in the other classes for this purpose. The results showed that all pass and honours students obtained at least 40 per cent in classes 1, 2 and 3, whereas none of the failed students scored more than 40 per cent in these classes. Thus there was no difference in the result—so far as pass and fail were concerned—using this assessment based on the classification as compared with the overall percentage method. There was, however, a considerable difference in the number of honours grades awarded, for different taxonomic gradings, one of which is as follows:

	Honours	Pass	Fail
Overall percentage grading	25	93	82
Taxonomic grading	40	78	82

The conclusion appears to be that considerable care is needed, particularly at the upper end of the scale, if examining is to provide an accurate assessment of what is sought from students.

SALARIES OF UNIVERSITY TEACHING STAFFS IN BRITAIN

IN a written answer in the House of Commons on March 14, the Chief Secretary to the Treasury, Mr. H. Brooke, stated that the Government had considered the recommendations of the University Grants Committee for recurrent grants for the five-year period 1962–67. It had also considered substantial increases in the salaries of university teaching staffs with the determination to effect the most rapid development of higher education that is consistent with other claims on the nation's prospective economic resources in the next five years and with the Government's policy for incomes. These considerations had made it necessary to depart from the Committee's recommendations and he had informed the Committee that, subject to annual approval of the Estimates by Parliament in the normal way, it should plan on the basis of the following recurrent grants from August 1 next: 1962–63 £56 million; 1963–64, £60·5 million; 1964–65, £65·5 million; 1965–66, £70·5 million; 1966–67, £76·5 million. The grant for the present university year is £49·5 million. These figures exclude provision for rates, which will be financed by special grants outside the quinquennial settlement, and the cost for the universities of the Government's recent request for an increase in the intake of medical students had yet to be finally settled and would also be additional to these figures.

Mr. Brooke reaffirmed the undertakings given last year on the rapidly increasing provision for non-recurrent grants and added that the awards to university students from public funds was also continually growing. In sum, the aggregate cost of the universities to public funds had increased from £50 million to £104 million during the past five years and might increase by at least £50 million in the next five years.

The universities' estimates, including those of the seven new universities authorized during the present quinquennium, indicated that, given adequate financial support, they would be prepared to work to an expansion of the total student population from about 111,000 in 1961–62 to about 150,000 in 1966–67. Mr. Brooke had asked the University Grants Committee to inform the universities that it was the Government's wish that the additional funds should be applied so far as possible to achieving the figure of 150,000. The Government's aim was to continue the expansion to about 170,000 places by 1973–74. These recurrent grant figures are based on rates of academic salaries which came into effect in January 1960. The Government had taken carefully into account the strong arguments advanced by the University Grants Committee for a substantial increase in these salaries, along with the need to expand the teaching staff in relation to the increase in student numbers, but did not feel able to authorize an increase of more than 3 per cent in the bill for academic salaries. Scales based on an aggregate increase of this order would be authorized from April 1, 1962, and would cost about £1 million extra in the first full year. The scales would be reviewed afresh next year. Provision for university expansion was only part of the resources that the Government was devoting, on an increasing scale, to higher education; and all these programmes might need to be re-examined in two or three years' time in the light of the report of the Robbins' Committee on Higher Education. The Government's proposals for the first year of the new quinquennium and for the increase in salaries would necessitate a supplementary estimate of some £4·5 million for 1962–63.

RHEOLOGY OF CLAY SOILS

AT the meeting of the Cambridge Rheology Club held on February 5, Mr. W. J. Thompson (Department of Engineering, University of Cambridge) spoke on the "Rheology of Clay Soils". He pointed out that it is current practice in soil mechanics to use the triaxial apparatus to study the shear characteristics of a soil. In this apparatus a cylindrical sample of soil can be subjected to any desired combination of axial and hydrostatic stresses, the sample being either allowed to drain during the deformation process (drained test) or be maintained at a constant volume (undrained test). The allowable shear strength value used in soil mechanics computations is taken as some proportion of the maximum shear stress τ_m obtained in a constant axial strain-rate triaxial test, although corresponding tests with constant axial stress have indicated that for stresses $> 0{\cdot}8\ \tau_m$ a state of structural disintegration is obtained. Consideration should therefore be given only to the region of the stress-range ($< 0{\cdot}8\ \tau_m$) within which the soil would behave in the field. Present shear theories are generally based on the results of constant strain-rate tests and neglect time considerations.

The existence of a yield-point in the shear deformation of clays, as investigated by Bingham and Scott

Blair, is not generally accepted. Tests by Gueze and Tan with a torsion plastometer, and by Haefeli, have indicated the existence of yield-point and at higher stresses a pronounced tendency for the development of a state of structural disintegration as in triaxial tests. It is considered by several workers that the apparent yield-point is due to measurement inaccuracies and that the true flow-curve is of the thinning thixotropic form observed by Ostwald. Experimental evidence appears to confirm the conclusion reached by Reiner that volume deformation is generally a combination of elastic, viscous and plastic processes. The rate of the water expulsion occurring with volume decrease in saturated soils is governed by Darcy's law.

The large strain behaviour associated with shear-failure may be considered as of an essentially plastic nature, this being generally assumed in the determination of allowable footing loads which correspond to much smaller strains. Simple viscoelastic models are proposed for small strain volume change and shear deformation caused by stresses $< 0{\cdot}8\ \tau_m$, based on available experimental evidence. On the assumption of isotropy, these models can be combined for any stress and strain conditions. The predicted behaviour for one-dimensional compression, in which a laterally confined cylindrical sample is subjected to a uniform axial stress, is in reasonable, but not exact, agreement with the observed data. The generally used analysis of one-dimensional compression data is based on the assumption that the structure of the clay particle behaves as an isotropic elastic material for any given increment of load. The inclusion of anisotropic considerations is of excessive mathematical complexity.

The study of the rheology of clay soils is still at a preliminary stage, and much detailed experimental work remains to be done before further progress can be made. The use of apparatus enabling precise measurement of the imposed conditions is essential.

The next meeting of the Club will be held at the Technological Research Station, Spillers, Ltd., Station Road, Cambridge, on May 7, when Mr. R. G. King (Farol Research Engineers) will speak on "Viscoelasticity and the Weissenberg Rheogoniometer". The instrument will be available for the two days following the meeting for practical demonstrations on materials which members of the Club or visitors are invited to submit. Notice of this should be given in advance to the Secretary of the Club at the above address. H. G. MULLER

PHENOLIC ALKALOIDS

A SYMPOSIUM organized by the Plant Phenolics Group was held at the School of Pharmacy, University of London, on January 9, and was concerned with the alkaloids of the opium poppy and of ipecacuanha.

Under the chairmanship of Prof. W. O. James a paper on the distribution and significance of alkaloids in *Papaver somniferum* was presented by Prof. J. W. Fairbairn (School of Pharmacy, University of London). The work in his laboratory was chiefly a study of the occurrence of alkaloids in relation to the origin and distribution of laticiferous vessels and an analysis of the latex composition using samples drawn at short intervals from unripe capsules. It was confirmed that alkaloids were restricted to laticiferous vessels and also shown that this anastomizing system always occurred in the phloem, but was absent from stamens, ovules, seeds and seedlings up to the cotyledon stage. Latex from capsules had been assayed for morphine, caoutchouc and total solids, and it was shown that rapid changes in the composition of the latex took place in short periods of time such as 2 hr. Similar rapid changes had also been shown to take place in the fruit of *Conium maculatum* (Fairbairn and Suwal). From anatomical and qualitative chemical results it had been suggested that the alkaloids might be products of photosynthesis, secreted into the laticifers and so removed from metabolic circulation. This 'drain-pipe' theory now appeared unlikely owing to the rapid alkaloidal variation, and further work might raise the status of the latex from that of a slowly growing heap of 'metabolic sewage' to that of a living entity, actively participating in the metabolism of the plant.

Biosynthesis of Opium Alkaloids. An account of the studies which have been carried out in several laboratories was given by Dr. A. R. Battersby (University of Bristol), who was responsible for recent work at Bristol involving the feeding of growing plants with tyrosine, labelled specifically with carbon-14. Unambiguous degradation of carefully purified alkaloids, isolated from the plant, had shown that the molecule of papaverine was specifically labelled at positions 1 and 3 and morphine at the corresponding positions 9 and 16. These results were interpreted as showing that two aromatic units with a two-carbon side-chain (*Ar*–C–C) constitute building blocks in the carbon(16) skeleton of the alkaloids studied. Supporting evidence was available by synthesis of norlaudanosoline specifically labelled with carbon-14, which was incorporated into papaverine and morphine by feeding experiments. Furthermore, results of controlled degradation of the latter radioactive alkaloids showed little doubt that the rings *A* and *C* of morphine are joined by a phenolic coupling reaction between two aromatic rings. Rate studies on the incorporation of activity into the two alkaloids from carbon-14 carbon dioxide (Rapoport *et al.*) and from specifically labelled tyrosine (Bristol workers), together with other evidence, had shown that the alkaloids are formed in the order thebaine–codeine–morphine. Concluding his account of recent work, Dr. Battersby was able to show that the present evidence was in keeping with the view that the opium alkaloids are products of the shikimic–prephenic acid pathway, the two *Ar*–C–C units produced by this route being used to provide the sixteen carbon atoms required for skeletons of the bases. It was probable that the units were different, yet derivable from tyrosine in the living plant.

Chemical Structure and Pharmacological Activity. In the afternoon session Prof. W. B. Whalley (School of Pharmacy, University of London) introduced Dr. N. J. Harper (Chelsea College of Science and Technology), who discussed the relationships between structure and pharmacological activity of morphine and related substances. He stated that morphine had been modified chemically in many ways in attempts to reduce undesirable side effects, namely, liability to produce addiction and depressant effect on the respiratory centre, but such modifications had not yet resulted in a compound which had significantly replaced morphine as a clinical agent. The alternative approach of synthesizing fragments of the morphine molecule had resulted in the production of the morphinans and benzomorphans which represented the greatest achievements in this direction. These studies clearly emphasized the importance of the phenolic, alcoholic and basic groupings. Stereochemical factors played an important part in analgesic activity since, with enantiomorphs, one isomer was always more active than the other and in some synthetic analgesics the most active enantiomorphs were related to D-(–)-alanine. Various studies had led to the hypothesis that in the mediation of analgesic activity a three-dimensional fit at a 'receptor site' was involved and stereochemical implications could be important in the separation of desirable and undesirable pharmacological effects. It was probable that the chief factor limiting the intensity of response and duration of effect of morphine and the synthetic analgesics was biotransformation, and in these transformations the phenolic grouping played an important part. *N*-dealkylation also had important pharmacological implications following from two provocative hypotheses; one relating it to analgesic action, and the other to the development of tolerance.

Chemistry and Biosynthesis of Ipecacuanha Alkaloids. Dr. H. T. Openshaw (Wellcome Research Laboratories, Beckenham, Kent) dealt chiefly with the chemistry of the alkaloids and said that they formed a group of inter-related isoquinoline alkaloids of unique structure, the biogenetic relationship of which to the benzylisoquinoline group was of particular interest. The structure of emetine was established by classical methods of degradation, but in its final stages this investigation received powerful support from the developments in biogenetic theory then current; in turn, the biogenetic theory was strongly upheld by the discovery in ipecacuanha of a postulated intermediate in the biosynthesis. Considerable significance was also attached to the stereochemistry of the alkaloids and their relationship to that of the cinchona and indole series. The absolute configuration of each of the four asymmetric centres in emetine had been established, and attention had been paid to the problem of stereospecific synthesis of the alkaloid. A feature of special interest in the chemistry of emetine was its conversion by mild oxidation into a series of dehydrogenation products, the structure of which had been elucidated.

Pharmacologically, emetine was of considerable importance for its amœbicidal activity, and of special interest in that all the stereoisomers and closely related compounds arising from the synthetic work, only one other substance, 2-dehydroemetine, showed comparable activity.

The symposium therefore proved to be well balanced and showed the value of studies on the location and variation in alkaloids of opium, as a background to results obtained using tracer techniques. Classical chemical investigations, in particular with stereochemical considerations, were of benefit to pharmacology and the work on the ipecacuanha alkaloids formed an interesting comparison with studies on morphine and its related compounds.

S. B. Challen

THE NATIONAL PARKS COMMISSION

THE twelfth annual report of the National Parks Commission, for the year ended September 30, 1961, besides providing the customary concise and very readable illustrated account of the activities of the Commission during the year, progress in the national parks and with the long-distance routes, is marked by two notes of anxiety*. First, the Commission is anxious that amending legislation to the National Parks Act of 1949 should not be long delayed. Apart from the practical benefit expected to follow the adoption of the recommendations submitted to the Minister, the Commission believes that reappraisal in Parliament of the working of the Act would give welcome encouragement to those whose duty it is to administer the Parks and would help to produce in the public mind a clearer conception of the whole idea of national parks. The second note of anxiety relates to the continuing proliferation of aerial masts throughout the countryside, usually on remote and unobstructed high ground. These increasing coverage demands appear as yet to be entirely unco-ordinated, and while the Commission recognizes the need, it is far from satisfied that the problem of mast-sharing receives adequate consideration.

The magnitude of the development issue is shown by the fact that during the year nearly 450 proposals for development affecting landscape beauty in national parks, in areas of outstanding natural beauty, or elsewhere were referred to the Commission by Government departments, park or other local planning authorities, or by amenity organizations. One notable success during the year recorded in the report is the decision of the North-Western Electricity Board to place the entire Upper Borrowdale scheme underground at its own expense, notwithstanding the offer of contributions from the Friends of the Lake District and the official consent to the placing of certain sections of the line overhead. Regarding areas of outstanding natural beauty, the report comments that review of the work done by the local planning authorities indicated that while the preservation of these areas from unsightly development was receiving full attention, insufficient use was made of the opportunities for positive action for the enhancement of natural beauty which would earn payment of the special 75 per cent rate of Exchequer grant. Some progress is reported with the long-distance routes, notably with the Dorset Coast Path, on the South Downs route, and field work on a North York Moors and a Yorkshire Court Path. One of the two remaining major problems of the Pennine Way has been solved, completing the Path within the Peak District National Park.

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* National Parks and Access to the Countryside Act, 1949. Twelfth Report of the National Parks Commission for the year ended September 30, 1961. Pp. vi + 91 + 8 plates. (London: H.M.S.O., 1962.) 7s. net.

IMMUNOLOGICAL FUNCTION OF THYMUS AND BURSA OF FABRICIUS

Thymus Lesions in an Auto-immune Disease of Mice

A STRAIN of mice, *NZB/BL*, has been shown by Bielschowsky *et al.*[1] to develop a hæmolytic anæmia of auto-immune type, usually around 6–7 months of age. Through their co-operation we have had this strain of mice available for investigation during the past two years and have fully confirmed their findings. As judged by the occurrence of a positive direct Coombs test on the red cells, the condition can be transferred to young isologous mice by transfer of splenic cells from an older animal showing the typical signs but not from similar numbers of cells from mice in the pre-symptomatic stage[2].

It is well known that in about 70 per cent of human cases of myasthenia gravis the thymus contains numerous lymph follicles with typical germinal centres[3] and that in a significant proportion of early cases thymectomy cures or greatly alleviates the disease. Simpson[4] and Strauss *et al.*[5] have considered the condition a manifestation of auto-immune disease. We have, therefore, recently examined thymus sections from most of the mice of the *NZB/BL* strain being killed for transfer experiments or for other reasons. More than half have shown appearances which we interpret as lymph follicles with germinal centres and which have a close resemblance to the histological findings in typical human cases of myasthenia gravis (Fig. 1).

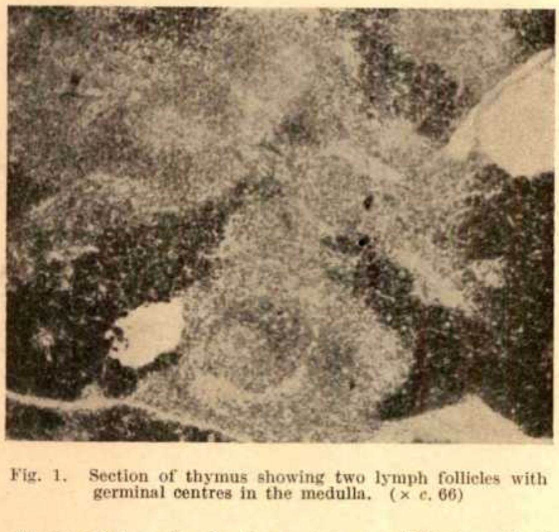

Fig. 1. Section of thymus showing two lymph follicles with germinal centres in the medulla. (× c. 66)

Many of the mice in the series were killed routinely when moribund, and in these there was severe involution of the thymus from age and stress, usually with complete atrophy of the cortex. Some of these showed an excess of lymphoid cells in the medulla; but the most striking feature was that in all mice more than 9 months of age in which the thymus cortex was still clearly shown, lymph follicles in the medulla were present. The usual appearance was of a complex area showing two or more germinal centres with incomplete surrounding crescents of mature small lymphocytes. It was characteristic to see small areas or cords of close-packed small lymphocytes, presumably representing over-filled intrathymic lymphatic channels.

These findings will be more fully described and their significance discussed in another publication. Here we wish merely to consider them briefly in relation to recent work[6] which suggests that the thymus is an essential primary source of stem cells for the development of immunologically competent cells in spleen and lymph nodes. It has been claimed[7] that auto-immune disease is a manifestation of the activity of 'forbidden clones' of cells unresponsive to the normal homeostatic controls which prevent the development of cells reactive against body constituents. If the site of origin of such forbidden clones is the thymus, they might well be expected to show their forbidden quality by proliferation as a germinal centre in a region where such activity never normally takes place.

We are also interested in the possible analogy between the condition manifested in our strain *NZB/BL* as auto-immune disease and the emergence of lymphoid leukæmia in mice of the high-leukæmia strain *AKR*. In the latter the first appearance of the forbidden (malignant) clone is almost regularly in the thymus and the incidence of leukæmia is greatly reduced by thymectomy at an early age.

Active experimental work based on these ideas and analogies is in progress.

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Dissociation of Immunological Responsiveness in Fowls with a Hormonally Arrested Development of Lymphoid Tissues

IN recent months there has been evidence of an increasing interest among immunologists in the role of the thymus in the development of immunological functions. Several workers[1-3] have independently expressed the view that precursors of immunologically competent cells arise wholly or mainly in the thymus, moving out during the first weeks of life to colonize other lymphoid organs such as spleen and lymph nodes, where they become available for the various types of immunological function.

Experimental evidence supporting this view has been presented by Miller[2], in showing that thymectomy of one-day mice allowed them to accept homografts for up to 60 days. Archer and Pierce[4] have also shown that thymectomy of rabbits at 5 days removed the ability to produce antibody in response to a standard antigenic challenge later in life.

In chickens, removal of the bursa of Fabricius in the first week of life similarly prevents any antibody response to appropriate challenge.

The work to be reported in this communication is concerned with studies on 'hormonally bursectomized' chickens. Prevention of the development of the bursa was achieved by the administration of 2·5 mgm. of testosterone propionate (B.D.H.) to the allantoic cavity of the 12-day chick embryo, the bursal changes resulting from this having been previously described[5]. Chickens treated in this manner are termed bursaless in this communication.

The resulting changes in other lymphoid tissues are more varied. The thymus appears histologically normal in about 60 per cent of bursaless chickens. The remainder show a variety of changes, ranging from a moderate atrophy of the cortex (30 per cent) to a group with complete cortical atrophy and severe degenerative changes in the medulla (10 per cent). In most bursaless chickens, the weight of the spleen is about half the normal value, and histological examination shows a relative absence of lymphoid tissue and plasma cells, although active lymphoid follicles are visible. It is only in bursaless chickens with a complete atrophy of the thymic cortex that these follicles are greatly reduced in number. Small lymphocytes in blood were present in normal amounts in most bursaless chickens, and again it was only in those with severe thymic atrophy that the number was reduced to about one-third.

Our initial experiments involved a repetition of the work of Mueller *et al.*[6], in challenging hormonally bursectomized chicks with several standard antigens, and the results obtained were in complete agreement, no antibody at all being produced. As many of these chicks had a morphologically normal thymus, it was decided to determine if other types of immunological reactivity persisted in these birds. Studies were made on: (1) the ability of the blood to produce lesions on the chorioallantoic membrane[7]; (2) the rejection of skin homografts; (3) the tuberculin reaction as an example of delayed hypersensitivity. These tests, together with the response to bovine serum albumin, were carried out on normal, bursaless, and bursaless chickens with atrophic thymuses. Birds in this last group are relatively rare as most die early in life, and only a few figures are at present available. The results are presented in Table 1.

Table 1. IMMUNOLOGICAL RESPONSES OF NORMAL AND BURSALESS CHICKENS

Type of reaction	Normal chickens	Chickens treated with testosterone *in ovo*	
		Bursaless	Bursaless with atrophic thymus
CAM lesions:	*2,000 ± 800*	*1,900 ± 800*	*2,600 ± 100*
Large lymphocytes per focus	(35)	(10)	(2)
Homograft rejection:	*7 ± 1 day*	*7 ± 1 day*	Grafts intact at time of death* (2)
Average time of rejection	(12)	(12)	
Delayed hypersensitivity:	*positive*	*negative*	N.T.
Tuberculin reaction	(3/3)	(3/3)	
Antibody production:			
Incidence of positive titres	*100 per cent*	0 per cent	0 per cent
Mean reciprocal titres	320 (50)	<10 (40)	<10 (10)

N.T., not tested. Figures in brackets show the number of birds tested. Results in italics indicate positive (normal) reactivity.

* 20 and 24 days after grafting.

It can be seen from the results that the types of immunological response of which the bird is capable depend on the extent to which the primary lymphoid organs, thymus and bursa, have developed in the treated chickens during the first weeks of life.

The influence of the hormone is not a simple quantitative reduction of all immunological responses, but rather a selective eradication of some, leaving others intact, which in turn may be absent in more severely affected animals.

The dissociation between the different types of immunological reactivity achieved in this system indicates at least two basic levels of immune response, related functionally in the fowl to different primary lymphoid organs. In the order of disappearance of these functions under the influence of the hormone, the first level is associated with the production of circulating antibody and delayed hypersensitivity. The common denominator between these responses is that they are both concerned with extrinsic microorganismal and similar antigens. The bursa of Fabricius appears to be primarily responsible for these functions. The second level of immune response is concerned with the recognition of histocompatibility antigens. The ability to produce lesions on the chorioallantoic membrane probably represents the basic form of graft versus host reactivity, and the presence of the thymic cortex is essential if this is to be developed into the full complex of the mechanism of homograft rejection, and perhaps of graft versus host reactions as induced by one of the standard methods.

It seems to be more than coincidence that the order of maturation of the different immunological functions in the fowl follows the same order as the resistance to elimination by hormonal treatment. This order, according to reports in the literature and some of our own results, is as follows: Simonsen phenomenon, homograft rejection[8], and circulating antibody production. It seems possible that this order of maturation reflects the evolutionary development of immunological reactions[9].

The presence of both bursa and thymus makes the fowl a very convenient model for examining this concept of different levels of immunological activity. It would be more difficult to undertake this in mammals, if, as we suspect, the mammalian thymus has combined the functions of the avian bursa and thymus.

Further results and a more complete discussion will shortly be published elsewhere.

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GENETIC NATURE OF TOLERANCE OF *HYPOMYCES SOLANI* F. *CUCURBITAE* TO PENTA- AND TETRA-CHLORONITROBENZENE

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IT is well known that a number of different fungi, when grown in the presence of chlorinated nitrobenzenes, give rise to sectors the growth of which is less inhibited by the fungistat than that of the original isolates[1-4]. Such relatively insensitive strains have been considered to arise by saltation[5] or mutation[2], but the nature of these changes has not so far been examined by means of genetic analysis. This might have been because of the absence of suitable sexual cycles in the particular organisms used. In many other cases of appearance of resistance to fungicides the mechanism of such an adaptation has been left a matter for speculation by the lack of data on genetic behaviour. Most investigators seem to believe that resistance which is not lost after serial transfers on a medium lacking the fungicide is mutational, while others[6] have presented some evidence that persistent adaptation does not always involve a change of a nuclear gene. In the work presented here the mutational origin of tolerance of *Hypomyces solani* f. *cucurbitae* Snyd. and Hans. to pentachloronitrobenzene (PCNB) and one of the isomers of tetrachloronitrobenzene (TCNB) has been established by crossing tolerant and sensitive strains and demonstrating a Mendelian segregation in the progeny.

The fungus is a well-known plant pathogen, causing root-, stem- and fruit-rot of cucurbits; it is heterothallic and occurs in four sex forms: hermaphrodites, females, males and neuters[7,8]. Different strains of race 1 of this organism[9], which had been maintained by single conidial transfers, were used in this work. The first PCNB-tolerant strains were obtained from fast-growing sectors which appeared in colonies on agar plates containing 0·3 per cent PCNB, a few days after mass transferring. All these tolerant strains grow almost twice as fast as their parent isolates in the presence of PCNB. Furthermore, they are all tolerant to 1,2,4,5-tetrachloro-3-nitrobenzene, a sample of which was given to me by Dr. W. A. Kreutzer of the Shell Development Co. In the presence of this latter material the difference between tolerant and sensitive strains is more clear-cut since tolerant strains grow at least ten times as fast as their parents. Tolerant strains have also been produced in the presence of TCNB. In one case an ascospore isolate of the sensitive type produced a tolerant sector 10 days after the germinated ascospore was transferred to a medium containing 0·2 per cent 1,2,4,5-TCNB. All these strains are also tolerant to PCNB.

A number of different tolerant strains was crossed with sensitive ones of the opposite mating type, some of the resultant perithecia were chosen at random and ascospores were germinated on water agar plates. Germinated random single ascospores were then plated on a medium containing 1,2,4,5-TCNB. The results of these experiments are summarized in Table 1. In crosses 1–3, protoperithecia of sensitive strains were fertilized with conidia of tolerant ones. In crosses 4 and 5 the protoperithecia of tolerant were fertilized with conidia of sensitive strains. In both cases tolerant strains reappeared in about 50 per cent of the progeny (Fig. 1). This demonstrates that, at least for the strains used, tolerance is controlled by a single gene. Small differences in the degree of tolerance of different segregants suggest the possible presence of modifying genes. The results presented in Table 2 show that ascospores from every one of the nine perithecia from cross No. 4 of Table 1, which were studied, segregated to tolerant and sensitive. This was also the case with all the other crosses. Therefore, the tolerant strains used apparently contain the gene for tolerance in all their nuclei. Evidence has also been obtained that tolerance segregates independently of sex. When protoperithecia of the sensitive strain 12 were fertilized with conidia of the sensitive strain 4, no tolerant ascospore isolates were obtained though 104 asco-

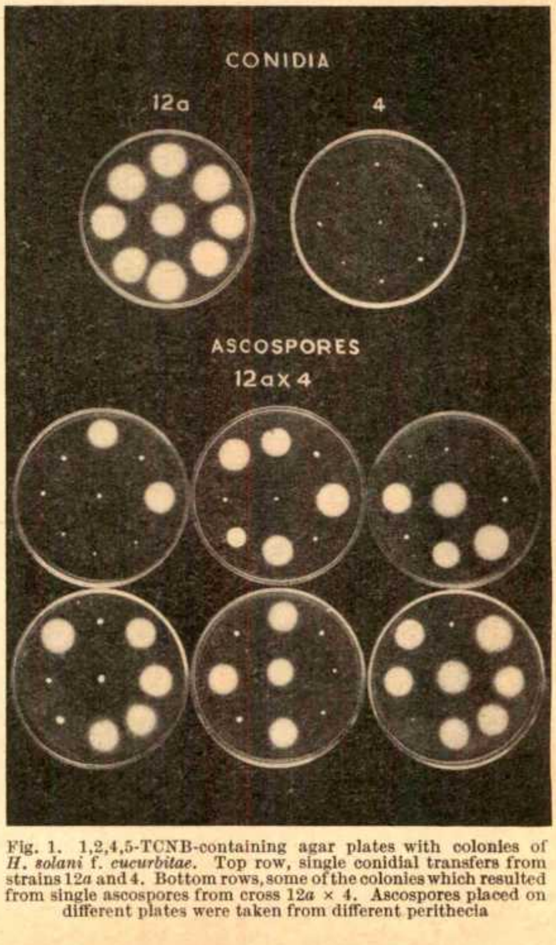

Fig. 1. 1,2,4,5-TCNB-containing agar plates with colonies of *H. solani* f. *cucurbitae*. Top row, single conidial transfers from strains 12*a* and 4. Bottom rows, some of the colonies which resulted from single ascospores from cross 12*a* × 4. Ascospores placed on different plates were taken from different perithecia

Table 1. SEGREGATION OF PROGENY FROM CROSSES BETWEEN STRAINS OF *H. solani* f. *cucurbitae* TOLERANT AND SENSITIVE TO PCNB AND 1,2,4,5-TCNB

Cross No.	Parents	Ascospores tested	Tolerant (T)	Sensitive (S)	Ratios T : S
1	14* × 10*a*†	225	115	110	1 : 0·957
2	9 × 30*a*	162	81	81	1 : 1
3	12 × 4*c*	178	92	86	1 : 0·935
4	12*a* × 4	194	100	94	1 : 0·940
5	12*c* × 4	272	130	142	1 : 1·092
		1,031	518	513	

* Strains represented by a number only are sensitive.
† Strains represented by a number and a letter are tolerant and have been derived from sensitives labelled with the same number.

Table 2. SEGREGATION FOR TOLERANCE TO PCNB AND 1,2,4,5-TCNB IN PROGENY FROM A CROSS BETWEEN STRAINS 12*a* AND 4 OF *H. solani* f. *cucurbitae*

Perithecium No.	Ascospores tested	Tolerant	Sensitive
1	18	8	10
2	16	8	8
3	34	14	20
4	18	10	8
5	36	24	12
6	18	4	14
7	18	13	5
8	18	9	9
9	18	10	8
	194	100	94

spores from different perithecia were tested. On the other hand, all 120 ascospores from a cross between two tolerant strains, 12*c* and 4*c*, that were studied, produced tolerant colonies. This indicates that the two strains crossed apparently contain the same gene for tolerance. The possibility that different tolerant strains might contain genes for tolerance at different loci is now being investigated.

This is believed to be the first instance in which tolerance of a plant pathogenic fungus to a commercially used fungicide has been shown to be transmitted through the sexual cycle and its genetic nature established by means of genetic analysis.

By inoculation tests strains 14 and 10*a*, and also 20 of the ascospore isolates which were obtained from the cross 14 × 10*a* and of which 10 were tolerant and 10 sensitive, were all found to be pathogenic to ripe banana squash fruits. Passage through such a host was not found to alter the tolerance or sensitiveness of the strains.

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ELECTROPHORESIS OF SERUM AND SOLUBLE LIVER PROTEINS ON CELLULOSE ACETATE

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WE have been working on the electrophoresis of soluble liver proteins in order to find a satisfactory method for the study of biopsies from patients with liver disease. We began by using rat liver and have varied the duration of the electrophoresis, the site of application of the sample, and the buffer systems during the course of this work. When veronal buffer (pH 8·6) was used with an overnight run (16 hr.), a better separation of the protein bands was obtained when the sample was applied 2·5 cm. from the anode end of the strip ('anode application') than when it was applied the same distance from the cathode end ('cathode application'). We shall describe the work we have done on 'anode application' of serum and soluble liver proteins to elucidate the patterns obtained with this unorthodox procedure.

Liver. There are several different methods for the production of clear, concentrated and undenatured solutions of soluble cell proteins from solid organs[1-7]. We have used the following method for rat liver. It was perfused with 0·15 N saline at 37° C. with the rat still living under ether anæsthesia[8]. The organ was then removed, cut up into small slices and homogenized in a Tower's hand homogenizer, in the cold, with a volume of saline equal to the weight of the liver. The homogenate was then centrifuged at 60,000g for 1½ hr. in a refrigerated centrifuge. The clear supernatant was brought to a pH of 5·1–5·2. At this pH precipitation of the major enzymatic fractions occurs[9], the removal of which prevents denaturation of the remaining soluble proteins in the clear supernatant obtained by a final centrifugation at 3,000g. We have routinely applied the sample 2·5 cm. from the anode and 2·5 cm. from the cathode ends of alternate cellulose acetate strips in a horizontal Shandon tank in more than 100 electrophoretic runs. Fig. 1 is an example of the pattern obtained with an overnight run using veronal buffer. There are four main bands with 'cathode' and five with the unorthodox 'anode application'. 'Anode application' results in migration of the sample away from the application site towards the cathode and improved separation of each fraction. The use of a water seal to cut down evaporation makes little difference to the result.

Serum. In contrast to the results obtained with liver, 'anode application' of human serum using the

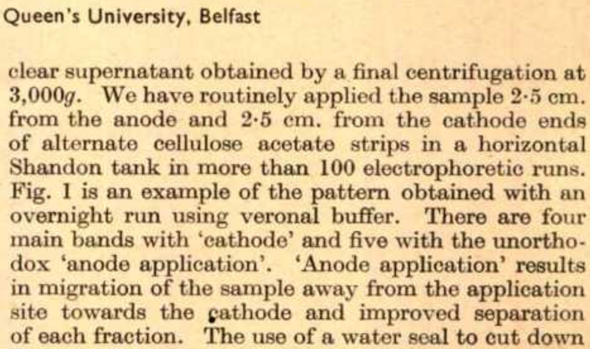

Fig. 1. Electrophoretic separation of the soluble liver cytoplasmic proteins of the rat. The 'cathode end' is on the left. Veronal buffer, pH 8·6, μ = 0·1, 90 V., 0·4 m.amp./cm., 16 hr. Upper strip = 'anode application'

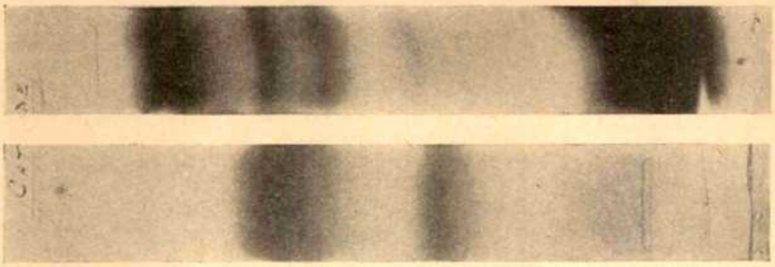

Fig. 2. Electrophoretic separation of human serum using the same conditions as in Fig. 1. The 'cathode end' is on the left. Lower strip = 'anode application'

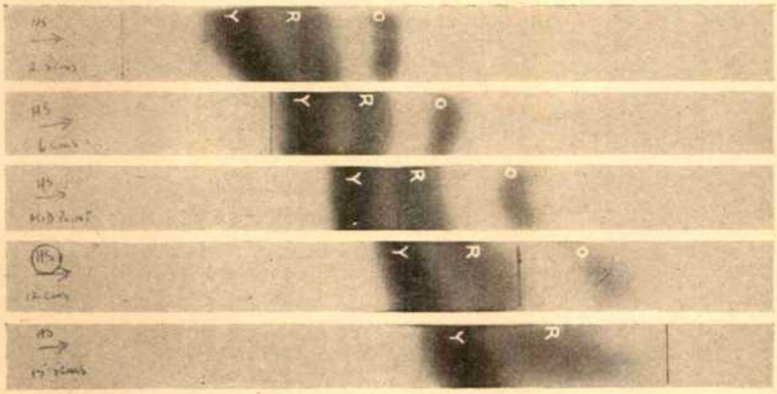

Fig. 3. Electrophoretic separation of fluorescein conjugated crude globulin fraction of immune rabbit serum with variation of application points. The 'cathode end' is on the left. Veronal buffer, *p*H 8·6, $\mu = 0{\cdot}1$, 150 V., 2 hr. The sites of the coloured bands (R = red, Y = yellow, and O = orange) have been marked and the strip then stained with lissamine green. Protein staining bands correspond with the coloured bands

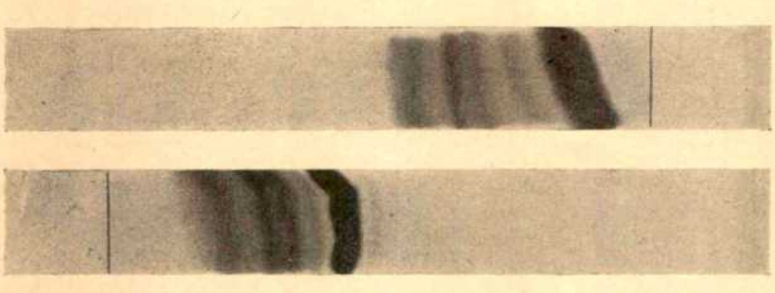

Fig. 4. Electrophoretic separation of human serum using *tris*–veronal buffer, 180 V., 0·4 m.amp./cm., 3 hr. The 'cathode end' is on the left. Upper strip = 'anode application'

same conditions (16 hr.; veronal buffer) produces only two bands instead of the usual pattern (Fig. 2).

We used a fluorescein conjugated crude globulin fraction of immune rabbit serum (Wellcome rabbit precipitating anti-guinea pig serum) to observe the migration of protein bands during electrophoresis. This fraction was obtained by the mixing of equal volumes of serum and saturated ammonium sulphate at 4° C. followed by centrifugation and solution of the precipitate in a little distilled water. The globulin fraction was dialysed at 4° C. against 0·15 N saline overnight and then conjugated with fluorescein isothiocyanate using the method of Marshall, Eveland and Smith[10]. On electrophoresis the conjugate split into three coloured bands: red, yellow and orange. These coloured bands correspond exactly with the protein fractions stained in the usual way. The effects of application of this conjugate at different sites along the strip are shown in Fig. 3. When the sample is applied 2·5 cm. from the anode end, the orange band, after an initial short movement towards the cathode, moves away towards the anode end of the strip, while the yellow and red bands move towards the cathode. Fig. 3 shows that the three protein fractions bear a consistent relation to each other wherever the sample is applied, but when the sample is applied nearer to the anode end the yellow and red bands start to separate 'backwards' towards the cathode. When the top and bottom strips in this figure are compared it is seen that the bands which show least movement forwards on 'cathode application' show the greatest movement 'backwards' on 'anode application'.

The study of fluorescinated globulins helped us to evaluate the pattern obtained with liver and serum (Figs. 1 and 2). The slowly moving globulin fractions in each case are those which migrate towards the cathode on anode application. All fractions in our preparation of rat liver behave in this way; but in the case of serum only two bands are obtained, as the faster moving fractions have moved off the strip towards the anode. The pattern obtained with the 'anode application' may be due to the fact that endosmosis and evaporation have a greater effect on the migration of some globulin fractions under these conditions than does the current.

The pattern with 'anode application' of serum can be changed by altering the electrophoretic conditions. Using *tris*–veronal buffer and a short 3½-hr. run we found that all fractions, including albumin, migrate towards the cathode (Fig. 4). Liver behaves similarly with the veronal and *tris*–veronal systems, but orthodox application with *tris*–veronal gives the better results.

Application of the sample near the anode end of a cellulose acetate strip may be of value in studying electrophoretic separation of protein fractions from other tissues provided that satisfactory buffer systems and timing of the run can be arranged to suit this unorthodox method.

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NERVE SUPPLY OF THE PULMONARY BIFURCATION BARORECEPTOR AREA AND ITS SIGNIFICANCE

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KOCH[1] was the first to interpret the significance of the anatomical location of the mammalian baroreceptor areas. He pointed out that the carotid and aortic baroreceptors are found in surviving areas in the mature animal of the visceral arch vessels of the embryo and each of these areas was innervated by the corresponding visceral arch nerve. The carotid sinus formed from visceral arch III is supplied by the glossopharyngeal nerve. The aortic arch pressoreceptor area derived from visceral arch IV is innervated by a branch of the superior laryngeal nerve, the nerve of visceral arch IV.

Recent investigations have revealed an aggregation of sensory endings, probably pressoreceptors at the bifurcation of the main pulmonary artery of the cat, which on stimulation cause reflex changes in heart-rate, vascular resistance and respiration[2-4]. As the anatomical nerve supply to this sensory area located on a vessel derived from visceral arch VI has not been described, dissections were carried out in ten cats to determine this. The nomenclature of Mizeres[5] will be adopted in this description. It was found that the recurrent cardiac nerve and sometimes the cranio-vagal cardiac nerves carry the main innervation of the pulmonary bifurcation and the adjacent parts of the main pulmonary artery and its right and left branches.

The recurrent cardiac nerve (*r.c.*) is a moderately large nerve arising from the right recurrent laryngeal nerve (*r.l.*) as it loops around the right subclavian artery. It usually receives a contribution of varying size from the vagal trunk (*v.*) and another from the stellate ganglion. The nerve passes dorsally to the anterior vena cava, laterally to the brachiocephalic artery and arch of the aorta, to the pulmonary bifurcation where it divides into anterolateral and posterolateral branches. The anterolateral branch is the smallest. These branches fan out over the anterior and posterior aspects of the main pulmonary artery (*p.a.*) and communicate with plexi around the right and left pulmonary arteries and the pretracheal plexus. Some fibres continue to the heart and the coronary plexi. During its course it communicates freely with the cranio-vagal cardiac nerves (*c.v.c.*). The right vagal cardiac nerves arise from the right vagus trunk caudal to the origin of the right recurrent laryngeal nerve. They fall into two groups—the cranial and caudal vagal cardiac nerves. These vary in size, number and course. Some of the smaller divisions may be seen only with the aid of a dissecting microscope. They supply twigs to the right pulmonary artery plexus, the antero- and postero-lateral branches of the right recurrent cardiac nerve at the pulmonary bifurcation and to the plexus formed by the ventral branch of the vagus, anterior to the pulmonary root, and then terminate in the atrial wall. Small twigs, variable in size and position and sometimes absent, are supplied to the pre-tracheal plexus and the plexus around the right and left pulmonary artery by the right stellate cardiac nerves, the ventromedial cervical cardiac nerve, the left recurrent laryngeal nerve and the ventral branch of of the left vagal trunk. Other twigs are supplied from a diffuse plexiform network of fibres from the ventrolateral cardiac nerve and the left stellate cardiac nerve.

Of all these nerves, the recurrent cardiac nerve is undoubtedly the most important and constant. Nonidez[6] states that in the dog the recurrent cardiac nerve contains pre-ganglionic, afferent and sympathetic post-ganglionic fibres. The recurrent cardiac nerve is a branch of the right recurrent laryngeal nerve, the nerve of visceral arch VI (ref. 7). It is therefore of considerable interest that the main nerve supply to the pulmonary bifurcation sensory area, part of visceral arch VI, is derived from the recurrent laryngeal nerve, the nerve of visceral arch VI. As the most cephalic part of the pulmonary

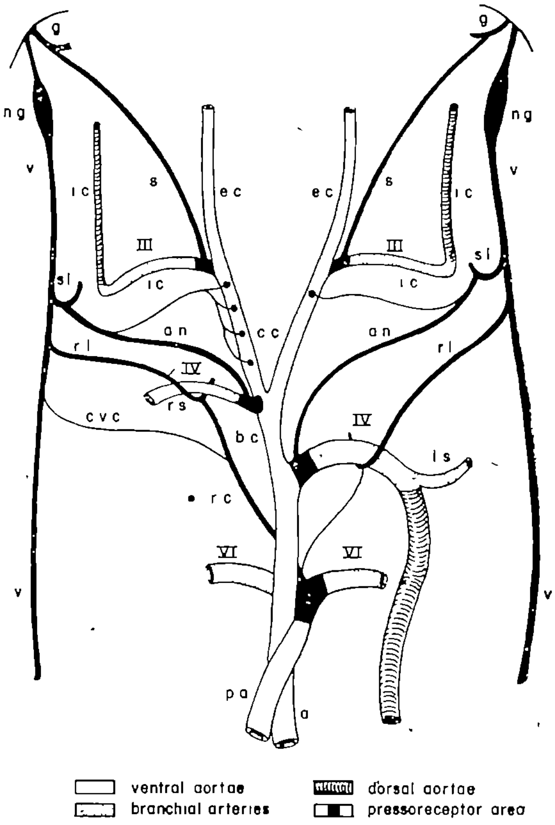

Fig. 1. Diagrammatic representation of the localization and innervation of the pressoreceptor areas of the cat in relation to the embryological derivation of the vessels

a, Aorta; *a.n.*, aortic nerve; *b.c.*, brachiocephalic artery; *c.c.*, common carotid artery; *c.v.c.*, cranio-vagal cardiac nerve; *e.c.*, external carotid artery; *g.*, glossopharyngeal nerve, *i.c.*, internal carotid artery, *l.s.*, left subclavian artery; *n.g.*, nodose ganglion; *p.a.*, pulmonary artery; *r.c.*, recurrent cardiac nerve; *r.l.*, recurrent laryngeal nerve; *r.s.*, right subclavian artery; *s*, sinus nerve; *s.l.*, superior laryngeal nerve; *v*, vagus

artery is formed from the posterior and right lateral parts of the bulbus cordis[8], it is reasonable that this vessel should be predominantly supplied from the right visceral nerve VI.

In addition to this pulmonary area, other pressoreceptor areas in the cat have been described since the publication of Koch's monograph. The bilateral common carotid areas at the level of the origin of the superior thyroid arteries[9], small discrete areas on the right common carotid[6] and others at the bifurcation and adjacent parts of the wall of the brachiocephalic trunk[10] have been found. All are supplied by branches that emerge from the superior laryngeal or aortic nerves.

In Fig. 1, the probable embryological origin of some of the larger arteries of the upper thorax and neck of the cat is represented. The ascending aorta, pulmonary artery, brachiocephalic trunk and the common and external carotid arteries are derived from the embryonic ventral aortæ. The vessels of visceral arch III are represented by the internal carotid arteries (not patent in this animal); those of visceral arch IV by the subclavian arteries and the arch of the aorta; those of visceral arch VI by the right and left pulmonary arteries[11].

It is of interest that in the mature animal a pressoreceptor area is found near the junction of each surviving visceral arch artery, with an artery derived from the ventral aortæ. The carotid sinus lies at the junction of the internal carotid and common carotid arteries, the brachiocephalic areas at the junction of the subclavian and brachiocephalic arteries, the aortic area at the junction of the aortic arch and ascending aorta and the pulmonary artery bifurcation area at the junction of the right and left pulmonary arteries with the main pulmonary artery. These areas include all the known pressoreceptors except those in the walls of the common carotid arteries and the main pulmonary artery, which are derived from the embryonic ventral aortæ. Nonidez[6] states that the afferent endings found in the ascending aorta are probably proprioceptor in type.

The presence of pressoreceptors in the ventral aortæ and at the junction of all visceral arch vessels and the ventral aortæ is most significant in relation to the comparative anatomy, embryology and physiology of this area[12]. It is in keeping with the hypothesis of Koch, which now deserves greater credence, as it may now be applied to all known pressoreceptor areas in the cat.

This work was supported by grant *H*6730, U.S. Public Health Service.

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EXISTENCE OF AN ERYTHROPOIETIC LABILE IRON POOL IN ANIMALS

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During recent years the use of radioiron (iron-59) as a tracer has elucidated some fundamental aspects of the physiology and biochemistry of iron kinetics. Pollycove and Mortimer[1] have proposed mathematical models to explain the major pathways of iron kinetics in man (Fig. 1). The intercompartmental rate constants from compartment i to compartment j, α_{ij} are calculated from the measured slopes (r_1, r_2, r_3), intercepts (A, B, C), and fraction of radioiron irreversibly fixed in maturing erythrons. The amounts of iron in the labile pools are calculated from these intercompartmental rate constants and direct measurement of total plasma iron. Iron for hæmoglobin synthesis is the product of the fraction of erythropoietic labile iron pool irreversibly fixed in maturing erythrons, α_{23}, and the amount of iron in the erythropoietic labile iron pool. The intercompartmental rate constant α_{23} determines the rate at which iron in the erythropoietic labile pool is converted into hæm iron.

The concept of a labile erythropoietic iron pool[1] is of chief importance to these models. So far, no physiological or biochemical entity corresponding to such a labile iron pool has been demonstrated. In the present series of experiments, an attempt was made to examine some components of iron kinetics in the dog and rabbit, with the view of demonstrating the existence of an erythropoietic labile iron pool.

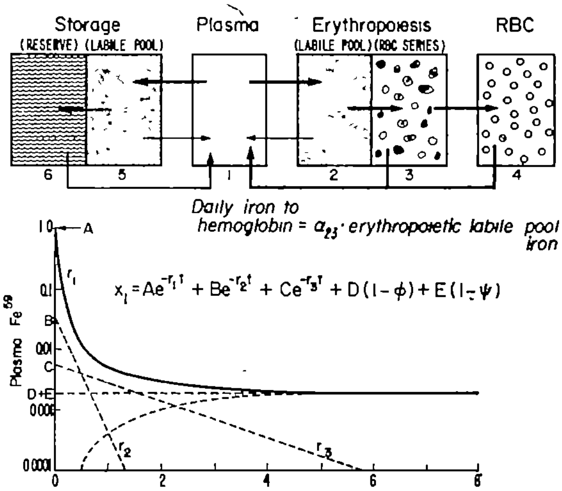
Storage
(RESERVE) (LABILE POOL)
Plasma
Erythropoiesis
(LABILE POOL) (RBC SERIES)
RBC
6
5
1
2
3
4
Daily iron to hemoglobin = α_{23} · erythropoietic labile pool iron
$x_1 = Ae^{-r_1 t} + Be^{-r_2 t} + Ce^{-r_3 t} + D(1-\phi) + E(1-\psi)$
1.0
A
r_1
0.1
B
0.01
C
D+E
0.001
0.0001
r_2
r_3
Plasma Fe59
0
2
4
6
8
Days

Fig. 1. General iron kinetics model

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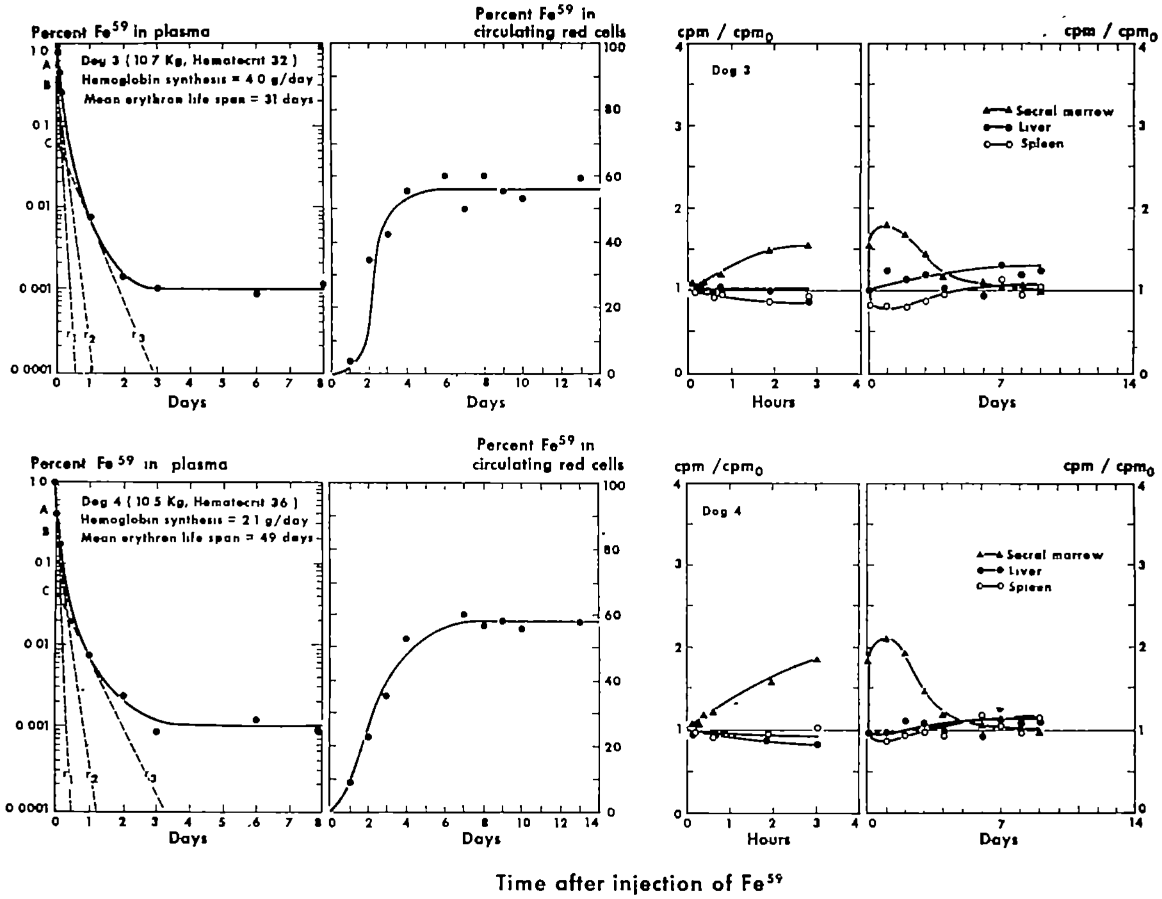

Fig 2. Iron kinetics data of dogs 3 and 4. Intercompartmental rate constant a_{32} is calculated from measurements of the slopes r_1, r_2, r_3, their intercepts A, B, C, and the fraction of radioiron fixed in maturing erythrons, f

Graded doses of 2·5 per cent aqueous solution of phenylhydrazine were injected subcutaneously in rabbits to produce reticulocytosis. For each experiment 30–100 μc. of radioiron (supplied by Abbot Laboratory, Oak Ridge), as ferrous citrate specific activity 3–12 μc./μgm., were incubated at 37° C. for ½ hr. with sufficient quantity of fresh plasma from dog/rabbit, to ensure binding of all iron to the β_1-globulin transferrin. After incubation, a measured amount of plasma labelled with iron-59 was injected intravenously in the dog/rabbit and blood samples were collected from the dog/rabbit at different time intervals depending on the nature of the experiments. The experimental animals were then killed and samples of blood, bone marrow, ribs, rib-marrow and pieces of liver and spleen collected for biochemical studies. Radioactivity in the different biological samples was measured by counting 1–2 ml. aliquots in a scintillation well counter.

Complete iron kinetics investigation were made in two dogs for a period of ten days as described by Pollycove[2] and Pollycove and Mortimer[1]. By applying the mathematical model to radioiron measurements of plasma, red cells, liver, spleen and sacral marrow, the intercompartmental rate constant was calculated for transfer of iron from the erythropoietic labile iron pool to erythron iron irreversibly fixed for hæm synthesis (Fig. 2).

The results from eight experimental dogs (dogs 1, 2, 5–10) showed that marrow radioiron was associated with cellular elements. Doubly crystallized hæm was prepared from marrow aspirations of femur, marrow scrapings from ribs and intact rib homogenates of dogs after hæm synthesis was arrested with 5 × 10^{-4} M lead acetate. Analysis of results showed that soon after injection of labelled plasma,

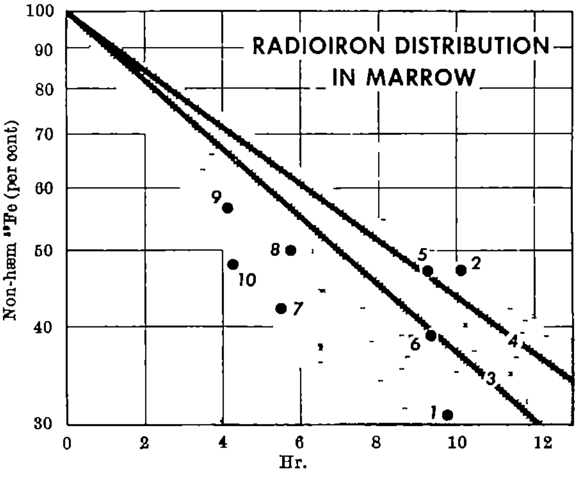

Fig. 3. Radioiron distribution in marrow. Points for dogs 1, 2, 5–10 were obtained from direct analysis of red marrow including measurement of iron-59 specific activity of double crystallized hæm. The slopes for dogs 3 and 4 are the calculated intercompartmental transfer-rates from the erythropoietic labile iron pool to irreversibly fixed erythron iron, a_{32}. These rate constants were calculated by analysis in accordance with the mathematical model (Fig. 1) of plasma radioiron data shown in Fig. 2

Table 1. DISTRIBUTION OF RADIOIRON (IN MARROW CELLS) BETWEEN NON-HÆM AND HÆM IN CELL MEMBRANE AND CELL INTERIOR

Data correspond to times after injection and distribution of radioiron in intact cells of the dogs shown in Fig 3

Dog	Non-hæm ^{59}Fe (per cent) Cell membrane	Cell interior
6	61	21
7	80	--
9	59	9
10	68	22

Table 2. TRANSFER OF RETICULOCYTE RADIOIRON TO PLASMA OR ACD AFTER 1 HR. INCUBATION AT 37° C.

Reticulocytes were initially labelled by *in vivo* transfer of iron-59 from plasma transferrin

Incubate	Total iron binding capacity (μgm./100 ml.)	Reticulocyte ^{59}Fe transferred (per cent)
Phenylhydrazine plasma	418	3·9
Post-venesection plasma	329	3·2
Normal plasma	186	2·6
Acid–citrate–dextrose solution	0	1·7

marrow radioiron occurs chiefly in non-hæm form, 8 hr. after injection (6 hr. after most of the radioiron initially in the plasma had accumulated in marrow) approximately half the marrow radioiron was present as erythron non-hæm radioiron (Fig. 3). Afterwards, increasing amounts of erythron radioiron occurred in hæm as non-hæm radioiron decreased. The calculated rates of transfer of radioiron from labile non-hæm iron to fixed hæm iron in dogs 3 and 4 (Fig. 2) are in good agreement with the direct marrow analyses in the other eight dogs (Fig. 3). The non-hæm radioiron was largely associated with red cell membrane, while hæm radioiron was present almost entirely in the interior of the red cell (Table 1). In the case of phenylhydrazine-treated rabbits with 90 per cent reticulocytes, reticulocytes labelled with iron-59 were obtained by venesection 30 min. after the intravenous injection of a tracer amount of transferrin-bound radioiron. Subsequent incubation of these washed (three times with unlabelled plasma) labelled reticulocytes with unlabelled rabbit plasma and acid citrate dextrose solution demonstrated considerable transfer of reticulocyte radioiron back to plasma and significantly less to the acid citrate dextrose solution (Table 2). The data on rabbit erythrocytes and canine marrow indicate that a labile erythropoietic iron pool exists on the membranes of immature erythrons, and that this iron is derived from plasma transferrin-bound iron and transfers back to plasma, as well as transferring irreversibly into erythrons for hæm synthesis. Further work on man is being continued.

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VARIATION IN GONADOTROPHIC HORMONE-LEVEL DURING ŒSTROUS CYCLE

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SEVERAL investigators have attempted to bring anœstrous animals into œstrus followed by successful fertilization. The administration of pregnant mare serum or pituitary follicle-stimulating hormone followed by human chorionic gonadotrophin was successful in bringing ewes into heat, but only few of these animals produced lambs[1]. The administration of pregnant mare serum to mature rats and subsequent injection of human chorionic gonadotrophin was not very successful in inducing full œstrus and subsequent pregnancy after mating[2]. These facts called for the study of gonadotrophic hormone status during naturally occurring œstrous cycles.

Forty-eight mature female rats of an average weight of 180 gm. were used in this experiment. Vaginal smears were procured from these animals every 12 hr. for 20 days. They were then divided into six groups representing: procœstrus, early œstrus, late œstrus, metœstrus, early and late diœstrus. The animals were killed by decapitation under light ether anæsthesia. The blood collected from each animal was allowed to clot and then centrifuged at 4,000 r.p.m. for 15 min The sera from rats of the same groups were pooled. Steroid hormones were removed from the sera by using the procedure of Szego and Roberts[3]. The remaining serum proteins were then redissolved in saline to bring it back to the original volume of serum. The pituitary glands of each group of rats were weighed with a torsion balance then dried with acetone. Saline suspensions of the pituitary glands were prepared to contain 0·5 mgm. of fresh weight in every 0·1 ml. saline.

The total gonadotrophic hormone content of the sera and pituitaries was determined by using the chick method of assay[4]. Two groups of 15 chicks each, corresponding to every rat group, were given daily doses of 0·3 ml. serum or 0·5 mgm. pituitary for 4 days. One group was kept as control injected with saline. The weights of testes of chicks were used as a criterion of the gonadotrophic hormone content of the test material. The results were then expressed in terms of I.U. pregnant mare serum from a standard curve established under the same experimental conditions.

The follicle-stimulating hormone content of 0·5 ml. serum and 0·5 mgm. pituitary were assayed by the use of the luteinizing hormone augmentation method[5]. Two groups of 5 immature mice were allotted to each group of rats, one receiving serum while the other received pituitary saline suspension.

The luteinizing hormone content of 0·5 ml. serum and 0·5 mgm. pituitary was determined by Soliman's method[6]. Two groups of 5 immature female mice were used for each of the experimental rat groups. The follicle-stimulating and luteinizing hormone levels were expressed in I.U. from standard curves established under the same conditions, using standard preparations of pregnant mare serum and human chorionic gonadotrophin. The data were analysed statistically using the t or F values.

The total gonadotrophic hormone contents of the pituitaries did not show any significant variation during the œstrous cycle as judged from the analysis of variance. The total gonadotrophins present in the blood reached their highest level during the phases of procœstrus and early œstrus, then its level dropped during the other phases of the cycle (Table 1).

Table 1. TOTAL GONADOTROPHIC HORMONE IN RAT PITUITARY GLANDS AND SERUM DURING THE ŒSTROUS CYCLE

Phase of the œstrous cycle	Pituitary: Testes wt of chicks (mgm./100 gm. body-wt.)	Pituitary: I.U. (per mgm.)	Serum: Testes wt. of chicks (mgm./100 gm. body-wt.)	Serum: I.U. (per ml.)
Procœstrus	47·67 ± 4·00	7·24	40·55* ± 4·75	4·32
Early œstrus	51·42 ± 7·25	9·55	39·18* ± 3·72	3·91
Late œstrus	37·61 ± 2·61	3·49	28·60 ± 0·70	1·81
Metœstrus	49·01 ± 7·48	7·99	31·86 ± 3·70	2·29
Early diœstrus	44·13 ± 6·28	5·61	31·58 ± 3·24	2·25
Late diœstrus	44·79 ± 3·20	5·88	28·31 ± 4·76	1·77

± Standard error.

* Significantly different from late œstrus group at 5 per cent level of probability.

The pituitaries of procœstrous and œstrous rats had the highest concentration of follicle-stimulating hormone, and minimal amounts were present in the pituitaries of rats during the other stages of the cycle. In the blood there were two peaks of follicle-stimulating hormone, one being attained during early œstrus and the other during early diœstrus (Table 2).

Table 2. FOLLICLE-STIMULATING HORMONE CONTENTS OF RAT PITUITARIES AND SERUM DURING THE ŒSTRUS CYCLE

Phase of the œstrous cycle	Pituitary: Ovary wt of mice (mgm./100 gm. body-wt.)	Pituitary: I.U. (per mgm.)	Serum: Ovary wt. of mice (mgm./100 gm. body-wt.)	Serum: I.U. (per ml.)
Procœstrus	33·17 ± 3·00	0·73	27·16 ± 2·28	0·47
Early œstrus	31·71 ± 2·59	0·65	34·21 ± 3·45	0·78
Late œstrus	23·05 ± 1·32	0·35	18·33 ± 1·61	0·25
Metœstrus	22·05 ± 2·12	0·32	17·60 ± 3·48	0·23
Early diœstrus	24·56 ± 1·32	0·39	31·54 ± 2·45	0·64
Late diœstrus	24·61 ± 1·41	0·30	23·92 ± 2·14	0·37
F value	5·21		3·78	
Degrees of freedom	5, 29		5, 29	

± *S.E.*

The highest level of luteinizing hormone was observed in the pituitary glands during late œstrus. In the blood the luteinizing hormone level had two peaks, the higher one during late œstrus and the second during procœstrus.

It is concluded that the level of gonadotrophic hormone in the pituitary does not essentially parallel its concentration in the blood[7]. The luteinizing hormone level in the blood is relatively high at procœstrus, probably to augment the action of follicle-stimulating hormone, thus stimulating the growth of the Graafian follicles and secretion of œstrogen[8]. During late œstrus there is an increased rate of synthesis and release of luteinizing hormone to induce ovulation.

Table 3. LUTEINIZING HORMONE CONTENTS OF RAT PITUITARIES AND SERUM DURING ŒSTROUS CYCLE

Phase of the œstrous cycle	Pituitary: Average No. of corpora hæmorrhagica per mouse	Pituitary: I.U. (per mgm.)	Serum: Average No. of corpora hæmorrhagica per mouse	Serum: I.U. (per ml.)
Procœstrus	0·60 ± 0·13	0·42	1·33 ± 0·20	0·94
Early œstrus	1·20 ± 0·16	0·81	0·50 ± 0·33	0·38
Late œstrus	2·40 ± 0·40	3·04	2·67 ± 0·33	4·09
Metœstrus	0·60 ± 0·13	0·42	0·40 ± 0·11	0·84
Early diœstrus	0·20 ± 0·02	0·27	0·40 ± 0·11	0·34
Late diœstrus	1·60 ± 0·13	1·26	0·00	0·00
F value	3·20		7·40	
Degrees of freedom	5, 29		5, 29	

± *S.E.*

Table 4. SLOPES AND FIDUCIAL LIMITS OF THE STANDARD LOG.-DOSE RESPONSE CURVES

Type of hormone	*b*	Fiducial limits at 5 per cent level	Average log.-dose $\overline{X}$	Average response value (mgm./100 gm. body-weight) $\overline{Y}$
Total gonadotrophins	31·68 ± 2·35	5·03	1·94	50·19
Follicle-stimulating hormone	31·67 ± 1·27	2·68	1·95	67·67
Luteinizing hormone	2·09 ± 0·48	0·99	2·04	3·56

± *S.E.*

The standard pregnant mare serum and human chorionic gonadotrophin preparations were kindly supplied by the National Institute for Medical Research, London.

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LACK OF RELATIONSHIP BETWEEN MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION AND THE DICOUMAROL-SENSITIVE FLAVOENZYME 'DT DIAPHORASE' OR 'VITAMIN K REDUCTASE'

By LENNART DANIELSON and LARS ERNSTER

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IT has recently been reported by Martius[1] that mitochondria from pigeon liver, a tissue previously[2] found to be devoid of the dicoumarol-sensitive flavoenzyme 'vitamin K reductase', exhibit phosphate/oxygen ratios with pyridine nucleotide-linked substrates which are one unit below the generally accepted value for mitochondria from various other animal tissues. With succinate as substrate, the phosphate/oxygen ratio was normal. These findings were taken by Martius as further support for his concept[3] that vitamin K reductase constitutes an obligatory link in the aerobic oxidation of pyridine nucleotide-linked substrates in intact, phosphorylating mitochondria.

In 1958, the occurrence in rat-liver of a highly active flavoenzyme catalysing the oxidation of reduced di- and tri-phosphopyridine nucleotide by artificial electron acceptors was detected in our laboratory[4]. The enzyme, called 'DT diaphorase', was afterwards partially purified, and its properties, among them a high sensitivity to dicoumarol, were investigated in some detail[5]. Investigations were also carried out on the relation of DT diaphorase to the terminal respiratory chain, using both isolated mitochondria[6,7] and liver slices[8]. In this work, unequivocal evidence has been obtained that DT diaphorase does not take part in the main enzymatic pathway of the aerobic oxidation of reduced pyridine nucleotides in rat-liver.

Until recently, DT diaphorase was believed to differ from vitamin K reductase, in spite of some conspicuous features in common between the two enzymes. This belief was based primarily on the fact that DT diaphorase exhibits no significant activity with vitamin K_1 as electron acceptor[5], whereas this compound has repeatedly been reported to serve as a highly active electron acceptor for vitamin K reductase[3,9]. Recently, however, Märki and Martius[1] have revised this property of vitamin K reductase, after having found that authentic samples of vitamin K_1 react only very slowly with their enzyme. In view of this finding, and of some other newly reported properties of vitamin K reductase[1], we were then led to conclude[7] that vitamin K reductase very probably is identical with DT diaphorase, and consequently, that Martius's concept[3] regarding the role of vitamin K reductase in cellular respiration and phosphorylation also may require revision. Singer and associates[10] have recently extracted a flavoenzyme from beef-heart electron transport particles which they on good grounds could identify with the flavoenzyme component of the phosphorylating mitochondrial diphosphopyridine nucleotide oxidase system. This flavoenzyme is specific for diphosphopyridine nucleotide and insensitive to dicoumarol, and thus distinct from DT diaphorase or vitamin K reductase, which further makes the validity of Martius's concept[3] doubtful.

The present article reports further data relevant to these problems, obtained in experiments with isolated pigeon-liver mitochondria. It is shown that these mitochondria are devoid of DT diaphorase, in accordance with the findings of Märki and Martius[1] concerning vitamin K reductase. At variance with Martius's findings[1], on the other hand, the present data reveal that pigeon-liver mitochondria exhibit phosphate/oxygen ratios with pyridine nucleotide-linked substrates which clearly exceed the value of 2, and are thus quite comparable with those usually obtained with mitochondria from other sources. It is also shown that dicoumarol uncouples phosphorylation from respiration in pigeon-liver mitochondria in the same fashion as it does in mitochondria from other sources, suggesting that this effect of dicoumarol is unrelated to its inhibitory effect on DT diaphorase or vitamin K reductase.

Liver mitochondria from pigeon (*Columba domestica*) were prepared by using essentially the same procedure as is routinely used in this laboratory[11] for preparing mitochondria from rat-liver. The mitochondria were washed twice with 0·25 *M* sucrose, whereby special care was taken in removing a sticky, dark-brown sediment which usually appeared as a tightly packed sediment beneath the more fluffy mitochondrial pellet. The final mitochondrial pellet was suspended in 0·25 *M* sucrose to contain mitochondria from 1 gm. wet-weight liver (about 26 mgm. mitochondrial protein) per ml.

Table 1. COMPARISON BETWEEN RAT- AND PIGEON-LIVER MITOCHONDRIA WITH REGARD TO THE DT DIAPHORASE DEPENDENT BY-PASS OF THE AMYTAL-SENSITIVE SITE OF THE RESPIRATORY CHAIN

Additions	Respiration, µatoms oxygen Rat	Pigeon
None	5·49	4 98
Vitamin K_3	5·47	5·06
Dicoumarol	5 32	4·63
Amytal	0·22	0 00
Amytal + vitamin K_3	5·25	1 04
Amytal + vitamin K_3 + antimycin A	1·57	1·05
Amytal + vitamin K_3 + dicoumarol	1 18	1·48

Each Warburg vessel contained: 10 µmoles glutamate, 20 µmoles *tris* buffer (*p*H 7·4), 20 µmoles orthophosphate (*p*H 7·4), 4 µmoles $MgCl_2$, 2 µmoles adenosine triphosphate, 24 µmoles glucose, an excess of yeast hexokinase, 50 µmoles sucrose, and mitochondria from 200 mgm. liver. The amounts of the additions were as follows: 5 × 10^{-2} µmoles vitamin K_3, 10^{-2} µmoles dicoumarol, 1 0 µmole amytal, 1 µgm. antimycin A, and 1·0 µmole KCN. Final volume, 1·0 ml Temperature, 30° C. Time measured, 20 min. The values for rat are quoted from Conover and Ernster (ref. 6).

Respiration was measured with the conventional Warburg technique. The composition of the reaction mixtures is specified in the table legends. After the manometer readings were completed, the samples were fixed with 0·2 ml. 5 *M* sulphuric acid, and phosphate uptake was determined by the distribution of phosphorus-32, as described by Lindberg and Ernster[12]. Phosphate/oxygen ratio was computed by dividing the number of micromoles inorganic phosphate disappeared by the number of microatoms oxygen consumed. Oxygen consumption for the thermoequilibration period, 5 min., was calculated by direct extrapolation. All chemicals were commercial products; dicoumarol was purchased from AB Ferrosan, Malmo, Sweden.

The absence of DT diaphorase from pigeon-liver could be demonstrated in several ways. In rat-liver, DT diaphorase is most abundant in the cell-sap and

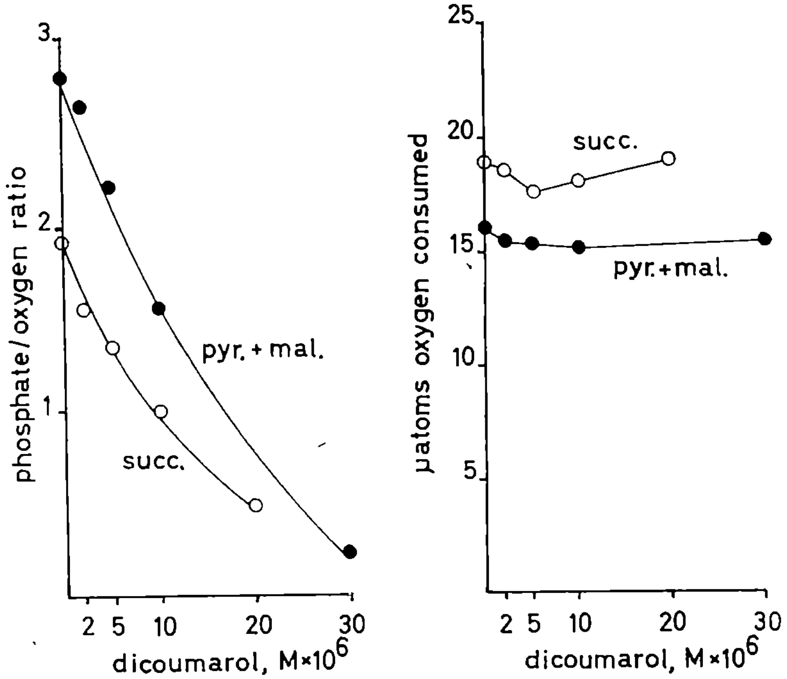

Fig. 1. Uncoupling of oxidative phosphorylation in pigeon-liver mitochondria by dicoumarol. Conditions as in Table 2. The substrates used were succinate or pyruvate + malate

Table 2. RESPIRATION AND PHOSPHATE/OXYGEN RATIOS EXHIBITED BY PIGEON-LIVER MITOCHONDRIA WITH VARIOUS SUBSTRATES

	Substrate											
	Succinate		Pyruvate + L-malate		Citrate		α-Ketoglutarate		L-Glutamate		DL-β-Hydroxybutyrate	
Exp. No.	µatoms oxygen	P/O	µatoms oxygen	P/O	µatoms oxygen	P/O	µatoms oxygen	P/O	µatoms oxygen	P/O	µatoms oxygen	P/O
1	18·0	1·62	16·6	2·80	12·6	2·54	14·4	2·91	12·4	2·88	0·5	—
2	21·6	2·00	12·5	2·42			12 3	2·32	6·7	2·36	0·1	—
3	18·9	1·95	16·7	2·61					14·9	2·08	0·4	—
4	19·5	1·55	17·4	2·51								
5	17·3	2·10							5·1	2·62		

Each Warburg vessel contained mitochondria from 200 mgm. liver, 125 µmoles sucrose, 20 µmoles substrate, 50 µmoles potassium orthophosphate (pH 7·4), 100 µmoles potassium chloride, 16 µmoles magnesium chloride, 2 µmoles adenosine triphosphate, 60 µmoles glucose, and an excess of yeast hexokinase, in a final volume of 2 ml. Temperature, 30° C. Time measured, 20 min.

constitutes the sole diaphorase element of this cell-fraction[5]; consequently, DT diaphorase in this fraction can be simply assayed by measuring the reduction of 2,6-dichlorophenolindophenol by reduced di- or tri-phosphopyridine nucleotide. In the mitochondrial and microsomal fractions, which account only for a minor portion of the total rat-liver DT diaphorase, and in which also other diaphorases are present, DT diaphorase can be demonstrated by measuring the difference in dye-reduction by reduced triphosphopyridine nucleotide in the absence and presence of 10^{-6} *M* dicoumarol[5]. All these tests in the case of pigeon-liver gave completely negative results. A more accurate method for ascertaining the absence of DT diaphorase from pigeon-liver mitochondria was based on the previous finding[6,7] that, in rat-liver mitochondria, addition of vitamin K_3 establishes a by-pass of the amytal-sensitive site of the respiratory chain; this by-pass involves DT diaphorase as indicated by its high sensitivity to dicoumarol (Table 1, left column). With pigeon-liver mitochondria, incubated under identical conditions, no bypass of the amytal-sensitive site of the respiratory chain occurred on the addition of vitamin K_3 (Table 1, right column).

Results from five experiments concerning respiration and phosphate/oxygen ratios of pigeon-liver mitochondria with various substrates are summarized in Table 2. Succinate, pyruvate + malate, citrate and α-ketoglutarate were oxidized at relatively high and consistent rates, whereas the rate of respiration with glutamate varied between medium-high and low from one experiment to another. No respiration under the prevailing conditions was consistently obtained with β-hydroxybutyrate. The phosphate/oxygen ratios ranged between 1·55 and 2·10 with succinate as substrate (average value 1·84) and between 2·08 and 2·91 with pyridine nucleotide-linked substrates (average value 2·50). These values are in perfect agreement with those commonly obtained with mitochondria from rat-liver and other tissues, and fail thus to substantiate the occurrence of a systematic deviation from the normal pattern as suggested by Martius[1]. It may also be mentioned that mitochondria from pigeon-breast muscle which likewise lack vitamin K reductase[9] have been reported by Azzone and Carafoli[13] to exhibit normal phosphate/oxygen ratios.

The absence of DT diaphorase from pigeon-liver mitochondria provided a possibility for deciding the previously raised question[5,7], whether the well-known uncoupling effect of dicoumarol on oxidative phosphorylation[11] is related to its inhibitory effect on DT diaphorase. If this were the case, phosphorylation in pigeon-liver mitochondria should be insensitive to dicoumarol. This proved clearly not to be the case. As illustrated by the results in Fig. 1, dicoumarol uncoupled oxidative phosphorylation in these mitochondria both with succinate and with pyridine nucleotide-linked substrates, and the dependence of the uncoupling effect on the dicoumarol concentration was roughly the same as has been reported[14,15] for rat-liver mitochondria. Furthermore, the half-uncoupling concentration of dicoumarol was the same with succinate and with pyridine nucleotide-linked substrates, about 10^{-5} *M*, which is consistent with the earlier conclusion of Cooper and Lehninger[15] that dicoumarol attacks the three respiratory chain phosphorylations to equal extents.

In summary, the results reported in this article substantiate the previous conclusions that vitamin K reductase is identical with DT diaphorase[5,7], and that this enzyme does not constitute an obligatory link in the aerobic oxidation of pyridine nucleotide-linked substrates in intact, phosphorylating mitochondria[6,7]. It is also evident from the results that dicoumarol can uncouple oxidative phosphorylation in mitochondria lacking DT diaphorase, and consequently, that this effect of dicoumarol very probably is unrelated to its inhibitory effect on DT diaphorase even in those mitochondria in which DT diaphorase is present. This appears to eliminate the possibility, discussed in earlier papers[5,7], that DT diaphorase still might have an accessory function in the coupling of respiration to phosphorylation even though it does not constitute part of the respiratory chain. Hence, the function of DT diaphorase in mitochondria, and in the cell, is still not clear. However, the fact that the enzyme is absent from the pigeon may serve as a valuable basis for future comparative metabolic studies aiming at an elucidation of its physiological function.

This work was supported by a grant from the Swedish Cancer Society.

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X-RAY INACTIVATION OF INDUCIBLE ENZYME SYNTHESIS AND THE EFFECT OF OXYGEN AND GLYCEROL

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IRRADIATION can not only destroy the reproductive integrity of bacteria and inactivate enzymes, but it can also destroy the ability of bacteria to produce adaptive enzymes. For example, relatively small doses of X-rays could prevent the adaptive formation of formic hydrogenase in *Escherichia coli*[1]. The dose to inactivate the pre-formed enzyme β-galactosidase[2] is about 100 times greater than the dose required to inhibit the adaptive synthesis of the same enzyme[3]. In *Bacterium cadaveris*, Pauly[4] has shown that both the rate of induction and the final adaptive lysine decarboxylase activity formed, fell off exponentially, with increasing X-ray dose. Pauly also found that this effect of X-rays was dependent on oxygen (see Gray[5]). The present article confirms the exponential X-ray inactivation of the inducibility of another adaptive enzyme, concerned in the oxidation of histidine, in a *Pseudomonas*, and demonstrates that both colony-forming ability and the ability to synthesize an adaptive enzyme respond similarly to the modifying agents oxygen and glycerol.

The *Pseudomonas* used in these experiments was isolated from soil and identified as being very similar but not identical to *Ps. ichthyosmia*. The irradiation was 200-kV. X-rays from a Marconi *KX* 10 X-ray tube using a 1·6-mm. aluminium filter and dose-rate between 1,000 and 2,200 r./min. The irradiation vessel was the water-jacketed 'Perspex' vessel used previously[6].

For experiments on cell survival, cultures were grown for 24 hr. at 25° C. in unaerated nutrient broth. Shortly before irradiation the suspension was diluted one in fifty in 0·015 *M* phosphate buffer at *p*H 7. After irradiation samples were suitably diluted in phosphate buffer and plated out in nutrient agar. Visible colony counts were made after 48 hr. incubation at 25° C. The fraction of cells surviving to form a visible colony is shown as a function of dose in Fig. 1.

For experiments on the ability to form the adaptive enzymes concerned with histidine oxidation, the bacteria were incubated with aeration by shaking in a medium consisting of: NaH_2PO_4 5·45 gm.; NaCl 0·9 gm.; $MgSO_4$ 0·1 gm.; NH_4Cl 2·0 gm.; $FeSO_4$ 0·05 gm.; glucose 10 gm.; water to 1 litre, and adjusted to *p*H 7 with 5 *N* potassium hydroxide. Repeated sub-culture on this medium produced poor growth, but by using a cell suspension in 2 ml. of nutrient broth as an inoculum, good growth of unadapted cells was obtained. After 24 hr. incubation at 25° C. the bacteria were centrifuged, washed with water, re-centrifuged and made up in 0·15 *M* phosphate buffer *p*H 7 to a thick suspension containing 10^{10} cells per ml. Portions of this suspension were irradiated in the same manner as for cell survival. 1 ml. of irradiated or control cells was put in each Warburg flask with 1 ml. of 0·15 *M* phosphate buffer *p*H 7, and ½ ml. of 0·04 *M* l-histidine was tipped in from the side bulb. The oxygen consumed was measured at 25° with air in the gas phase.

In preliminary experiments, by using different concentrations of histidine and bacteria, it was found that on addition of inducer the endogenous respiration continued simultaneously and independently of histidine oxidation and was practically unaltered by the doses of radiation used. The endogenous respiration, which amounted to about 6 per cent of the maximum rate of consumption of oxygen by adapted cells, was therefore subtracted from all other readings. The rate of oxygen uptake in excess of endogenous respiration increased slowly after adding histidine. After 7–8 hr. the rate became constant until the histidine was exhausted, when it dropped

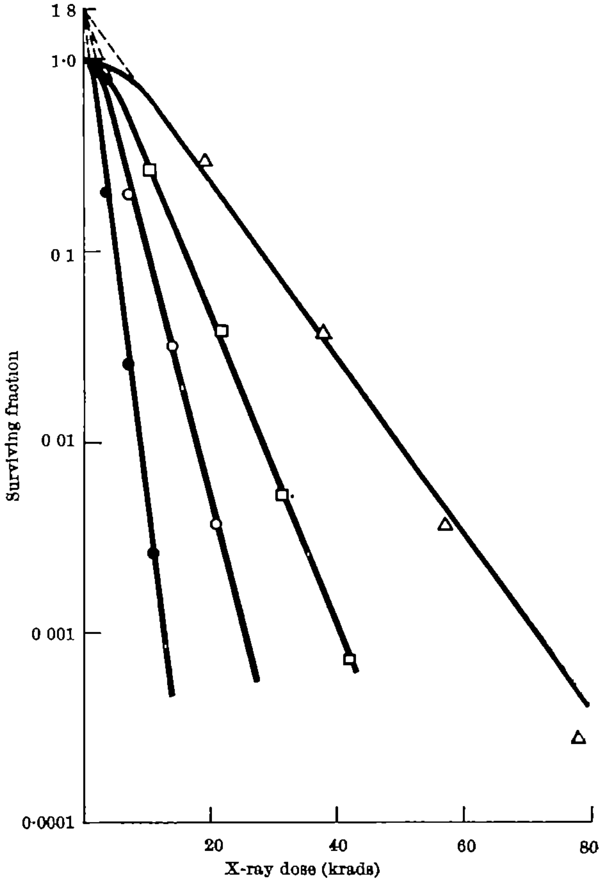

Fig. 1. Loss of colony-forming ability of *Pseudomonas* after 200-kV. X-rays

●, Irradiated in 100 per cent oxygen; ○, irradiated 0·11 per cent oxygen in nitrogen; □, irradiated in nitrogen containing 0 00085 per cent oxygen; △, irradiated in *M* glycerol in nitrogen

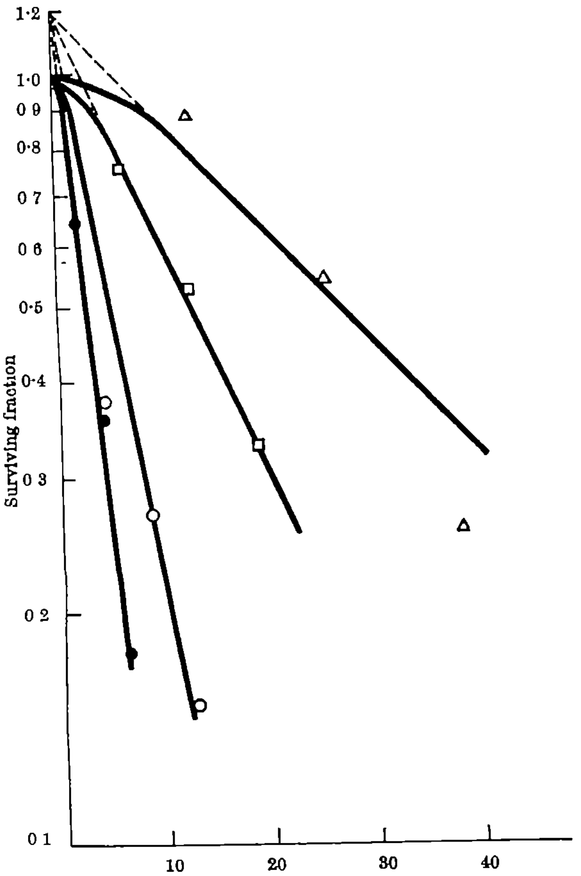

Fig. 2. Loss of ability of *Pseudomonas* to synthesize inducible histidine-oxidizing enzymes. Symbols as for Fig. 1

sharply to a new low value still slightly greater than endogenous. During this time the viable cell count increased by about 25 per cent. The theoretically complete oxidation of each molecule of histidine requires 5 molecules of oxygen. The mean uptake was 50·6 per cent (standard deviation of the mean 0·6 per cent) of the theoretical amount and was independent of histidine concentration in the range measured (2 mM–0·4 mM). The rate of formation of the enzyme was, however, reduced by high histidine concentrations. When cells were irradiated before the addition of histidine the time course of the adaptation process was found to be unaltered, but the fully induced activity was reduced. The drop in this maximum rate of oxygen uptake as a percentage of the controls was used as measure of the X-ray damage.

In Fig. 2 the log fraction of fully induced enzyme activity is plotted against X-ray dose. The scales of Figs. 1 and 2 have been adjusted for easier comparison, and demonstrate that the modifying effect of 0·1 per cent oxygen, nitrogen and *M* glycerol in nitrogen are broadly similar. For a more detailed comparison the slopes of the linear part of the curves measured in krads for a 37 per cent change, are listed in Table 1. The ratio of the 37 per cent doses for colony-forming ability and for loss of adaptive enzyme formation is about 2 in all cases. For the adaptive enzyme measurements, rather high concentrations of bacteria were necessary. When the cell suspension was gassed with 100 per cent oxygen the effects of small changes in oxygen tension are negligible and the exact concentration of dissolved oxygen is not important. When the cells were gassed with nitrogen, respiration helped to maintain anoxia. As a check against possible air leaks, oxygen in the gas emerging from the irradiation vessel was measured by a Hersch cell[7]. Observations were only accepted when the emerging gas contained less oxygen than 20 parts per million. For irradiations at small oxygen concentrations, bacteria were bubbled with gas from a cylinder containing 0·11 per cent oxygen (obtained from the British Oxygen Co.). The difficulty of holding a high concentration of respiring cells at an accurately known low oxygen concentration is considerable[8], and is increased when irradiations are carried out at high dose-rates, on account of the radiochemical utilization of dissolved oxygen[9]. Thus, starting with a suspension in equilibrium with 0·11 per cent oxygen, 10^{10} cells/ml., consuming 10 µl./ml./hr., would render the suspension anoxic in 0·2 min. due to respiration alone, and similarly a dose-rate of 2,000 r./min. would render the suspension anoxic in 0·15 min. due to the radiochemical changes alone. In practice, bacteria at 10^{10}/ml., bubbled with 30 ml./min. of 0·11 per cent oxygen, showed almost anoxic sensitivity when irradiated at 2,000 r./min. Halving both the concentration of bacteria and the dose-rate raised the sensitivity to the level shown in Fig. 2.

Glycerol is known to protect the colony-forming ability of bacteria from X-ray damage. The hyperprotection dose-factor (the amount of protection above the anoxic level) for *M* glycerol was 1·6 compared with 1·9 previously reported for *Serratia marcescens*[10].

Comparable studies of loss of capacity for adaptive enzyme synthesis were complicated in several ways. In the first place, *M* glycerol increases the rate at which *Pseudomonas* becomes adapted to histidine without, however, affecting the maximum level of enzyme synthesis. This is in contrast to the effects of glucose and of excess of histidine, which delayed the adaptive process. The effect of glycerol on the rate of adaptation is not sensitive to radiation. Secondly, in the presence of *M* glycerol the oxygen consumption by *Pseudomonas* is about ten times as great as that due to normal endogenous respiration. Moreover, the additional oxygen consumption, unlike endogenous respiration, is depressed by irradiation, but not apparently to a degree which is related in any simple exponential manner to dose. This effect is therefore difficult to allow for. The points on the glycerol line in Fig. 2 were obtained by subtracting from the rate of oxygen uptake of fully induced cells, the respiration-rate of the same number of cells which had received the same dose of irradiation in the presence of glycerol. Unfortunately, this is a small difference between large numbers with corresponding loss in accuracy. The magnitude of the error is difficult to assess, and although the hyperprotection

Table 1

	Loss of colony-forming ability	Loss of ability to form adaptive enzyme	Ratio enzyme colony
37 per cent dose in oxygen	1·64 krads	3·25 krads	1·98
37 per cent dose in 0·11 per cent oxygen	3·42 krads	5·60 krads	1·64
37 per cent dose in nitrogen	5·47 krads	14 4 krads	2·63
37 per cent dose in *M* glycerol	8·57 krads	30·1 krads	3·5
m = nitrogen/oxygen	3·34	4·41	
$K = \dfrac{(O_2)(Dm - D_n)}{D_n - D}$	4·5 μM	1·8 μM	
Hyperprotection by *M* glycerol	1·6	2·1	
Extrapolation No.	1·8	1·2	

(O_2), oxygen concentration in solution in µmol./l.; D, 37 per cent dose at the oxygen concentration (O_2); D_n, 37 per cent dose in nitrogen.

of glycerol against X-ray damage to enzyme-inducing capacity is certain, the magnitude of this protection cannot be given with any great precision.

Hitherto, colony-forming ability has been recognized as one of the outstandingly radiosensitive functions of micro-organisms. The observations which are reported here show that, in the case of *Pseudomonas*, the capacity for adaptive enzyme synthesis for histidine oxidation is destroyed by doses which are only about twice as great as those in a comparable loss of colony-forming ability. They show, moreover, that the enhancement of sensitivity by oxygen and the lowering of sensitivity by glycerol is roughly the same for both indices of damage in this micro-organism. Thus, similar radiochemical mechanisms are probably involved in the initiation of both forms of radiation damage. If loss of colony-forming ability is caused by damage to deoxyribonucleic acid (DNA), then a large fraction of this DNA must be left intact if induced enzyme synthesis is to continue. This is supported by the recent finding of Eisenstadt and Novelli[11], who have shown that a cell extract of X-ray inactivated *E. coli* would only synthesize β-galactosidase if specific DNA from induced non-irradiated *E. coli* was added.

From the shape of the survival curves in Figs. 1 and 2 it can be inferred that chemical changes initiated by at most a few ionizing particles are sufficient to destroy the colony-forming ability of a single micro-organism, and the activity of a single centre controlling enzyme synthesis. The question as to whether one or more such control centres are present in each bacterium is at present under investigation.

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FACTORS CONCERNED IN THE INITIATION OF GROWTH AND MOULTING IN *LOCUSTA MIGRATORIA* L.

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IN general the life-cycle of an insect is linked to the changes which occur in its environment. Changes in temperature, humidity, photoperiod, and food supply all have their effects on the rates at which processes occur in insects. At extremes, these can lead to a cessation of growth and moulting which may not be resumed until conditions become more favourable, or until some definite stimulus is applied. Thus, in *Rhodnius prolixus* Stal. a blood meal is necessary at the beginning of each instar before further growth and moulting can occur[1], while in *Hyalophora cecropia* (L.) chilling of the pupal brain is necessary before diapause is broken and growth resumed[2]. Both these cases represent adaptations to life in harsh environments. There is, however, no information about the processes which ensure a continuous sequence of moulting cycles in insects which normally feed continuously throughout the greater part of each stadium, and do not encounter conditions liable to interrupt their growth. This article describes those processes which in such an insect, here represented by *Locusta migratoria* L., ensure a continuous succession of growth and moulting cycles throughout its developmental period. Only the conclusions with the main facts which support them are given ; full experimental evidence will be published elsewhere.

An examination of the neuro-endocrine system of the insect was made, using Gomori's chrome hæmatoxylin and phloxine stain for the detection of neurosecretory material. Particular attention was paid to the medial neurosecretory cells, the chiasma formed by their axons, the nervi corporis cardiaci I, and the corpora cardiaca. Studies of the insects made at daily intervals throughout the third, fourth and fifth stadia showed no sign of cyclical changes anywhere throughout the system during this period. Neurosecretory material was found to be present in small amounts in the cell bodies, in their axons, and in the corpora cardiaca, where it was distributed in those regions close to the aorta. This picture is compatible with the continuous active formation and secretion of material throughout the period investigated, and this is taken to be the normal condition in the growing locust. It contrasts markedly with that found in the mature adult, where there is an accumulation of neurosecretory material in the cell bodies, their tracts and corpora cardiaca.

Experiments have shown that the further growth and moulting of the locust was completely stopped by the cutting of the frontal connectives or the removal of the frontal ganglion at any time during the first 50 per cent of the stadium (Fig. 1). Cutting the recurrent nerve behind the frontal ganglion, the outer œsophageal nerve, the ventral nerve cord in front of the first abdominal ganglion, or of only one of the frontal connectives, has no effect over that of control operations on the further growth and moulting of the insects, which proceeds normally.

An examination of the neuro-endocrine system of locusts in which the frontal connectives had been cut, or the frontal ganglion removed 6 hr. after ecdysis to the third instar, was made when they were 211 hr. old. This, compared with operated controls at the end of the third instar, that is 125 hr. after the operation, showed the following points. In the experimental locusts there was a marked accumulation of neurosecretory material in the corpora cardiaca, the nervi corporis cardiaci I (Fig. 2), and the chiasma, but scarcely at all in the protocerebral cells.

The effects of the removal of the frontal ganglion or the cutting of the frontal connectives cannot be attributed to starvation for the following reasons :

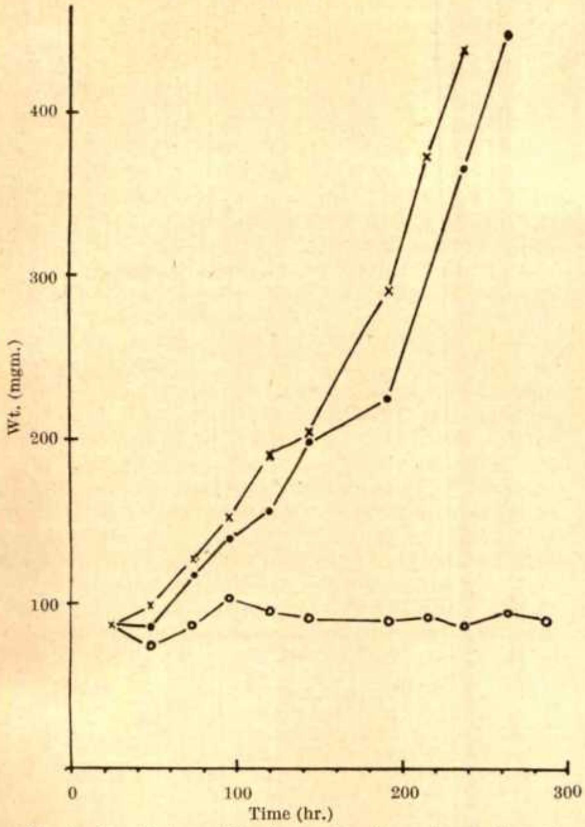
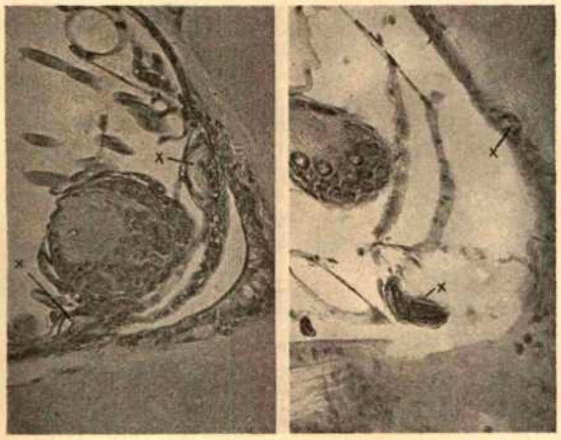


Fig. 1. Growth in weight of normal and experimental locusts throughout the third instar. ×, Unoperated controls; ●, operated controls; ○, with the frontal ganglion removed. The unoperated controls moulted to the fourth instar at 120 hr., the operated controls at 175 hr., those with the frontal ganglion removed did not moult again

the animals were observed to feed regularly, and the gut on dissection was always found to contain freshly eaten grass. The weights of the locusts remained constant at the value they had at the time of the operation ; in starved animals it always fell, or in partially starved animals decreased or increased slowly, but never remained constant. The oxygen consumption of the experimental animals was the same as that of normal insects, that of starved animals was always lower.

Examination of the frontal ganglion both by dissection and in serial sections showed that in addition to the recurrent nerve originating at its posterior boundary and the frontal connectives antero-laterally, there are two pairs of smaller nerves also present. One pair is just in front of the recurrent nerve and the other just behind the frontal connectives. Methylene blue preparations show that these nerves are distributed to the muscles of the pharynx where they end in stretch receptors (Fig. 3). These are placed in line on the pharyngeal wall. No sign of motor endings could be found in connexion with these nerves. (See also ref. 3 for *Oryctes*). Investigations also failed to reveal any signs of neurosecretory cells in the frontal ganglion.



a *b*

Fig. 2. Photomicrograph of the brain, nervi corporis cardiaci I (marked with an ×); and the hypocerebral ganglion of normal and operated locusts. *a*, A normal insect showing the virtual absence of neurosecretory material in the nervi corporis cardiaci I; *b*, a locust in which the frontal ganglion had been removed showing the accumulation of material in the nervi corporis cardiaca I

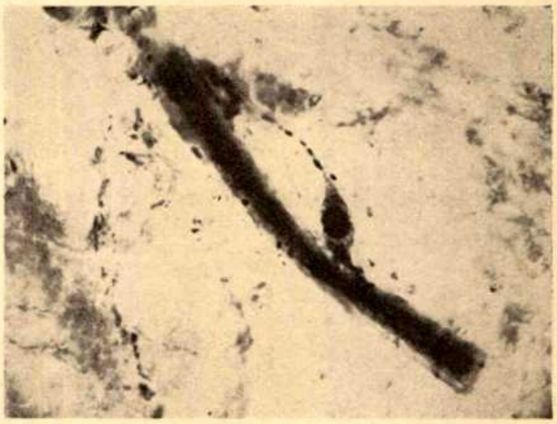


Fig. 3. Photomicrograph of a stretch receptor from the pharyngeal regions of *Locusta migratoria*. Methylene blue preparation

The anatomical arrangements of these parts make it possible for nervous impulses arising from these stretch receptors to pass to the brain via the frontal ganglion and the frontal connectives. It is postulated that in normal circumstances this does in fact happen, and that the continuous activity of the neurosecretory cells depends on the continuous arrival of these impulses. Under normal conditions the impulses arise through the movement of the pharynx. This occurs while feeding throughout a stadium and while swallowing air into the gut throughout ecdysis[4].

Accepting the implications of Thomsen's[5] work on *Calliphora erythrocephala* Meig., that the neurosecretory cells are concerned in protein metabolism, then this mechanism will ensure that the production and release of the hormone is correlated with the intake of food ; a situation similar to that described by Strangeways-Dixon[6] for *Calliphora*. The cutting of the frontal connectives or the removal of the frontal ganglion of the locust causes cessation of the production and/or release of the neurosecretory material (Fig. 2), and hence a cessation of protein metabolism. This would account for the immediate cessation of growth which has been shown to follow from this operation.

The continuous secretion of neurosecretory material poses the problem of how this can produce cyclical moulting in the animal. In the locust one of the first signs of the action of the growth and moulting

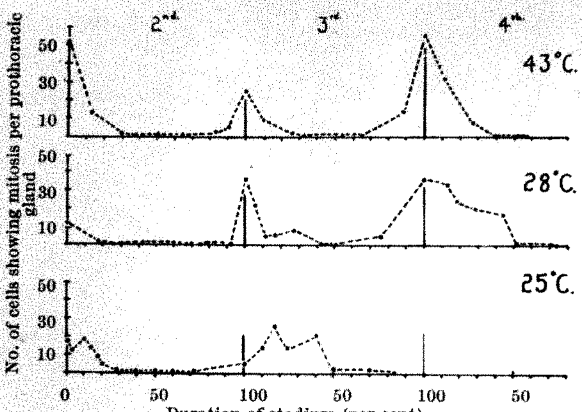


Fig. 4. Graph showing the numbers of mitoses per thoracic gland at different temperatures throughout the second, third and fourth stadia. Each figure represents the mean of at least five glands; during a stadium the low figures are not zero but represent values such as two divisions found in five glands examined

hormone in each cycle is the appearance of mitotic divisions in the cells of the prothoracic gland[7]. Examination of the prothoracic gland throughout the second, third and fourth stadia showed that the peak of frequency of the mitotic figures coincided with the moment of ecdysis (Fig. 4). At temperatures below 26° C. there is a shift of this peak into the following stadium ; but temperatures between 27° C. and 43° C. are without effect on the time of its occurrence. At all temperatures, however, the first appearance of mitotic figures in the prothoracic gland always occurs some hours before ecdysis. The most conspicuous events happening to the locust at about this time are the emptying of the gut of food and the swallowing of air preparatory to ecdysis.

On the basis of the above events the following hypothesis is proposed. That the neurosecretory material is constantly produced and secreted throughout the growth of the locust. During a stadium it is used for the metabolism of proteins associated with the intake of food. At an ecdysis the hormone is still produced but is not used, so that its blood titre will rise. If the prothoracic gland requires a rather higher titre of the hormone than is normally present in the blood, then this is the moment when it will be stimulated. After ecdysis when the animal commences to feed, the titre of neurosecretory material will fall and the prothoracic gland cease to respond. In this manner a cycle of growth and moulting will follow each ecdysis as quickly as environmental circumstances will permit. Thus continuous growth is assured and is independent of any external stimulus being applied at the beginning of each instar.

Since the vast majority of insects are continuous feeders throughout their growth, it is perhaps reasonable to suggest that mechanisms of this sort, which will ensure the fastest possible sequence of moulting cycles, will be widespread throughout the group.

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DISTRIBUTION OF THE KANGAROO-TICK *ORNITHODOROS GURNEYI* IN ARID SOUTH AUSTRALIA

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THE kangaroo-tick *Ornithodoros gurneyi*, an Argasid, lives buried in the sand in places where the red kangaroo *Macropus rufus* 'camps' in the shade during the heat of a summer's day.* In South Australia *O. gurneyi* lives a precarious existence, entirely dependent on the behaviour of the kangaroo, in some of the continent's most inhospitable country. A knowledge of how it maintains itself should throw light on more general problems of population ecology in arid regions. For this reason a survey of the tick's distribution was made as a preliminary to a more extensive investigation of its ecology.

Ticks, caught in the field, have been maintained and bred in the laboratory. When fed on rat's blood, adults and nymphs feed fairly rapidly, many completing their feed within 2 hr., but larvæ require three days, on the average, to complete their meal. When fully fed, all stages drop from the host. In the field it is likely that most nymphs and adults, after feeding on kangaroos resting in the shade, would fall back to the place whence they came. But larvæ would be carried for about three days, for the kangaroo is active, and does much of its feeding at night.

From an agricultural point of view, South Australia can be divided into three main regions: farmland, pastoral land, and desert. Fig. 1 shows the 10-in. isohyet of average annual rainfall, which roughly divides the wetter southern region, devoted to farming, and grazing on improved pastures, from the dry pastoral areas where sheep and cattle graze on natural bush 'pasture' on large stations. To the north of this line, the country is either divided into stations or it is virtual desert, uninhabited and ungrazed. Within the pastoral area a wire-mesh, dingo-proof fence, running from the eastern border westwards and then southwards to the Great Australian Bight, separates the southern sheep-country from the dingo-infested northern cattle country (Fig. 1). The boundaries of the pastoral country are also shown in Fig. 1.

The sheep stations are fenced and sub-divided into paddocks, most of which have permanent or semi-permanent water. Many of the cattle stations are unfenced or partly fenced, and waters are often far apart. The whole area is extremely hot in summer, and the low rainfall is unreliable. Droughts of three

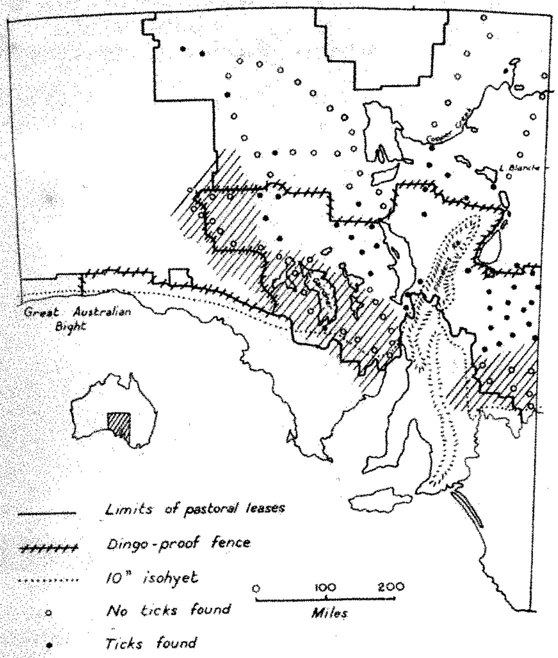

Fig. 1. Showing the places in South Australia in which *Ornithodoros gurneyi* was observed during the survey

or four years duration, with only an inch or so of rain a year, are common.

The vegetation of the station country is very variable, but for present purposes two broad categories can be recognized. Most of the northern areas carry a sparse cover of shrubs and bushes, with trees along the water-courses or in specially favourable situations. In the south and west the land is wooded, carrying dense stands of mulga, *Acacia aneura* and *A. brachystachya*, or myall, *A. sowdenii*, or more open stands of black-oak, *Casuarina cristata*, with bluebush, *Kochia sedifolia*, as an underbrush (hatched area in Fig. 1).

To-day, the red kangaroo does not occur in the farming areas of South Australia, except perhaps as a very rare immigrant, and then the chance of its surviving the farmers' guns must be remote indeed. It is widespread, however, in the pastoral country, despite the fact that the owners of many sheep stations pay a bounty on scalps, and commercial shooting for meat is profitable. The red kangaroo occurs in all the open country south of the dog-fence; but the density of the population varies considerably from place to place. In the timbered areas in the southern and western parts of the sheep country the red kangaroo becomes scarcer as the density of the trees increases. Eventually, as the vegetation becomes scrub, its place is taken by the great grey kangaroo *Macropus major*. On the cattle stations, red kangaroos are scarce as a rule; but local concentrations often occur where food is plentiful. In the area north of the Flinders Ranges as far as Cooper's Creek and west to Lake Blanche, red kangaroos are fairly numerous. They are very rare in the far north-east, but denser in the north-west.

In Fig. 1 are shown all the places in which I have found kangaroo-ticks, and also all the places in which I searched over extensive areas and yet did not find them. The method used in the survey was to drive through the country, stopping frequently and sieving sand from places where I thought a kangaroo might have camped. Ticks were retained in the sieves. Only a very small section of the pastoral area was covered in this way, the search being limited by time and the inaccessible nature of much of the country. Nevertheless, a pattern can be seen in the distribution of ticks shown in Fig. 1.

Ticks were found only in places where there was a resident, though perhaps small, population of kangaroos. The large areas in the north where ticks were not found were places where there were no kangaroos or kangaroos occurred in widely dispersed and shifting groups. But it cannot be said that ticks occur wherever red kangaroos were constantly present, because ticks did not occur in the wooded areas in the southern part of the pastoral country, and yet red kangaroos were more plentiful there than in some of the more open areas where ticks were found. The tick population is confined to the open country and does not penetrate among the trees. The explanation of this, I think, is to be sought in the behaviour of the red kangaroo. In open country, particularly on wide plains where there are few shady bushes, each suitable bush is likely to be used frequently as a camping-place for kangaroos. In such places ticks can almost invariably be found, even when kangaroos are quite scarce. But as the density of suitable camping-places increases, the probability of any one being used at a particular time, decreases, until, when the trees become quite dense, the chance that a tick will get a meal vanishes and a population of *O. gurneyi* cannot persist.

This hypothesis should take into account the density of the kangaroo population; but in the pastoral country kangaroos are unlikely to become sufficiently numerous to enable ticks to survive in thickly wooded areas. There is also a possibility that, even in thickly wooded country, there are places where kangaroos are especially likely to camp. There is some evidence for this. In places where the trees are fairly dense one can often find a broad spreading shrub, throwing a deep shade, where there is evidence that many kangaroos have camped. In such places ticks usually occur. But similar places in thickly wooded country do not show signs of having been used frequently, and ticks do not occur in them.

Yet ticks must frequently be carried in among the trees as larvæ. During the three days they are feeding they are likely to be carried long distances by a large and agile animal like the red kangaroo.

In Fig. 1 ticks are shown in one place south of Lake Gairdner, an area predominantly covered by large myall trees. This one spot represents a few bushes beside a water-course in a large, isolated, treeless area.

This preliminary investigation has posed many problems, some of which it is hoped to take up later. For example, how long can ticks survive in the field and how sparse must the kangaroo population become before ticks are unable to survive, even in treeless country ? How rapidly and over what distances can ticks disperse ? Is there some mechanism whereby larval ticks drop from the kangaroo only when it is camping ? If there is not, the death-rate among larvæ must be extremely high. What keeps the red kangaroo so scarce in large areas of the cattle country ? It may be that dingoes prey on them heavily; but there are extensive areas in South Australia where sizeable populations of red kangaroos exist despite the presence of dingoes. It seems more likely that in the cattle country the kangaroo population is living in much the same way, and at much the same density, as it did before the land was settled, and that the present concentration of red kangaroos on the sheep stations is due to the better supplies of water, and perhaps to the fact that sheep-fences may act as a partial barrier to their dispersal in dry times. This implies that the kangaroo, which is regarded as somewhat of a pest on the sheep stations, is being unwittingly 'farmed' by the graziers.

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AN INSECT-SPREAD BACTERIAL WILT EPIPHYTOTIC OF BLUGGOE BANANA

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BACTERIAL wilt of bananas (moko disease), caused by a specific race of *Pseudomonas solanacearum*, occurs in various countries of tropical America and has been known since 1910[1]. Its origin on plants of the indigenous musaceous genus *Heliconia* in jungle lands in Costa Rica has been recorded[2]. The disease is distributed, however, in areas where *Heliconia* species are either absent or healthy. Movement on propagating material and spread by tools are well known but do not entirely explain distribution and new outbreaks of the disease[1].

An epiphytotic of moko disease occurred in 1961 in the Ulua valley of Honduras in the bluggoe cooking banana, an *ABB* type[3] known as chato or moroca, that has provided new insight into the disease. The bluggoe cooking banana is planted as a food crop by the farmers and occurs scattered in small patches around houses both in and away from the large commercial Gros Michel banana plantations. Larger plantings exist; these are used for cattle and hog feed. It has been growing at least twenty years in this area unaffected by moko disease until early 1961. When the bluggoe moko disease was brought to our attention in October 1961 it was found that an insect-spread epiphytotic was at its height with the majority of the plants in the valley already diseased. Diseased bluggoes now occur over an area 30 by 10 miles. In contrast to root-infected or tool-infected plants which show symptoms on suckers and leaves, the disease symptoms consisted of blackened and shrivelled flower buds and peduncles. Internally, the fruit of all the fingers of such affected bunches was rotted and blackened. In an area where farmers had noticed diseased fruit 9–12 months previously, symptoms on plants were now systemic. Apparently this area was a primary centre of infection for the subsequent epiphytotic.

Large numbers of bees (*Trigona* sp.), wasps, and other insects normally frequent banana flowers and moist surfaces on the peduncle. Fifty species of insects have been collected from inflorescences from one location and up to 100 bees and wasps per hour have been counted frequenting a single inflorescence. Of 700 bees (*Trigona corvina* Cockerell) and wasps collected in a diseased patch during a 20-day period, 5 per cent were carrying the pathogen as ascertained by direct isolation techniques. Bagging experiments showed that disease did not occur if insects were excluded from the inflorescences.

Bacteria ooze from cushions on diseased peduncles and from bases of bracts in milky white droplets (Fig. 1) for approximately a 10-day period starting about 15 days after infection occurs. Healthy plants are infected when bacteria are carried by insects from such oozing peduncles to moist cushions on the peduncle from which male flowers have recently abscissed. (After the fruit has been produced by the female flowers the bud produces a 'hand' of male flowers each day which absciss at the base of the ovary leaving a 'cushion' on the peduncle of receptacular tissue with the sub-tending bract scar.) A newly exposed cushion can be infected for only about two days after flower abscission; however, the bud exposes approximately one new cushion a day until the fruit is harvested—a period of three months. De-budding experiments have shown that infection does not occur if the bud is broken off before the first cushion of male flowers is exposed.

Once a single infected inflorescence is present in a patch of bluggoe bananas all the hanging inflorescences rapidly become infected. The infection of neighbouring patches is rapid, but distances of more than one mile which are free of bluggoe act as substantial barriers to dissemination. Transmission has occurred, however, in isolated instances to bluggoes as distant as five miles, where secondary centres of the epiphytotic have become established. The

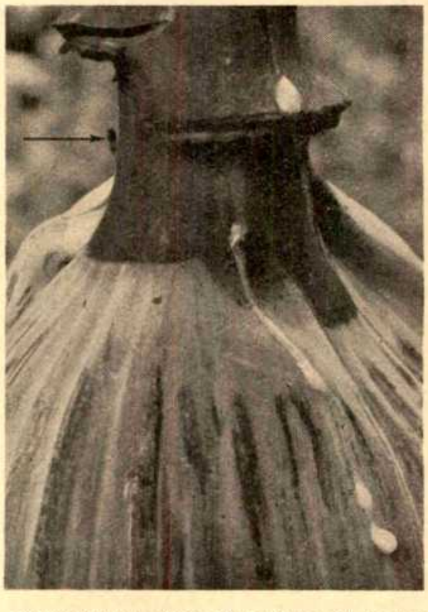

Fig. 1. Diseased bluggoe bud and peduncle with bacterial ooze and insect (arrowed)

agent responsible for long-distance spread is not known, but it may be different from the insects which disseminate locally.

The insect-disseminated pathogen eventually produces typical moko disease symptoms throughout the plant. By invading roots and rhizomes and by dripping from infected buds, it becomes an inhabitant of soils to which it is not indigenous, and may, as a result of tool transmission or root infection, spread to *Heliconia* or other plantains and bananas nearby. Horn plantain (*AAB*) is usually healthy even when growing adjacent to bluggoe patches which are completely diseased, since the male axis on this variety degenerates early, precluding bud infection. However, in the oldest infected bluggoe areas horn plantains are becoming diseased following root infection or pruning tool transmission.

Inoculum present in bluggoe patches has been carried by insects to commercial banana plantations and sporadic cases have appeared in many farms of Gros Michel that have never before had moko disease. In some instances undetected inflorescence-infected Gros Michel plants have become sources for subsequent spread on pruning tools. Previously, such appearances of the disease in new areas could not be explained. The practice in commercial banana production in Central America of removing the buds from the male axis has by coincidence greatly reduced chances of dissemination of the disease via insects. We believe there are other as yet undetermined factors which contribute to the reduced incidence of inflorescence infection of commercial bananas.

The strain of the banana race of *P. solanacearum* which is causing the bluggoe epiphytotic is distinguishable on the basis of colony morphology from another banana strain that has caused most of the moko disease in commercial bananas in the Ulua Valley for the past six years, and from the banana strain that causes moko disease in other parts of Central America. It is thus possible to determine if new disease cases in commercial banana farms are related to the insect spread epiphytotic in bluggoe bananas.

The senior author recently visited Colombia where, in the Department of Tolima along the Magdalena River, moko disease of bluggoe (there known as cachaco) occurs. The disease situation is similar to the epiphytotic in Honduras. During the past one to two years spread by insects has occurred over a distance of 60 miles from a small disease source known to have existed for about five years. In Venezuela, the disease also occurs on bluggoe and on other varieties in areas around Lake Valencia where the pathogen is not indigenous since *Heliconia* species are not present. It is suggested that the disease there also is insect spread and has been disseminated from more humid lowland areas via the bluggoe flower-infection chain.

After having seen the epiphytotic on bluggoe in Honduras it is now possible to understand the earliest reports of moko disease from Trinidad, which state that around the turn of the century the moko (bluggoe) plantain was virtually wiped out on the island whereas other varieties, although susceptible, were only occasionally diseased. What undoubtedly occurred was an insect-disseminated epiphytotic in this variety.

We are now able to propose a hypothesis which covers many aspects of the biology of this disease. (1) It has evolved on *Heliconia* in certain specific areas of the jungles of tropical Central and South America, where some of the strains are sufficiently *Musa*-adapted to infect bananas or plantains via the roots. (2) The disease in bananas spreads slowly via root infection, more rapidly via contaminated tools and to new areas on diseased rhizomes. (3) In commercial banana plantations insects occasionally transmit the bacteria from diseased inflorescences or from cut surfaces to healthy inflorescences, sometimes at a distance. (4) Once the disease reaches bluggoe, insects initiate infection cycles which rapidly move the pathogen into new areas, into higher and drier soils where *Heliconia* do not occur. (5) The bluggoe moko disease begins initially as an epiphytotic, later subsides, and becomes endemic in the survivors.

We suspect that originally, in the jungle, spread among *Heliconia* species may also have been occurring through flower infection, and that epiphytotics in *Heliconia* may have occurred in this manner from more localized centres of origin. Although we have no proof for this hypothesis, we have been able to infect *H. latispatha* by spraying bacteria on to the inflorescence.

It may be expected that moko disease can eventually appear in any warm area of tropical America where there is a continuous chain of plantings of bluggoe cooking bananas back to wet lowlands that have *Heliconia* species diseased with the banana strain of *P. solanacearum*. It is interesting that although bluggoe is a significant food source in many areas outside America—Philippines, South India, East Africa and elsewhere[3]—no report of moko disease has appeared from those areas, nor is *Heliconia* indigenous to them.

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METABOLIC REGULATION OF THE ALKALINE EFFECT OF NITRATE UTILIZATION IN PLANTS

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IN earlier investigations a practically complete survey of the ion equivalents involved in uptake by *Lolium perenne* L. was obtained[1–5]. A subdivision was also made as to form of chemical combination of nitrogen[4], sulphur[6] and phosphorus[7] in order to throw light on utilization and requirements for normal growth.

In the present article the regulation of the electroneutrality is considered more in detail. The ion balance of uptake showed that at adequate supply with nitrogen after four weeks re-growth, total cation absorption was about 1·8 equiv. K + Na + Mg + Ca and total anion absorption about 3·2 equiv. $NO_3 + Cl + H_2PO_4 + SO_4$ per kgm. dry matter produced[4,8].

It follows from electroneutrality that the deficit of neutral cations: $3 \cdot 2 - 1 \cdot 8 = 1 \cdot 4$, is an apparent uptake of acid H^+ ions. If grown on an initially neutral salt solution, which is partially exhausted at the completion of the uptake, an excess of 1·4 equiv. of the neutral cations supplied would remain in the solution for each kgm. dry plant matter produced. This excess is now balanced by OH^-, the former partner ion of the H^+ withdrawn by the uptake.

Consequently, the apparent H^+ uptake remains behind in the solution as its equivalent of the strongly basic hydroxides of the cations supplied. This is the external alkaline effect, and the conditions for its occurrence will be dealt with after discussion of the internal balance of uptake and utilization.

The apparent H^+ uptake is large relative to the total uptake and weight of the plant and should result in a pronounced internal acidification. Since the titratable acidity of the plant material is very small, it appears that the apparent H^+ uptake is only a numerical quantity in the balance of uptake which is neutralized as it is absorbed. Although the *p*H of the plant sap is favourable for the buffer action of a number of constituents their internal concentration is usually so low that this must become overshadowed by some other process which eliminates the internal acidity due to the uptake.

The ionic equations:

$$(N^{5+}O_3)^- + 8H^+ + 8e \longrightarrow (N^{3-}H_3) + 2H_2O + OH^-$$

and

$$(S^{6+}O_4)^{=} + 8H^+ + 8e \longrightarrow (S^{2-}H_2) + 2H_2O + 2OH^-$$

show that the reductive assimilation of the non-basic nitrate and sulphate anions transfers their ionic charges to strongly basic OH^- anions.

Kjeldahl nitrogen was found to be equivalent to 2·7 nitrate equivalents and unchanged nitrate was present at 0·45 equiv./kgm. dry matter. Since nitrate participates in some 60 per cent in the Kjeldahl modification used, it must have been assimilated at a rate of about 2·4 equiv./kgm. dry matter. From other evidence[6] it follows that sulphate assimilation must have been near to $0 \cdot 06 \times 2 \cdot 4 = 0 \cdot 14$ equiv./kgm. dry matter.

This shows that the conversion of nitrate and sulphate to the non-ionic organic form issues as about 2·5 equiv. alkali/kgm. dry matter. This total internal alkali (a_t) due to the balance of utilization is considerably in excess of the apparent H^+ uptake or external alkaline effect derived from the balance of uptake. Hence, the complete balance of uptake and utilization can easily account for the absence of internal acidity.

A considerable portion of the total internal alkali (a_t) becomes neutralized by the external alkaline effect (a_e) or apparent H^+ uptake. The remainder is called here the internal alkalinity $a_i = a_t - a_e$. It originates from the reductive assimilation of nitrate and sulphate anions which, together with the other ions, have been absorbed as the nitrates and sulphates of potassium, sodium, magnesium and calcium, the neutral cations supplied, and should be present in the plant as the corresponding hydroxides.

In the numerical example given here the internal alkalinity $a_i = 2 \cdot 5 - 1 \cdot 4 = 1 \cdot 1$ equiv./kgm. dry matter. In spite of this high value the plant material does not show excess titratable alkali. Apparently a_i is a numerical value of the balance of uptake and utilization which is neutralized by the metabolism as soon as it is formed.

There is every reason for relating this directly to the metabolic synthesis of organic acid molecules. The naturally occurring plant acids (malic, citric, oxalic, etc.) are sufficiently strong (*p*K's 1·5–5·5) to exist almost wholly as their salts at the *p*H of the plant sap. We have only to test whether organic acid production proceeds at a rate comparable with the internal alkalinity derived from the balance of uptake and utilization.

For this purpose I have made a few analyses on organic acids in *Lolium perenne* grown under similar conditions as before. Fresh herbage samples, corresponding to 2·25 gm. dry matter, were repeatedly extracted with a total of 500 ml. boiling water, the extracts decationized and passed over an anion exchanger (strongly basic, OH^-). Elution of the acids other than oxalic was effected with 5 *N* formic acid and, after evaporation to dryness *in vacuo*, they were submitted to partition chromatography on silica gel according to modified prescriptions of Bulen, Varner and Burrel[9]. Oxalic acid was recovered by a second elution of the exchange column with 1 *N* sulphuric acid and determined by direct titration of the eluate with permanganate. A total of about 2·2 m.equiv. organic acids was developed off the partition column and 0·13 m.equiv. oxalic acid was obtained. Recalculation yields a value of about 1 equiv. of non-volatile organic acids/kgm. dry matter, which is very near to the above value for a_i.

Let us now consider the external alkaline effect in more detail. It results from the fact that the neutral anions supplied are absorbed in excess of the neutral cations supplied and is formally explained by the uptake of H^+ equivalent to the deficit of neutral cations absorbed. The water of the solution is a practically inexhaustible source of H^+, but its actual concentration is usually very low relative to the salt concentration. However, the external alkaline effect should not necessarily be connected with a true uptake of H^+. The internal alkali produced in excess might as well undergo an overall distribution in the plants and neutralize the acidity during its entrance in the root system. This brings us close to the view put forward earlier that instead of H^+ uptake there is a release of OH^- to the external solution in an amount equivalent to the excess of neutral anions absorbed[4], which passes into the plant merely by exchange. This view implies an external neutralization of the apparent H^+ uptake instead of its internal neutralization in the case of true H^+ uptake. Of course, the question as to whether or not uptake of H^+ occurs is not of practical interest because entrance of H^+ means numerically the same as the release of its equivalent OH^-.

In the absence of nitrate utilization by the metabolism the external alkaline effect fails to reach a level of significance because the acidity of the uptake remains unneutralized. So far as normal growth is concerned a continuous utilization of the nitrate absorbed is a condition to the progress of the external alkaline effect. The foregoing reasoning shows that it is also associated with sulphate utilization. The small partial effect thereof remains unrecognized because sulphate assimilation proceeds only together with the far more prominent nitrate assimilation[6].

The foregoing balance shows that the internal alkalinity, which is the total internal alkali not neutralized by the external alkaline effect, becomes completely neutralized by the synthesis of organic acid molecules. If in healthy growing neutral plants the permissible loading with excess internal acidity (H^+, H_3O^+ and other acidic cations as NH_4^+) would be low (for example, < 0·1 equiv./kgm. dry matter) any organic acid production in excess of the internal alkalinity cannot take place without disturbing growth, uptake and utilization by too excessive internal acidification. The response would be an increase in the uptake of neutral cations so as to reduce the acidity of the uptake or external alkaline effect. A too low production of organic acids would result in an accumulation of internal alkalinity (OH^-, HCO_3^- and other basic anions) beyond the permissible loading. The response would be a decrease in the uptake of neutral cations in order to augment the acidity of the uptake and external alkaline effect.

Thus the complete balance of uptake and utilization is essentially composed of and determined by the reciprocal actions of cation uptake, anion uptake, reductive utilization of nitrate and sulphate and synthesis of organic acids. A discussion whether each term has or has not an independent existence is beside the point since it cannot vary apart from the others and is bound up with the particular conditions of growth and uptake.

According to our present routine the balance is obtained by plant analysis on the cations $C = K + Na + Mg + Ca$ and the anions $A = Cl + P + S + N$, expressed as equiv./kgm. dry matter of the ionic forms supplied. In plants grown in soil other elements do not usually add to the uptake of equivalents in significant amounts. Silica is absorbed by grass up to a marked weight percentage, but due to the very weak acidic properties of silicic acid and its probably polymerized state its contribution seems negligible in the balance of equivalents. In addition, unchanged nitrate is determined. Its recovery as ammonia in the Kjeldahl procedure for total nitrogen should be known, or otherwise special precautions are taken for its complete recovery. In the normal case of nitrate supply assimilated nitrate (N_0) is obtained by subtracting nitrate from total nitrogen. Assimilated sulphate (S_0) is calculated as above. Calculation yields: the external alkaline effect $a_e = A - C$; the total internal alkali production $a_t = N_0 + S_0$; the internal alkalinity $a_i = a_t - a_e = N_0 + S_0 - A + C = C - A_i$ in which $A_i = Cl + \text{total } P + $ inorganic sulphate + inorganic nitrate, the anions still present in the form in which they have been absorbed. It is assumed that the ionic state of phosphorus is not changed by its utilization.

In neutral plants $C - A_i$, the excess of neutral cations to unchanged neutral anions should be present as the salts of organic acids. This has been demonstrated for a number of plant species (with a single exception) by Pierce and Appleman[10]. Their values for $C - A_i$ are somewhat too low, which might have been caused by the use of total sulphur values instead of sulphate, in view of the probably high nutritional status of the plants.

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ASYMMETRICAL RIPPLE MARKS AND THE ORIGIN OF CROSS-STRATIFICATION

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A CLEARER understanding and more satisfactory classification than hitherto of asymmetrical ripple marks formed in water has resulted from a previously unattempted quantitative morphological analysis of small current ripples and asymmetrical 'sand-waves', using published and unpublished data. The work, which will be reported later in full, has revealed for the first time in three-dimensional detail some part of the geological role these structures may have had in forming cross-stratification generally. Sorby[1] and Hülsemann[2] had previously shown the clear connexion between asymmetrical ripple marks and cross-stratification, but only in terms of two-dimensional vertical sections parallel to the current direction.

Asymmetrical ripple marks are features of loose grain beds, migrating down-current in societies by addition of sand in layers to the steep or lee-sides as erosion proceeds to varying degrees depending on sediment supply on the gentle or stoss-sides. The proposed new classification is based on their form and scale, and is primarily morphological (Table 1).

Table 1. CLASSIFICATION AND NOMENCLATURE OF ASYMMETRICAL RIPPLE MARKS

I. *Straight forms*	
(1) Straight small-scale ripple marks	Form-drag conditions
(2) Straight major ripple marks	Conditions other than form-drag
(3) Straight megaripples	
II. *Linguoid forms*	
(1) Linguoid small-scale ripple marks	Form-drag conditions
(2) Linguoid major ripple marks	Conditions other than form-drag
(3) Linguoid megaripples	
III. *Lunate forms*	
(1) Lunate major ripple marks	Conditions other than form-drag
(2) Lunate megaripples	

Three forms of asymmetrical ripple mark have been distinguished on the appearance of the crests in plan. The straight forms have essentially straight crests which lie parallel to each other in a society of ripples. The crest lines of the linguoid forms are generally strongly curved, turning through almost 180°, with closure always in the down-current sense in an assemblage. In the lunate forms, which resemble æolian barchans, the crests are also strongly curved, the closure being consistently up-current in a society.

Asymmetrical ripple marks have been found to occur within two apparently discrete size ranges. The straight and linguoid forms of small scale, the familiar current ripples of beaches and streams[3], have crest heights generally less than 3 cm. It has been found that the wave-length/height ratio decreases with increasing scale, as do all linear measures with diminishing grain size. No relation has been detected between ripple-height and water-depth.

The large-scale structures, among which the height varied from 5 cm. to 22·5 m., have been classed either as major ripple marks or megaripples, the distinction being made arbitrarily within the continuous variation of linear measures at a ripple height of 1 m. Straight forms at this scale are abundant in many shallow seas[4-6], rivers[7-9] and estuaries[3,10]. Van Straaten[11] has reported linguoid forms from ebb-flood channels, and they are known to occur as well in rivers like the Benue, Nigeria. Lunate structures of carbonate sand occur on certain submerged rock platforms of the Bahamas[5,12]. It has been found that among straight major ripple marks and megaripples the wave-length/height ratio increases with growing scale, in contrast with the small-scale forms. No relation was detected between grain size and scale. From an analysis of echograms and other depth profiles, the height of the ripple crests has been found to depend closely on the depth of the overlying water. The empirical logarithmic relation expressing this is:

$$D = 0{\cdot}8901 + 0{\cdot}8271H$$

where D is mean water depth (m.); H is mean ripple height (m.), the number of observations being 38, the correlation coefficient 0·91, the standard error of estimate 0·20, and the range of H from 0·075 to 7·80 m. The relation is based on data from the North Sea and British estuaries, and the Rivers Mississippi, Benue, Klaralven (Sweden) and Hii (Japan). The reason for expressing the relation as the regression of depth on height is explained here.

Small-scale asymmetrical ripple marks thus differ from the large-scale in the relation of: (1) wave-length/height ratio to linear scale; (2) linear scale to grain-size; (3) height to water-depth, in addition to being apparently discrete in size-range.

These morphological differences suggest a genetic distinction, some confirmation of which comes from Bagnold's[13] theoretical work. Bagnold showed that the structures classed above as small scale arose on a grain bed to provide between two critical flow intensities a certain 'form-drag' sufficient to balance a deficit in favour of the fluid between shearing transmitted from the flow and shearing resistance generated by grain encounters at the bed. The large-scale kinds, referred to as 'dunes', he suggested to form under a wider range of conditions of the whole flow, whenever suitable interaction occurred between a fluctuating current and an irregular bed. The relation between ripple-height and water-depth appears to substantiate Bagnold's prediction that their scale should depend on some major property of the entire flow. In the above light, the genetic significance of the different asymmetrical ripple marks has been noted in Table 1.

Numerous earlier investigations showed that ordinarily both small- and large-scale asymmetrical ripple marks shift down-current through simultaneous stoss-erosion and lee-deposition. Bearing in mind this unique mode of translation, it can be demonstrated using models how the three-dimensional internal structure of a deposit permanently left by a group of ripples will depend on the geometry of the moving bed. Demonstrations of this kind seem important in correctly understanding cross-stratification.

Fig. 1*A* represents an assemblage of straight ripple marks assumed to be moving under conditions of sediment supply allowing both permanent accretion beneath the rippled surface and stoss-erosion on the ripples. The effect on the sediment being built up of this moving pattern of parallel areas of erosion and deposition oppositely inclined is the structure in Fig. 1*B*. This consists of tabular layers of foresets deposited on the lee-sides of the ripples as they advanced, bounded by plane erosional surfaces swept out by the stoss-sides. Unless stoss-erosion was extreme, the thickness of each layer will compare with the height of the parent ripple, this depending on water depth for instances of large scale.

A structure identical with that deduced is familiar in the geological record[14] as planar cross-lamination, when the layers of foresets are a few cm. in thickness, and as planar cross-bedding when thicknesses measure decimetres or metres. Its essential features are, as in the model, plane erosional surfaces between successive layers, and foresets dipping consistently in the same general direction through each set of layers. Ancient sands marked by planar cross-stratification may thus have been deposited from societies of straight asymmetrical ripple marks. Wright's[15] explanation of the structure is unacceptable, since of the many modern point-bars examined none were found to have the characters he assumed The concepts underlying the formation of planar cross-bedding as explained experimentally by Sorby[1]

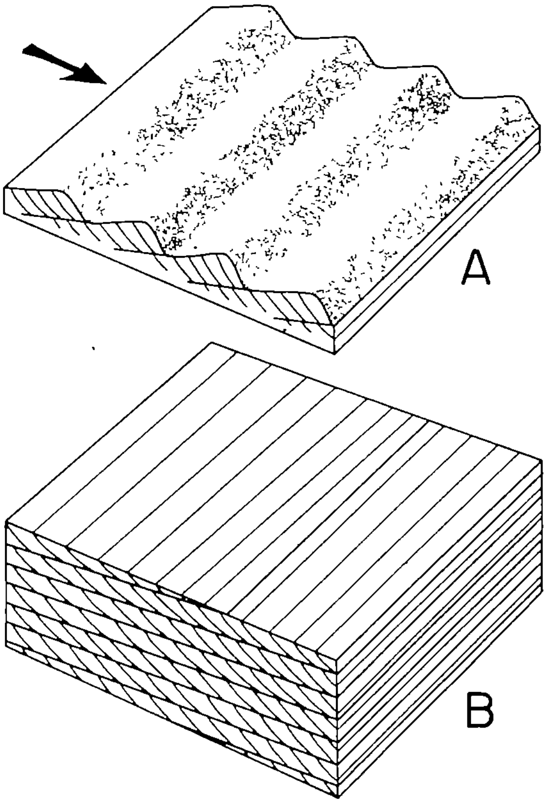

Fig. 1. Formation of planar cross-stratification from straight asymmetrical ripple marks. Arrow shows current. Erosion surfaces shown as thick lines, foreset planes as thin

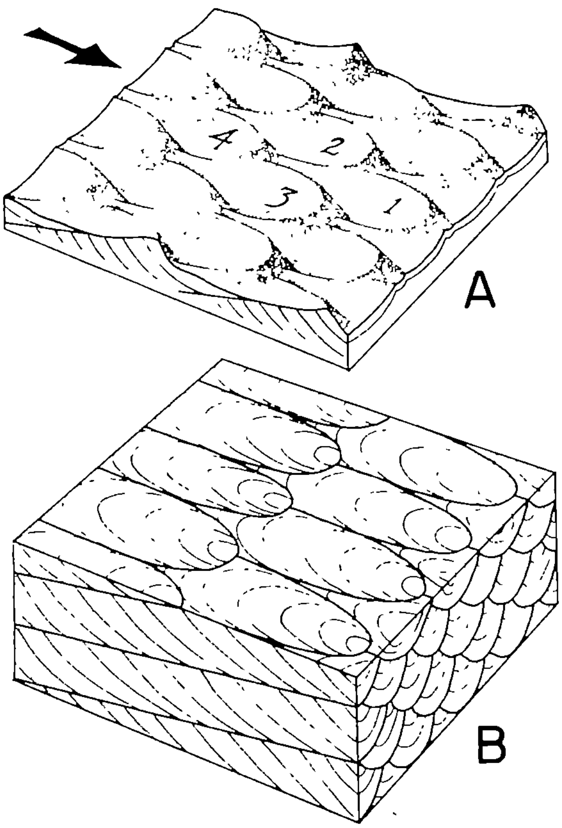

Fig. 2. Formation of trough cross-stratification from linguoid asymmetrical ripple marks. Arrow shows current. Erosion surfaces shown as thick lines, foreset planes as thin. Troughs of lower diagram slightly vertically exaggerated relative to ripples of upper one

and McKee[16] are difficult to accept in view of the behaviour of ripple marks.

In the above light the thickness of planar cross-bedding assumes importance as a guide to depth of deposition. Measurements of median thickness suggest, using the empirical relation above between ripple-height (cross-bed thickness) and water depth, the inter-tidal origin of the Lower Calcareous Grit of Berkshire (median, 12·5 cm.), sub-tidal to deeper sedimentation of the Folkestone Beds around Farnham, Surrey (median, 59 cm.; locally sets with median about 3 m.), in an environment perhaps resembling that of the southern part of the North Sea, and deposition from shallow streams for the Ditton Series of Shropshire (median, 19 cm.). Substantial rivers are implied by data from the Rough Rock[17].

Fig. 2*A* represents a society of linguoid asymmetrical ripple marks under the conditions assumed above. The arrangement of erosional and depositional areas is in this case more complex, the erosional ones being hollows crescentic in plan and distributed *en echelon*. The formation by this pattern of an internal structure in a sediment is most easily understood by considering the behaviour of the unit of four adjacent ripple marks distinguished in Fig. 2.

As ripple 1 advances its concave-upward stoss-side will sweep out erosively a concave trough-like surface. As speedily as this surface is eroded there will be deposited on it concave-upward foreset layers formed jointly on the advancing right-hand lee of ripple 2 and left-hand lee of ripple 3, the layers moulding themselves to the trough floor and dipping symmetrically inward toward its axis. The advancing stoss-sides of ripples 2 and 3 will partly truncate these layers and the upturned edges of the initial trough. Final truncation will occur as the stoss of ripple 4 advances over them. The resulting structure will thus comprise a long erosional trough filled with concave foresets bounded by similar erosional troughs. When all the ripples of the assemblage behave mutually in this way, the final structure is that shown in Fig. 2*B*.

An identical structure to this is widely known in the geological record[14] on the small scale as trough cross-lamination and on the large scale as trough cross-bedding. All the essential features of the model are reproduced, as shown by work on examples of both scales in the Lower Old Red Sandstone. Trough cross-stratification may therefore have arisen in the past from migrating societies of linguoid asymmetrical ripple marks.

This structure was formerly interpreted by Knight[18] and Sutton and Watson[19] as the result of channel cutting and then filling. Hamblin[20] correctly recognized that a relation existed between small-scale linguoid ripple marks and trough cross-lamination, but did not explain it.

Wurster[21] and Niehoff[22] accounted for trough cross-stratification by deposition from underwater 'barchan' ripples or dunes. The present circumstances of known underwater lunate ripple marks[5,12], which are very rare compared with straight-crested varieties in the same environment, suggests that they would not have formed permanent deposits. Like many æolian barchans, they are known only as isolated mounds on rock platforms where sand is in poor supply, becoming transformed into straight-crested ripples where supplies increase enough to cover the platforms entirely[5]. Were cross-stratified units ever to form from lunate ripples, it seems possible that they would be bounded by convex-upward surfaces.

Future work with models should reveal details of the cross-stratification patterns formed from asymmetrical ripple marks under other, more extreme, conditions of sediment supply.

I thank Prof. P. Allen for criticizing this article, Mr. R. G. C. Bathurst and the Directors of Netherlands Engineering Consultants for supplying data, and Dr. R. N. Curnow for aid with the statistical work.

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LETTERS TO THE EDITOR

RADIOASTRONOMY

Millimetre-Wave Radio Source in Taurus

RECENT microwave observations by Kuzmin and Salomonovich[1] and by myself of the radio emission from Taurus have revealed the presence of an unusual radio source located approximately 9 min. of arc east of Taurus-*A*. Kuzmin and Salomonovich observed the source with the 22-m. radiotelescope of the Lebedev Institute of Physics operating at 8-mm. wave-length and with a pencil beam-width of 2 min. of arc to the half-power points. While taking drift curves on Taurus-*A*, these workers detected a new source approximately 35^s east of Taurus-*A*. The reported flux density at 8-mm. wave-length is $1{\cdot}30 \times 10^{-24} \pm 25$ per cent W./m.^{2} (c.p.s.), the brightness temperature, T_B, is 7 ± 25 per cent ° K., the angular width in right ascension is 2·5 min. of arc and the flux density ratio to Taurus-*A* is 0·26. Kuzmin and Salomonovich conclude that the source is a thermal emitter with an emission measure of $2{\cdot}7 \times 10^6$, as would certainly be expected from a source initially discovered at 8-mm. wave-length.

The purpose of this communication is to point out that Kuzmin and Salomonovich's interpretation is inconsistent with the observations reported by Barrett at 1·8-cm. wave-length; however, it should be noted that the 1·8-cm. results were not available to Kuzmin and Salomonovich at the time. I have observed several strong radio sources with a 26-m. radiotelescope operating at 1·8-cm. wave-length with a 3·0 min. of arc pencil beam-width. In these observations of Taurus-*A* (Fig. 1), which included the region of the new source, no evidence of the source was detected, thereby enabling the establishment of an upper limit on the flux density of 0·05 times that from Taurus-*A* at 1·8-cm. wave-length. This upper limit, corrected for the finite source, size, and beam-width, is represented by the dotted curve of Fig. 1.

By assuming that the source is thermal, and using Kuzmin and Salomonovich's data, it can readily be shown that the optical depth of the source at 8-mm. wave-length is approximately 6×10^{-4}, and hence the optical depth at 1·8-cm. wave-length should be approximately 3×10^{-3}. Therefore, the source should be optically thin at both wave-lengths. If this were the case, the dashed curve of Fig. 1 would have been recorded. The observations clearly exclude this possibility. Indeed, observations at 3-cm. and 3·2-cm. wave-lengths, some of which were made by Kuzmin and Salomonovich, are not consistent with the interpretation of a thermal, optically thin source, since they should have revealed the source[3,4]. On the other hand, a comparison of the two results with Taurus-*A*, after correcting for the spectrum of Taurus-*A* and assuming a spectral index of $-0{\cdot}28$ (ref. 5), shows that the flux density of this new source at 1·8-cm. wave-length is no greater than one-fourth that at 8-mm. wave-length. Therefore, if the source is a thermal emitter, it must be interpreted as being one having an optical depth greater than unity at both 1·8-cm. and 8-mm. wave-lengths. This conclusion was pointed out by me[2]. To match the observed flux densities and still retain the concept of a thermal source requires optical depths of approximately 8·7 and 1·7 at 1·8 cm. and 8 mm., respectively, and leads to the absurd value of 8·5° K. for the electron temperature to give Kuzmin and Salomonovich's observed brightness temperature. We conclude that this source is not thermal, in the usual sense, if the observations are to be accepted.

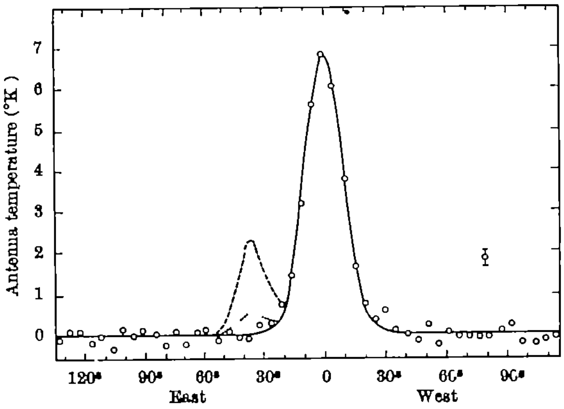

Fig. 1. Observations of Taurus-*A* at 1·8-cm. wave-length (solid curve). The dashed curve represents the result to be expected for the new source if it is thermal and optically thin; the dotted curve is the upper limit set by the 1·8-cm. observations

An interpretation of this source in terms of synchrotron radiation leads to a most unusual energy spectrum for the radiation electrons. Again, taking the spectral index of Taurus-*A* as $-0{\cdot}28$, it follows that the spectrum of the new source is at least as steep as $S \sim \lambda^{-1{\cdot}8}$. Since the flux-density spectrum from a distribution of relativistic electrons is given by $S \sim \lambda^{\frac{1-\gamma}{2}}$, where γ is the exponent defining the electron energy distribution, the observations imply an energy distribution of the form $N(E)\,\mathrm{d}E = \mathrm{k}E^{2{\cdot}6}\,\mathrm{d}E$, which is equally as unacceptable as the low value of the electron temperature indicated above.

Kuzmin and Salomonovich report an angular width in right ascension of 2·5 min. of arc from their preliminary observations, but this is sufficiently close to their minimum resolution of 2 min. of arc that I feel it does not rule out the possibility that the source is actually much smaller than this. An anomalously high first side lobe of their antenna pattern might reproduce their observed results, but this possibility seems to be excluded by their direct pattern measurements and their observations of Venus[6,7]. Therefore, if Kuzmin and Salomonovich's observations are valid, I conclude that this source is of a type that has not been encountered before in radioastronomy and that further measurements at short centimetre and millimetre wave-lengths are urgently required to confirm the existing observations and to extend the spectrum.

It should be pointed out that in this discussion I have used the values quoted by Kuzmin and Salomonovich. In particular, the brightness temperature of 7° K. obtained by them was derived from the Rayleigh–Jeans approximation to the Planck radiation law. However, it can easily be shown that for 8-mm wave-length and $T_B = 7°$ K. the requirement that $h\nu/kT \ll 1$ is not satisfied and values derived from the Rayleigh–Jeans law must be corrected approximately 11 per cent. This is less than their stated uncertainty, and I have ignored this correction because it does not alter the results; however, I believe that this is the first time the correction has assumed significant proportions in radioastronomy, and it is obvious that as the science of mm.-wave radioastronomy progresses the Rayleigh–Jeans approximation must be used with caution.

The programme of research in radioastronomy at Massachusetts Institute of Technology is supported in part by the U.S. Army Signal Corps, the Air Force Office of Scientific Research, and the Office of Naval Research.

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Interpretation of 'Surface Brightness' Measurements of Radio Sources

It is now recognized that most of the observed radio sources are extragalactic objects with radio powers greater than that of the Galaxy. It is, however, impossible to measure the distance of an individual source directly from radio observations alone, so that apart from the few sources for which optical red-shifts are available estimates of the radio luminosity function have had to be made by indirect means.

Some authors[1,2] have made observations of the radio surface brightness, and their results have been used to estimate the radio luminosity function assuming a typical linear dimension for the sources. Such discussions have not usually taken account of the change in the apparent surface brightness of a source with distance[3,4]. It is the purpose of this communication to direct attention to the fact that if this effect were considered, then for some cosmological models there is an ambiguity in the deduced radio luminosity; the Einstein–de Sitter model and the steady-state model are taken to illustrate the point.

The observed flux density per unit band-width from a source of luminosity $P(\propto \nu^{-x})$ situated at a distance r from the observer is $\dfrac{P}{r^2(1+z)^{1+x}}$, where z is the red-shift and P is the luminosity per steradian at the frequency of observation, not that of emission. The surface brightness may then be shown to be proportional to $\dfrac{P}{d^2(1+z)^{3+x}}$, where d is the linear dimension of the source. This result is similar to that given by Davidson[5] except that allowance has been made here for the spectrum of the source.

The formulæ quoted above apply both to the Einstein–de Sitter cosmological model and to the steady-state model, with the distinction that in the former r is equal to $\dfrac{2c}{H}[1-(1+z)^{-\frac{1}{2}}]$, and in the latter it is equal to cz/H, where H is the Hubble constant.

Observations at Jodrell Bank[6] and the California Institute of Technology[7] have indicated that for sources with a flux density at 159 Mc./s. of 10–20 $\times$ 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$, the most frequently observed surface brightness, expressed as an equivalent temperature, is about 4 × 10^6 °K.

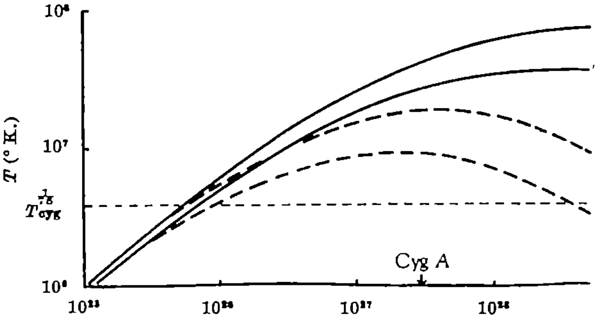

Fig. 1. Curves showing the relationship between the surface brightness temperature T and the source luminosity P for sources of given observed flux density. Two values of flux density are shown for: (*a*) the steady-state model (——); (*b*) the Einstein–de Sitter model (– – –). The upper line of each pair corresponds to a flux density of 20 × 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$ and the lower line to 10 × 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$. The curves are drawn for a value of the spectral index x = 0·8

The source Cygnus *A*, which has a flux density of 8,600 × 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$ at 159 Mc./s., is known to consist of two components each of 50 × 30 sec. of arc. A mean value for the brightness temperature of this source for the type of observation made at Jodrell Bank is about 3 × 10^8 °K. Thus the most frequently measured brightness temperatures are about 1/75 of that of Cygnus *A*. If it is assumed that the other sources have a similar physical size to that of Cygnus *A* it is interesting to use the observed distribution of brightness temperatures to estimate an associated luminosity function.

For any particular range of the flux density S, Fig. 1 shows the variation of brightness temperature which would be observed for sources of different radio luminosity; separate curves are drawn for two different values of the flux density (S = 10 and 20 × 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$). The luminosity is plotted in W.(c./s.)$^{-1}$ ster.$^{-1}$ at 159 Mc./s.; at this frequency the luminosity of Cygnus, ($P_{\rm cyg}$), is 3 × 10^{27} W.(c./s.)$^{-1}$ ster.$^{-1}$. The value for the source 3*C* 295 depends on the precise cosmological model adopted but probably lies in the range 3 × 10^{27} to 6 × 10^{27} W.(c./s.)$^{-1}$ ster.$^{-1}$.

It will be seen that at a typical value of surface brightness, $T = 1/75\ T_{\rm cyg}$, the Einstein–de Sitter model indicates two possible values of the source luminosity, and for an observed flux density of 10 × 10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$, these are 0·03 $P_{\rm cyg}$ and 11 $P_{\rm cyg}$.

If most of the sources so far observed have $P \simeq 0{\cdot}03\ P_{\rm cyg}$ (a value which would also agree with the predicted brightness temperatures according to a number of other cosmological models), then several hundred of the most intense sources would lie at distances less than that corresponding to $z = 0{\cdot}3$. If these sources are supposed to have an optical emission comparable with that of the Galaxy, they should be readily identifiable with visible galaxies.

The fact that most of them cannot be so identified[8,9] implies either: (*a*) that the optical emission is very much smaller than that of our own galaxy, in which case it is doubtful whether the corresponding physical dimension should be attributed to these sources in interpreting the measurement of angular diameter, or (*b*) that the sources are very much more distant and have powers comparable with or greater than that of the source in Cygnus. The latter interpretation would not require the hypothesis of a new class of radio source having a small optical emission.

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PHYSICS

Measurement of the Mobility of Negative Ions in Saturated Hydrocarbon Liquids with High Electric Fields

A considerable volume of experimental data concerning the problems of high field conductivity and the electrical breakdown in liquid dielectrics is now in existence, and many theories have been proposed to explain these results. Nevertheless it is rather doubtful whether it will be possible to choose the right one without an understanding of the negative charge carriers and their high field mobility. With the exception of the measurements of Chong[1], there are no mobility data at high field strengths. On the contrary, a number of papers concerning the mobility of ions in low fields have been published[2-5]. The most systematic investigations concerning this problem were carried out by I. Adamczewski.

In the quantitative theories of high field behaviour it has been necessary to use mobility values measured in fields of a few kV./cm. Such an extrapolation must, however, be treated with precaution because the ionic nature of the negative charge carriers is still uncertain[6-7]. The necessity for high field mobility measurements has been pointed out quite clearly[8,9]. In order to help resolve some of these problems, I have measured the negative ion mobility in a number of *n*-paraffins for field-strengths up to 300 kV./cm.

The negative ion mobility as a function of field-strength has been measured by a method based on the metal–liquid photoemission phenomenon. Fig. 1 shows the schematic diagram of the apparatus. In this method the mobility is obtained from the linear temporal increase of current in the liquid ionization chamber circuit and not by measurement of the current pulse duration as has been done by LeBlanc[4]. With the arrangement used in his work the high field measurements were not possible.

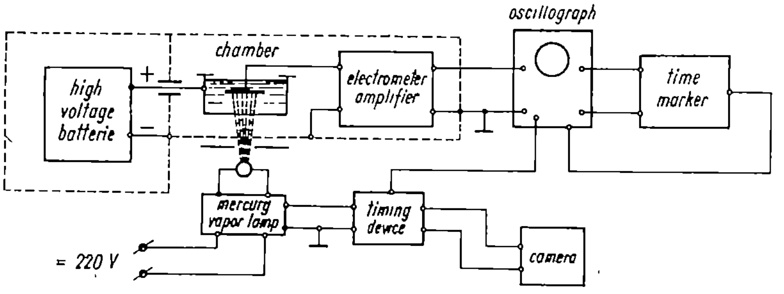

Fig. 1

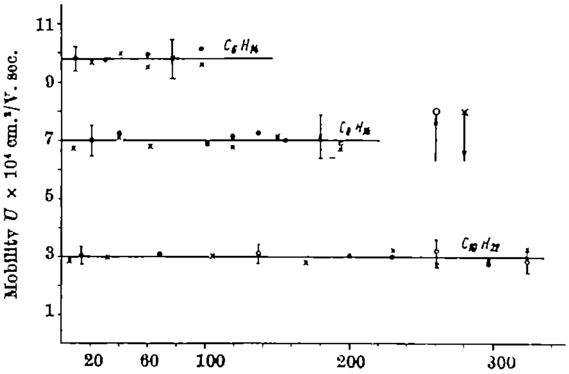

Fig. 2

For long ion transit times, the change in the sensitivity of photo-cathode as a result of 'fatigue' causes a distortion of the current pulse. In that case, better results are obtained by measuring the decay time of the current. These measurements can be carried out with the same apparatus except for a minor change in the working of the timing device.

The chamber used in the measurements differs in structure in several important details from other chambers described in the literature.

The cathode was illuminated through a transparent glass plate covered with a thin layer of stannum oxide. The transmission edge of the glass used lies significantly above the edge of the first absorption band of the hydrocarbons studied. In most measurements, the photo-cathode was a vacuum-deposited film of aluminium It was possible to arrange the electrodes exactly parallel by use of a ball joint with Wood's alloy. Adjustment of the spacing was carried out with the aid of an additional instrument with a micrometer screwed into the chamber. A glass vessel with a Schott *G*-4 filter, permanently connected to the chamber, was used for frequent rinsing of the chamber with the liquid sample.

Preparation of the liquid consisted of chemical processing, drying, fraction distillation and electrical cleaning. The final conductivity was about 10^{-19} Ω^{-1} cm.$^{-1}$.

The measurements of the current impulses were carried out with an electrometer amplifier of special construction connected to a direct current oscillograph. To reduce the time constant of the input circuit a system of negative feed-back was used.

In all measurements the source of ultra-violet light was a mercury vapour lamp with a *Q*400 bulb manufactured by Hanau.

The following liquids were selected for investigation: hexane, octane and decane. A series of liquid samples was studied with various electrode spacings, and for both increasing and decreasing electric stress. Changing the gap from 0·3 mm to 2 mm. gave the same result within the margin of error. In each liquid only one kind of negative ion was observed. The divergence among measurements resulting from changes in ambient temperature and from change of liquid sample remained, in most cases, within permissible limits.

The results of the measurements of the mobility of the ions as a function of the electric field strength are depicted graphically in Fig. 2. The range of the measurements was limited by the available high-voltage supply (10 kV.) and the rise-time of the light pulses (about 5 × 10^{-5} sec.). The results obtained indicate a lack of dependence on the field in the range studied. The possible experimental error introduced due to uncertainty in the electrode spacing was constant throughout each series of measurements, and considering only the dispersion of measured values the field independence is more clearly shown. Pyong-un Chong *et al.*[1], who measured the mobility in pure hexane and with the addition of 1 per cent ethyl alcohol for fields between 50 and 150 kV./cm., did not observe any dependence on the field either.

I thank Prof I. Adamczewski for his help and advice.

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Mobility of Ions in Liquid Dielectrics

The first extensive investigations concerning the mechanism of the movement of ions in liquid dielectrics were undertaken by Adamczewski[1,2]. He investigated systematically a group of liquid-saturated hydrocarbons ranging from pentane to nonane, and formulated the empirical dependence between the viscosity of the liquid (η) and the mobility of ions (u), $u = A\eta^{-3/2}$. This dependence has been established by measurements at constant temperature.

In connexion with the investigations carried out by Gzowski and Terlecki[3], as well as LeBlanc[4], in which the existence of rapid negative ions has been determined, it was of interest to verify the above-mentioned formula for these ions, and to investigate its applicability at different liquid temperatures.

In this work measurements of the mobility of negative and positive ions produced by means of X-rays in *n*-hexane, *n*-heptane, *n*-octane, *n*-decane and mixtures of *n*-octane–*n*-hexane and *n*-decane–*n*-hexane in the range of temperatures from 8° to 50° C. have been made, using the 'layer method'[3]. All the liquids investigated had a residual conductivity $k \leqslant 10^{-13}\ \Omega^{-1}$ cm.$^{-1}$. The viscosity was measured using Hoeppler and Arrhenius viscosimeters.

Characteristic differences between negative and positive ions were observed. The mobility of negative ions in all these liquids was found to be greater than that of the positive ions. The transit time of the front edge of the negative ion layer was sharply defined, which indicated the existence of ions with small diffusion velocity. The diffuse velocity spectrum for positive ions showed that several kinds of ions were present. Because of the low resolving power of the apparatus, the mobility of only the most rapid positive ions has been determined.

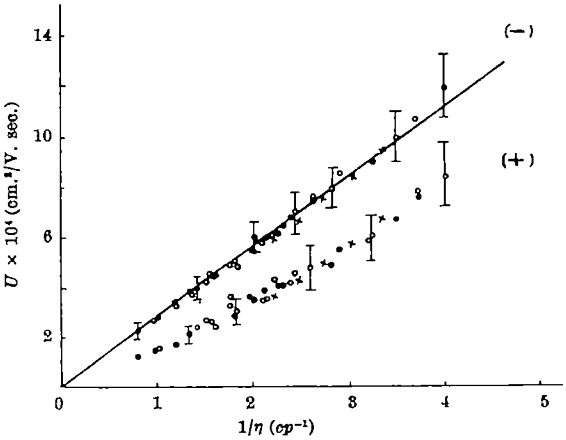

Fig. 1

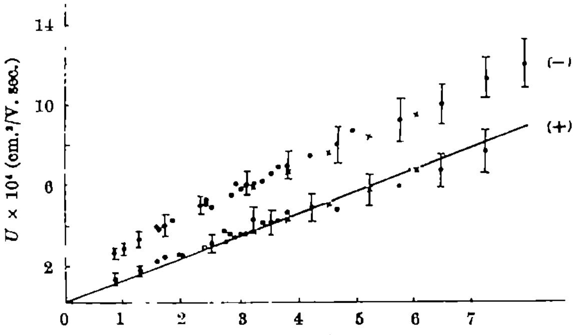

Fig. 2

Fig. 1 shows that the negative ion mobility may be expressed by the equation $u = B\eta^{-1}$, in agreement with Stokes's law, while Fig. 2 verifies that within the limits of the experimental error the positive ion mobility satisfies the equation $u = A\eta^{-3/2}$, where $A = 1 \cdot 2 \times 10^{-7}$ cm.^{2}/V. sec. poise$^{3/2}$. The crosses on both diagrams show results obtained for *n*-heptane. Detailed results and discussion will be published.

I thank Prof. I. Adamczewski for help in solving several problems connected with this work.

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Birefringence induced by Acoustic Waves in Polystyrene Solutions

When an ultrasonic wave passes through certain liquids and solutions they exhibit double refraction and behave as uniaxial crystals with their optic axes lying along the direction of propagation of the acoustic beam. This effect, which may be called acoustic birefringence, was first observed by Lucas[1,2] and may be considered to be the result of a velocity gradient caused by the wave. Acoustic birefringence is related to flow birefringence in that both effects are caused by a similar mechanism. However, it appears that in liquids and solutions of small or flexible particles the distribution function defining the number of particles in a definite state of equilibrium during the passage of the acoustic wave is a function of frequency, whereas in flow the function is constant. Thus acoustic birefringence is oscillating and usually a root mean square value is measured. Also the oscillating birefringence is not in phase with the acoustic wave, the difference being dependent on the viscosity of the liquid and some molecular parameter. Studies of the effect are being made in this laboratory both on pure liquids and solutions.

Some results for polystyrene in toluene and chloroform are reported here. The apparatus has been briefly described elsewhere[3]. The polystyrene was a commercial sample of 'Lustrex' supplied by Monsanto Plastics, Ltd. The solvents were of analar grade and were not further purified. All measurements were made at 25° C. The intrinsic viscosity of the polystyrene in units of 10^2 cm.^{3} gm.$^{-1}$, found by measurements with a U-tube viscometer, was approximately unity. The kinematic viscosities varied from 6 to 45 cs. according to the concentration. In Fig. 1 a graph of r.m.s. birefringence, $\overline{\Delta n}$, against the square root of the acoustic intensity, $(\overline{\omega})^{\frac{1}{2}}$, is shown for polystyrene in toluene. From this a linear variation is seen. Fig. 2 shows the variation of $\overline{\Delta n}/(\overline{\omega})^{\frac{1}{2}}$ with frequency for three concentrations. Similar curves are found for polystyrene in chloroform.

A theory for the effect for solutions of flexible macro-molecules has been put forward by Peterlin[4], who, for dilute solutions, gives the r.m.s. birefringence as:

$$\overline{\Delta n} = n_0 \eta_0 c \, [M]_{\text{sp}} \left(\frac{\overline{\omega}}{\rho v^3}\right)^{\frac{1}{2}} \frac{\omega}{(1 + \omega^2 \tau^2)^{\frac{1}{2}}}$$

in which n_0, η_0 are the refractive index and viscosity of the solvent respectively, ρ is the density of the solution, v is the velocity and ω is the angular frequency of the acoustic wave. $[M]_{\text{sp}}$ is the specific Maxwell constant the value of which depends on the model assumed for the molecule[5]. Kuhn and Kuhn[6,7], Stuart and Peterlin[8] and Hermans[9] show that for polymers it is a function of the anisotropy of the polarizability of the molecule, the degree of polymerization, and the size and molecular weight of the monomer. τ is the relaxation time and provides a measure of the phase lag between the birefringence and the wave.

The observations shown in Figs. 1 and 2 are in general agreement with Peterlin's equation and, assuming the equation to be true, values of τ and $n_0\eta_0 c[M]_{\text{sp}}$ have been calculated. The results are summarized in Table 1. It appears that the relaxation time τ varies with concentration and tends to a limiting value as the concentration approaches zero, but it does not vary significantly with solvents having approximately the same viscosity. These values of τ are considerably smaller than the value calculated from viscosity data using the equation of Zimm[10] for dilute solutions, namely:

$$\tau = \frac{42 \, \eta_0 \, [\eta] \, M}{RT}$$

in which $[\eta]$ is the limiting viscosity number or intrinsic viscosity, M the molecular weight, R the gas constant and T the absolute temperature. This equation gives a value for τ of $3{\cdot}6 \times 10^{-6}$ sec. The difference between these values is possibly due to the fact that the viscosity value is an orientation relaxa-

Table 1. SUMMARY OF RESULTS FOR POLYSTYRENE

No.	Concentration (gm./100 ml.)	$n_0\eta_0 c\,[M]_{\text{sp}}$ (10^{11})	Relaxation time τ (10^{-7} sec.)	$[M]_{\text{sp}}$ (10^{-10})
1	4	1·2	$0{\cdot}6_5$	3 7
2	5	1·4	0·8	3·5
3	8	2·9	1·1	4 3
4	10	3·6	$1{\cdot}0_5$	4·8
5	5	1·6	0·7	4·0
6	10	3·2	1·0	4·2

Toluene was the solvent for Nos. 1–4 and chloroform for Nos. 5 and 6.

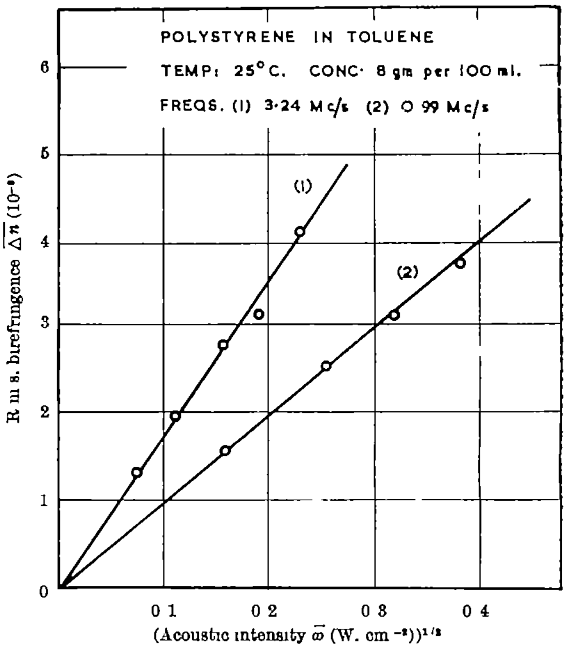

Fig. 1. Variation of r.m.s. birefringence with the square root of acoustic intensity for two frequencies

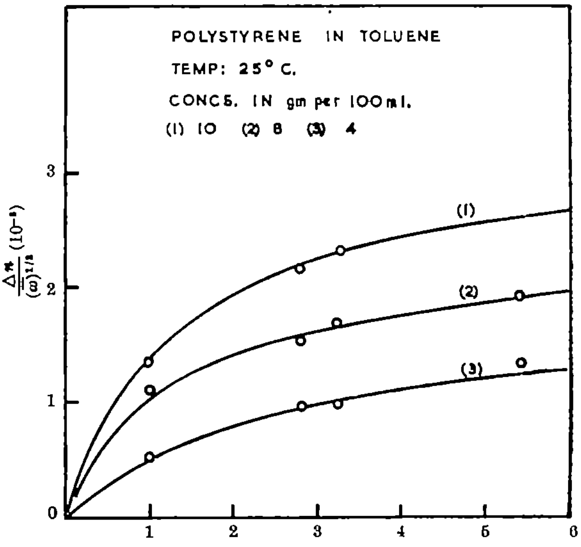

Fig. 2. Frequency variation of the ratio of r.m.s. birefringence to the square root of acoustic intensity for three concentrations

tion time, whereas the acoustic value is associated with molecular deformation. The existence of two relaxation times has already been suggested by Kuhn *et al.*[11] and by Cerf[12].

Measurements to be reported later have been made on polyvinyl chloride and cellulose acetate in *cyclo*hexanone.

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Electron Distribution and Characteristic Temperature Variation at Low Temperatures in Magnesium and Ruthenium

A number of X-ray investigations on metals have indicated that over and above a uniform background of electron distribution, there exists electron density concentration at particular sites in the lattice. Brindley and Ridley[1] attributed the deviations of the measured X-ray scattering factors of ruthenium from a smooth curve to the existence of electron concentration at the mid-point of all the basal triangles formed by ruthenium atoms. Brill *et al.*[2,3] discussed for magnesium a similar concentration at only those triangle mid-points which are surrounded by five magnesium atoms. A similar effect, enhanced at 90° K., was found by Griffith and Harris[4] in lithium. Ageev *et al.*[5,6] interpreted their results on copper and nickel on the basis of bridge electrons corresponding to covalent bonds. On the other hand, other investigations on aluminium, silver, copper, nickel, chromium, etc., do not support the concentration of electron density. Theoretically, it is known that high-temperature factors used to eliminate the termination effect in Fourier series or errors in Fourier coefficients can lead to secondary maxima which could be interpreted as electron concentrations or bridge electrons.

If the concentration of electron density at lattice points not occupied by atoms is a real effect, then one can consider it as an extra scattering centre which modifies the scattering from atoms of the unit cell[7]. It effects the scattering factors of low-angle reflexions to show systematic deviations from a smooth curve. Deviations in the intensity of low-angle reflexions from theoretical values of about 5 per cent in magnesium and 12 per cent in ruthenium are expected on the basis of the above-mentioned observations[1-4]. To verify this effect, accurate measurements of scattering factors of magnesium and ruthenium at 296° K., 90° K. and 5° K. have been made by means of a scintillation counter. The scattering factor curves of both metals have been found to be smooth and do not show the expected systematic deviations. Therefore, no evidence has been found to support the hypothesis of the existence of electron density maxima in magnesium and ruthenium lattices at points other than those occupied by atoms.

The characteristic temperature has been calculated from the measured scattering factors. For magnesium, the values of characteristic temperature are found to be 315° K., 320° K. and 375° K. at temperatures of 296° K., 90° K. and 5° K., respectively. For ruthenium, at the same temperatures, values are found to be 350° K., 350° K. and 390° K. respectively. The value of 390° K. for ruthenium at 5° K. is rather uncertain in view of the smallness of the temperature factor. In any case, both hexagonal metals show an increase in the characteristic temperature at low temperature. A similar rise has been found from specific heat measurements on various hexagonal metals[8]. The latest specific heat measurements[9] of magnesium give characteristic temperatures of 326° K., 326° K., 389° K., 406° K., at temperatures of 296° K., 90° K., 10° K., 0° K. (extrapolated), respectively. The elastic constants data[10] of magnesium if extrapolated yield at 0° K. a value of 388° K. for the characteristic temperature. This rise in the characteristic temperature is not expected on the basis of Debye's simplified continuum theory but it can be explained by means of a detailed lattice theory.

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CHEMISTRY

Magnetic Susceptibility Measurements of Iron (III) Form Ion Exchange Resin

It has been known for some time that the quantity of a tervalent metal sorbed on an ion exchange resin, in equilibrium with a solution of a salt of the same metal, varies, *inter alia*, with the *p*H value of the solution. In the case of indium (III)[1] and iron (III)[2] salt solutions it has been suggested that differences in uptake from the expected 0·333 gm. ion per equivalent of resin are caused by the presence in the solution of hydrolysis products.

According to Mulay and Selwood[3], the hydrolysed iron species $Fe_2(OH)_2^{4+}$, suggested by Hedstrom[4] and others, is diamagnetic and this fact has been used by Mulay and Selwood[3] to interpret the results obtained in investigations on the hydrolysis of iron (III).

For the present work, samples of cation exchange resin in the iron (III) form were prepared by equilibrating portions of the resin with solutions of ferric chloride having varying values of *p*H, and the magnetic moment of the iron sorbed on to the resin has been measured.

The iron form of the cation exchanger was prepared by passing, in large excess, solutions of ferric chloride (prepared from A.R. ferric chloride with the addition of A.R. hydrochloric acid where required) through a column of 'Zeo-Karb 225' resin in the hydrogen form. The resin was afterwards washed thoroughly with deionized water and, after air drying, was stored in a desiccator over calcium chloride.

Magnetic susceptibility measurements were made on a Gouy magnetic balance, allowance being made for the diamagnetism of the resin by running a blank magnetic weighing with the resin in the hydrogen form.

Table 1

Solutions					
[Fe]	[Cl]	pH	N_{Fe}	μ_r	μ_s
0·1	0·303	1·74	0·445	4·3	4·0
0·1	0·357	1·26	0·348	5·0	5·3
0·1	0·410	1·02	0·327	5·3	5·4
0·1	0·464	0·86	0·323	5·4	5·6
0·1	0·517	0·74	0·320	5·5	5·7
0·1	0·571	0·66	0·319	5·6	5·7

N_{Fe}, gm. ions of iron (III) per equivalent of resin. μ, magnetic moment in Bohr magnetons.

The results obtained are summarized in Table 1. The pH values (measured with a glass electrode and a calomel reference electrode on a commercial instrument) give some indication of the hydrogen ion concentration in the iron solutions examined. The amount of iron sorbed on the resin (N_{Fe} gm. ions per equivalent of resin) should be 0·333 for the saturation sorption of a tripositive ion. The increase in this value to 0·445 at pH 1·74 ([Cl$^-$] = 0·303) indicates sorption of ions having a positive charge of less than three associated with each iron atom. This can be accounted for by the presence of hydrolysed species such as $FeOH^{2+}$, $Fe(OH)_2^+$ and $Fe_2(OH)_2^{4+}$, possibly sorbed originally as chloro complexes. The decreases of the value of N_{Fe} to 0·319 at pH 0·66 ([Cl$^-$] = 0·571) would be accounted for by the sorption of unhydrolysed ferric ions (Fe^{3+}) together with an increasing number of hydrogen ions. The consideration that the decreasing sorption of iron is caused by changes in the quantities of hydrolysis products present is confirmed by the values of the magnetic moment for the iron present on the resin (μ_r). The increase in value of μ_r from 4·3 to 5·6 indicates that a diamagnetic ion is sorbed on to the resin from a solution low in free acid and that, as the acidity is increased, the iron becomes sorbed in the form of the spin free Fe^{3+} ion (magnetic moment of 5·9 Bohr magnetons). Included in Table 1 for comparison are values of the magnetic moment of iron in 0·04 M ferric perchlorate and 3 M sodium perchlorate solution (μ_s) calculated from the work of Mulay and Selwood[3]. Since the two sets of values for magnetic moment follow the same trend, it seems reasonable to draw the conclusion that hydrolysed species, as such, are present on the resin, and further, since the magnetic moments are similar in absolute value, that the various species are sorbed on to the resin in a ratio similar to that in which they exist in solution.

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Stepwise Hydration of Clay-Organic Complexes

IT is well known that the hydration of many expanding clays occurs in several discrete steps during which up to four monolayers of water are successively admitted between the unit layers of the clay[1]. It is also well known that organic molecules or cations are often adsorbed between the unit layers in a stepwise fashion[2]. In this communication we report a novel phenomenon, the stepwise hydration of an organic complex. So far, we have encountered this phenomenon in sodium Wyoming bentonite–α-picoline–water systems, in which as many as five discrete hydrates could be distinguished, and in sodium Wyoming bentonite–pyridine–water systems, in which two discrete hydrates were observed.

When a flake of dry sodium Wyoming bentonite is immersed in dry α-picoline, an adsorption complex with a c spacing of 15·9 Å. is obtained. On equilibration of the flakes with solutions of water in α-picoline of increasing concentration, the successive formation of hydration complexes with the following c spacings occurs:

Percentage of water in α-picoline	0	2	5	10	15	20	25	30	40	50
c spacing (Å.)	(15·9)	21·9	21·9	*d*	25·7	27·9	27·0	*d*	31·8	33·8

The X-ray patterns showed six or more integral orders, except at the water concentrations of 10 and 30 per cent, at which broadening of the lines and disorder of the patterns indicated that the next hydrate was not yet fully developed. Above a water content of 50 per cent, the flakes disintegrated spontaneously, and the X-ray patterns became diffuse.

When a flake of dry sodium Wyoming bentonite is immersed in thoroughly dried pyridine, a complex with a c spacing of 19·4 Å. is obtained. When the clay flakes are equilibrated with solutions of water in pyridine of increasing concentrations, the c spacing increases as follows:

Percentage of water in pyridine	0	2	5	10	15	20	25	30	35	40	45	50	55
c spacing (Å.)	(19·4)	*d*	*d*	23·3	23·3	23·3	*d*	*d*	*d*	29·3	29·3	29·3	29·3

At water contents of more than 55 per cent, the flakes disintegrate. The 23·3-Å. complex is also formed when the dry complex with the 19·4-Å. spacing is exposed to the laboratory atmosphere (relative humidity of about 50 per cent). (The formation of the 19·4-Å. complex, which has not been reported before, requires intensive drying of the clay and of the pyridine liquid, preferably with Linde 4A molecular sieve material. Greene-Kelly[3] observed the 23·3-Å. complex and recognized that the formation of this complex required the presence of traces of water in the system.)

Without presenting a detailed argumentation in this brief communication, we propose the following interpretation of the hydration phenomena.

In the completely anhydrous condition, the c spacing of the clay immersed in pyridine is 19·4 Å. Another complex observed in the earlier stages of adsorption from a pyridine solution in a hydrocarbon fluid has a c spacing of 14·8 Å. In the latter complex, the pyridine molecules are in an upright position, with the ring nitrogen and associated sodium exchange cations in a midway position between the unit layers (cf. Greene-Kelly[4]). In the 19·4-Å. complex, two layers of upright pyridine molecules are probably superimposed, possibly in a slightly tilted or partially overlapping position. The ring nitrogen and the sodium ions are probably again located in the plane midway between the unit layers. According to density determinations on the clay in pyridine, the pyridine molecules between the unit layers are in a closely packed condition.

On hydration, water molecules are likely to associate with the midway cations and ring nitrogen groups, causing a parting of the two superimposed, closely packed pyridine layers. The hydration is accompanied by an increase of the *c* spacing of 3·9 Å., which is more than the cross-section of a water molecule. Possibly, a re-orientation of the previously tilted or overlapping pyridine rings occurs, which is allowed by a decrease of the attractive force between the negative unit layers and the midway layer of cations with increasing distance. The formation of the second discrete hydration complex with a 6·0-Å. higher basal spacing can be explained by the entrance of two monolayers of water in the first hydration complex.

In the α-picoline system, when no water is present, only a complex with one layer of upright ring molecules is obtained with a *c* spacing of 15·9 Å. According to density determinations in liquid α-picoline, the interlayer molecules are less closely packed than in the liquid state, possibly because of the unfavourable packing geometry of these molecules in a monolayer. Probably for the same reasons, a two-layer complex analogous to the 19·4-Å. pyridine complex does not occur. Only in the presence of water are two-layer complexes formed, the 21·9-Å. complex being comparable with the first hydration 23·3-Å. complex of pyridine. The fact that the *c* spacing of the α-picoline hydration complex is smaller than the *c* spacing of the pyridine hydration complex in spite of the larger cross-section of the α-picoline molecules is probably a consequence of the less dense packing of the latter, which permits the water molecules to occupy midway positions without parting the two α-picoline layers.

In the formation of the next series of discrete hydrates, the irregularity of the successive steps in basal spacing would indicate that simultaneous re-orientations of the organic molecules occur.

These interpretations are still somewhat speculative, since we have no definite data on the composition of the various complexes. Although data on adsorption from solution are difficult to interpret unless certain assumptions on the relative adsorption of the two components are made, we shall attempt to obtain further information on the composition of the complexes from such data.

The X-ray diffraction patterns were obtained by Mr. W. K. Lumb, E. and P. Research Division, Shell Development Company.

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Manufacture of Alkali-free Corundum of Tabular Crystal Form

THERE are several modifications of aluminium oxide, the most important of which are α- and γ-forms. The latter cannot be considered as aluminium oxide proper, since it contains substantial quantities of alkali oxide. α-Aluminium oxide, corundum, constitutes the basic material of several ceramic products or plays an important part in the formation of some of these products[1].

γ-Aluminium oxide is formed at low temperatures by the dehydration of aluminium hydroxide. This modification is converted at temperatures exceeding 1,000° C. monotropically into the α modification. α-Corundum crystallizes in the ditrigonal-scalenohedral crystal class, forming squat or column-like elongated crystals. Until now, crystals of the tabular form could be obtained only when the basic material contained small quantities (0·2–1·5 per cent) of alkali oxide.

The bending strength and the compactness of products prepared from corundum are of importance in industry. Tabular corundum is used also as a colour pigment. α-Corundum in the tabular crystal form has several advantages from the technical point of view. Higher compactness, increased bending strength and higher sintering-rate may justify its use for the production of sintered corundum, for apart from being of tabular form it is also free of alkali. It is characteristic of the tabular form that the longitudinal and lateral dimensions of the crystal are are least 4–5 times its thickness. If a product of high purity is required, the addition of alkali oxide for the promotion of the development of the tabular form is to be avoided.

In a new process developed, tabular α-corundum is obtained as an end-product when aluminium fluoride (AlF_3) is hydrolysed with steam at a temperature above 800° C. Thermal treatment of aluminium fluoride is carried out expediently under fluid conditions. It is rendered fluid by a mixture of steam and air. Below 800° C. no pure corundum is obtained, because hydrolysis proceeds only when the equilibrium fluorine content is attained. Depending on the temperature, the fluorine content of the product thus obtained varies between 4·8 and 4·0 per cent. The end-product contains substantial quantities of aluminium oxy-fluoride besides α-corundum[2].

Hydrogen fluoride is also formed in the reaction, and this can be used for the manufacture of aluminium fluoride. The hydrolysis of aluminium fluoride begins at 300° C. Below 300° C. aluminium fluoride is formed from aluminium hydroxide or aluminium hydrate, when treated with hydrogen fluoride. This heterogeneous reaction is carried out most advantageously in the fluid state.

Fluidization is justified by an increase in reaction-rate in the fluid bed, as compared to the fixed bed. If the rate of a process is determined by external diffusion, this rate is substantially increased by the continual movement of the particles. The capacity of the fluidizing oven is further increased because the reaction zone occupies its greater part.

The absence in most cases of moving parts in the equipment constitutes another advantage of the process, and facilitates the handling of the equipment. Its working is easy, and little manpower is required for its operation.

Both the hydrolysis and the manufacture of aluminium fluoride became continuous operations by applying the fluidized process. Material handling was simplified by connecting pneumatic conveyors to the reactors. Owing to good heat transmission, uniform temperature is established in the fluid system.

Advantages from the industrial point of view are: (1) relatively low investment costs; (2) high capacity; (3) continuous operation; (4) uniform heat distribution in the bed; (5) high heat transmission-rate; (6) simple apparatus; (7) low labour requirement.

Hydrogen fluoride liberated during the production of corundum may be used for the manufacture of aluminium fluoride. Two vertical fluidizing ovens, which may be placed one above the other, are needed for the re-circulation of hydrogen fluoride. In the lower oven aluminium fluoride is fluidized at a temperature of above 800° C. in the presence of steam. α-Aluminium oxide of tabular form and hydrogen fluoride leave the unit. The latter may be introduced directly into the other oven, in which aluminium hydroxide or aluminium hydrate is fluidized at a temperature of 250–280° C. Aluminium fluoride, produced in this unit, may be used in the first unit for the production of tabular α-aluminium oxide.

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Mechanism of Ethylene Hydrogenation

ELEY[1], in his article on ethylene hydrogenation, points out several treatments for developing the half-hydrogenated state, which then is converted to ethane. Basically these are: (1) the Polanyi and Greenhalgh[2] treatment:

$$^{*}CH_2—CH_2{}^{*} + H^{*} \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} {}^{*}CH_2—CH_3 + H^{*} \xrightarrow{K_2} CH_3CH_3$$

which requires an essentially equivalent and ideal adsorption of hydrogen and ethylene on a uniform surface, and (2) the Twigg[3] mechanism, which postulates that the reaction of hydrogen on ethylene-metal can occur only by reaction of the hydrogen with chemisorbed ethylene:

$$^{*}CH_2—CH_2{}^{*} + H_2 \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} {}^{*}\,CH_2CH_3 + H^{*} \xrightarrow{K_2} CH_3CH_3$$

In the case of the nickel catalyst, which has been the most investigated metal, many of the results seem to favour, although they do not completely establish, the Twigg mechanism for the half-hydrogenated state.

I have conducted experiments with hydrogen and hydrogen–ethylene mixtures over electrodes impregnated with platinum in a fuel cell, and the results lend support to the Twigg mechanism. Equivalent electrodes, composed of 3 per cent platinum on carbon disks, were used as cathode and anode in a cell containing 20 per cent potassium hydroxide as electrolyte. This cell at 35° C. developed 1·00 V. open circuit, and yielded a current of 25 amp./ft.^{2} at 0·3 V. when hydrogen was fed to the anode and air to the cathode. However, when a mixture of gases containing 29·1 mole per cent hydrogen, 68·3 per cent ethylene, and 2·2 per cent ethane was used in place of hydrogen, 0·19 V. open circuit and less than 0·3 amp./ft.^{2} of current under load were obtained. This indicates that the participation of the hydrogen in the fuel cell reaction, that is, the oxidation to water, was virtually eliminated. However, the effluent from the fuel chamber contained 1·0 per cent hydrogen, 57·8 per cent ethylene and 40·8 per cent ethane, and indicated that the hydrogenation of ethylene was occurring smoothly. At 85° the hydrogen concentration fell to 0·1 per cent. Since the rate of the hydrogen–oxygen cell reaction is essentially zero at open circuit conditions, the potential should be maintained if anything approximating an equivalent amount of hydrogen is adsorbed even if the hydrogenation reaction is considerably faster than the oxidation. The sharp fall in open circuit potential and current density indicates that no hydrogen is adsorbed, hence the reaction of hydrogen on ethylene–platinum proceeds by the attack of the molecular hydrogen on the activated ethylene.

During the hydrogenation of isobutene, on the other hand, the hydrogen and olefine occupy approximately equal fractions of the surface[4]. Thus, while ethylene molecules can pack in such a way as to fill the surface and exclude chemisorbed hydrogen, the bulky nature of the methyl ethylenes necessarily leads to bare sites which can chemisorb hydrogen. In agreement with this I found that isobutene–hydrogen mixtures under the same cell conditions gave 0·57 V. open circuit and 2·8 amp./ft.^{2} under load.

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Correlation of Biological Activity of Phenoxyacetic Acids with Hammett Substituent Constants and Partition Coefficients

IN the 'two-point attachment' theory[1] on the mechanism of action for growth regulators of the auxin type we have assumed as a working hypothesis that the reaction between auxin and substrate is more chemical than physical in nature and that covalent-bond formation is a possibility. The need for an aromatic ring with at least one unsubstituted position for auxin activity[2] confirms our original belief that an *ortho* position in the phenoxyacetic acids is most suitable stereoelectronically to serve as one of the two points which attach the molecule to the plant substrate. The carboxyl serves as the second point, permitting the auxin to form a ring when joined to a plant protein. This reaction mechanism is supported by the work of Bonner *et al.*[3,4].

If a specific position on the aromatic ring is involved, and not the ring as a whole, then an examination of the effect of substituents should indicate what the ideal electronic character of this point should be. Early qualitative work[5] indicated that groups which lowered the electron density at the *ortho* positions gave increased biological activity in the phenoxyacetic acids. Calculations of pi-electron delocalizability have been of qualitative value in rationalizing auxin activity in the benzoic acids[6,7]. However, the use of Hammett sigma functions to establish a quantitative relationship between electron density at the *ortho* position and auxin activity have not been successful, although electron-attracting groups are more effective in promoting auxin activity than are electron-releasing groups.

$$\text{Solution} \xrightarrow{k_1} \text{site of action} \xrightarrow{k_2} \begin{array}{c}\text{one point}\\ \text{attachment}\end{array} \xrightarrow{k_3}$$

$$\begin{array}{c}\text{two point}\\ \text{attachment}\end{array} \xrightarrow{k_4 \rightarrow k_n} \text{growth}$$

The formulation of a precise relationship requires that several major steps in the auxin effect which may be rate controlling be considered. Growth here is measured as the elongation of 3 mm. segments of *Avena* coleoptiles when grown under conditions yielding a linear growth-rate[3]. This sequence indicates that the first possible rate-determining event in the auxin effect may be the penetration to the site of action where, after proper orientation, two point attachment occurs initiating a complex series of events ($4 \rightarrow n$) which are manifested in growth. Considering the large number of molecules with greatly different structures which effectively promote growth, it seems likely that the auxins act to initiate the growth process, but that they are not involved in steps $4 \rightarrow n$. That k_3 can control rate is shown by the inactivity of the 2,4,6-trisubstituted phenoxyacetic acids. That k_2 may be rate controlling is shown by the inactivity of many α,α-disubstituted phenoxyacetic acids.

It occurred to us that lack of success in applying the Hammett linear free-energy relationship to rationalize the effect of substituents on auxins such as phenoxyacetic acids was due to the failure to take into account k_1. The concentration of auxin at the site of action will depend on the rate of penetration of the various substances through the aqueous and organic portions of the plant cell. This rate of penetration might be approximately proportional to the partition coefficient of the auxin between water and a relatively non-polar organic solvent. That is, there should be an ideal hydrophilic–lipophilic character for a given type of auxin so that it would not tend to remain either in the aqueous phases or the organic phases of the cell, but move at an optimum rate to the site of action. To evaluate this hypothesis we have determined the partition coefficients (P) for a series of phenoxyacetic acids between 1-octanol and water at 30 ± 1°. The general procedure was to partition 20 mgm. of acid between 20 ml. of octanol and 200 ml. of water. For some of the auxins with high P values 25 ml. of octanol and 400 ml. of water were more suitable. For 3-OH and 3-COOH 150 ml. of octanol and 50 ml. of water were used. The octanol was saturated with water free of carbon dioxide and the water was saturated with octanol. For partitioning, the mixture was shaken vigorously for 1½ hr. after which the water layer was withdrawn and centrifuged at 2,500 r.p.m. for 1½ hr. Centrifuging was very important for complete separation of the octanol even in those samples in which no visible emulsification occurred. The amount of auxin in the water layer was determined coulometrically[8]. All operations were carried out under nitrogen to avoid contamination by carbon dioxide. For a number of acids a series of partitions was made over a range of concentrations. This permitted the calculation of the degree of association of the acids in the octanol layer. For acids as diverse as phenoxyacetic acid and 3-nitrophenoxyacetic only about 2·0 per cent association occurred at the concentrations used (of the order of 1×10^{-3} M). The association in the organic layer was ignored, and the expression used for the partition was $P = \dfrac{C_{\text{octanol}}}{C_{\text{H}_2\text{O}}(1-\alpha)}$. The degree of dissociation in the water (α) was calculated from the ionization constants, a number of which have been determined[9]. Those for which experimental values were lacking were calculated by the Hammett relationship[10]. It has been assumed that the rate of penetration of the auxin to the site of action will be a parabolic function of the partition coefficient. For simplicity in calculations we have used $\pi = \log P - \log P_H$, where P_x is the partition coefficient of the substituted phenoxyacetic acid and P_H that of phenoxyacetic acid. Modifying the Hammett equation to compensate for the relative rates of penetration we obtain $\log 1/C = k\pi + k'\pi^2 + \rho\sigma + k''$. C is the concentration in moles/litre of auxin which induces 10 per cent growth in the *Avena* cylinder test. It was observed for low concentrations of auxin (that causing less than 30 per cent elongation of the sections) that the rate of growth was proportional to log C. The evaluation of the constants k, k', k'' and ρ for the best fit to the experimental data was done with a Clary *DE*-60 computer. Sigma was chosen with respect to the *ortho* position, that is, for a substituent in the 4 position σ_m was used and for one at the 3 position σ_p was used. Compounds with large groups in the 4 position were not included since we have found Åberg's observation[11], that large substituents in the 4 position cause inactivity, to hold for a wide variety of groups.

The results given in Table 1, obtained from the expression $\log 1/C = 4{\cdot}08\pi - 2{\cdot}14\pi^2 + 2{\cdot}78\sigma + 3{\cdot}36$, show that an increase in activity of the substituted phenoxyacetic acid occurs with increase in sigma or as the partition coefficient is increased, sigma being held constant. Activity falls to zero with high values of P (compare 3-CH_3, 3-C_2H_5, 3-n-C_3H_7 and 3-n-C_4H_9, where the values for sigma are essentially constant). The most convincing evidence, other than that previously reported[1,2], for using the value of sigma at the *ortho* position is that a considerably better fit of the data with the above type of equation is obtained. Particularly striking is the comparative activities of the 3- and 4-F and 3- and 4-Cl acids. In each pair of isomers the value for π is almost the same for the 3 and 4 positions. The way in which the two halogens affect the electron density at the *ortho* positions by resonance is, however, quite different especially in the example of the fluorine. The dramatic difference in activity between the 3- and 4-fluorophenoxyacetic acids seems best explained in terms of the different electron densities at the *ortho* positions. Differences in steric and *H*/*L* factors would be the smallest possible. The result with chlorine is the same although less pronounced. In the case of the 4-F derivative the activity found is actually much greater than that predicted. At present it is not possible to offer a rationalization

Table 1

Substituent	σ	P	π	log 1/C (calc.)	log 1/C (obs.)
3-CF_3	0·55	330	1·09	6·8	6·5
4-Cl	0·37	168	0·80	6·3	6·4
3-I	0·28	325	1·08	6·1	6·3
4-F	0·34	43	0·20	5·0	6·3
3-Br	0·23	254	0·97	5·9	6·0
3-SF_5	0·68	1,190	1·64	6·2	6·0
3-Cl	0·23	178	0·82	5·9	5·7
3-NO_2	0·78	29	0·04	5·7	5·3
3-SCH_3	−0·05	105	0·59	4·9	5·3
3-C_6H_5	−0·15	200	0·87	4·9	5·3
3-*n*-C_3H_7	−0·15	890	1·52	4·2	4·7
3-OCH_3	−0·27	36	0·13	3·1	4·7
3-CN	0·63	16	−0·23	4·1	4·5
3-CH_3	−0·17	75	0·44	4·3	4·3
3-CH_3CO	0·52	22	−0·08	4·5	4·0
3-F	0·06	41	0·18	4·2	3·5
H	0·00	27	0·00	3·4	3·5
3-OH	−0·36	5	−0·73	−1·8	3·7
3-COOH	0·27	21	−0·13	3·5	3·0
3-*n*-C_4H_9	−0·15	3,100	2·08	2·4	0·0

for this or for 3-CH_3O and 3-OH, which are also poorly predicted. It is not surprising that the activity predicted at log $1/C = 2·4$ for 3-*n*-C_4H_9 is not realized. Most growth regulators are inactive at concentrations as high as 10^{-3} *M*.

Discussions with other investigators of the correlation of biological activity and chemical structure indicate that in both plant and animal systems where steric and metabolic factors can be ruled out, the use of Hammett functions and partition coefficients will be of significance in rationalizing structure and activity.

We are indebted to Prof. Alvin Beilby for advice in the coulometric determinations, to Mr. C. F. Geiger for the synthesis of several of the acids, to Prof. Donald McIntyre for instruction in curve fitting with the Clary computer, and to Prof. R. Nelson Smith for many helpful suggestions in the determination of the partition coefficients. We also thank Dr. W. A. Sheppard of the DuPont Co. for a sample of 3-pentafluorosulphurphenol and for the sigma values for the SF_5 group.

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RADIOCHEMISTRY

Beta-Scintillation Counting of Radioactive Tracers Insoluble in Toluene

Liquid scintillation in certain organic solvents is acknowledged in principle to be among the most sensitive methods of estimating radioactivity from weak β-emitters such as carbon-14, especially in low concentration. In the presence of water, however, the normally high efficiency of β-scintillation counting in toluene is seriously impaired[1]. Blending of aqueous samples with large volumes of liquid scintillator in toluene by addition of organic solvents[2] results in appreciable lowering of counting efficiency and in a tendency to precipitate the solute. Samples counted in suspension[3] require extrapolation to zero sedimentation. Packing of aqueous solutions with scintillator beads[4] is expensive, and accurate counting with anthracene suspensions[5] is time-consuming. Incorporation of aqueous samples into gels[6] is not always practicable for counting at low temperatures.

In this laboratory we have felt the need of a method for counting aqueous samples containing very small amounts of carbon-14. A new approach found useful consists of applying the aqueous sample to strips of lens tissue, which are then dried, cut into small pieces and loaded into the counting vessel. Sufficient liquid scintillator in toluene is added to immerse the whole mass of the tissue. The solvent pervades the tissue and renders it translucent. Among advantages of this procedure are: (1) simplicity, and economy in time and materials; (2) high sensitivity; (3) linear variation of response with activity of a standard; (4) small sacrifice of counting efficiency; (5) ability to recover the toluene-insoluble sample.

Up to 0·1 ml. of aqueous solution is uniformly pipetted on to 2 cm. × 12·5 cm. strips of lens tissue supported vertically. After drying in a current of warm air, further 0·1-ml volumes of sample may be applied. Each strip is cut into squares of about 0·2 cm., and these are lightly packed into ampoules. I have found 5 ml. dispensary ampoules useful containers (see also Hubscher *et al.*[7]). Scintillation fluid (2 ml. of 2 gm. *p*-diphenyloxazole per litre toluene) is run into each ampoule without entrapping air bubbles. The ampoules are pre-cooled at −20° C. in the dark for 30 min. (Sealing of the ampoules is unnecessary if they are counted within 6 hr.). For coloured samples, or samples containing much dissolved solid, glucose-1-^{14}C is used as internal standard to correct for quenching. For counting, one of the standard cuvettes for the *EKCO N*612 scintillation head is first placed in position above the shutter and the phototube with which optical contact is made in silicone fluid *MS* 200/2. Each ampoule containing the scintillator is then placed in turn in the centre of the cuvette in a pool of ethanol (3 ml.) which serves both as contact fluid and demisting agent.

The validity of this technique was assessed as follows: the voltage setting on the scaler was chosen so that the function $\frac{R_s^2}{R_b}$ was a maximum[8], where R_s and R_b were the observed counting-rates of sample and background respectively. Using our instrument the practical upper limit of this function at the 1-mμc. level was about 1,000. The counting efficiency $\left(\frac{\text{counts per second}}{\text{disintegrations per second}} \times 100\right)$ was then 39 per cent. The presence of lens tissue lowered this efficiency by no more than 3·4 per cent. To gauge reproducibility, the coefficient of variation among six replicate determinations was calculated for different low levels of activity, monitored in duplicate for 4,000 counts or 1,000 seconds, whichever was shorter. Counting samples for longer periods was rarely practicable where large numbers of samples were to be monitored. Activities were expressed as mμc, referred to a glucose-1-^{14}C standard based in turn on a toluene-reference standard, making due allowance for the presence of lens tissue.

The coefficient of variation of replicate counts of a single sample, expressed as a percentage of mean counts per sec., is found to be 5·9 for 0·01 mμc., 3·3 for 0·1 mμc. and 2·2 for 1 mμc. samples. The coefficient of variation of counts on replicate aliquots of the same sample are less than 2 per cent higher, showing that only a very small proportion of the total error is attributable to sample preparation.

I have found this technique particularly useful for the radioassay of biological fluids such as urine. It lends itself readily to the analysis of large numbers of samples in a variety of solvents. The samples can be recovered from the tissue by washing with toluene to remove scintillator, drying and extracting with water.

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BIOPHYSICS

Scattering Cross-Section of Carbon at Small Angle

In a previous communication[1] it was shown that, in the angular range $\alpha < 10^{-2}$ radian, the values of the scattering cross-section of carbon for 50-kV. electrons as calculated from electron scattering formulæ[2] do not satisfactorily agree with experimental results. The above theoretical calculation was based on Wentzel atomic radius as given by $R = a_{\mathrm{H}}/Z^{\frac{1}{3}} = 0{\cdot}29$ Å. However, if, according to Lenz[2], R is expressed as $((\mathrm{H})/6Z)^{\frac{1}{2}}$ with $(\mathrm{H}) = 16{\cdot}58\ a_{\mathrm{H}}^{2}$ on Hartree atom model and $(\mathrm{H}) = 7{\cdot}60\ a_{\mathrm{H}}^{2}$ from diamagnetic susceptibility value, the magnitude of the respective atomic radii becomes 0·36 Å. and 0·24 Å.

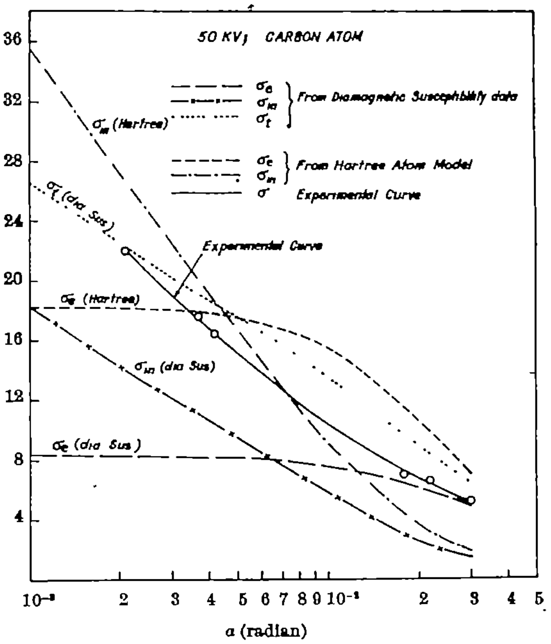

Fig. 1. Theoretical and experimental values of scattering cross-section of carbon for 50 kV. electrons at small angle; experimental points from De (ref. 1), theoretical curves from Lenz (ref. 2)

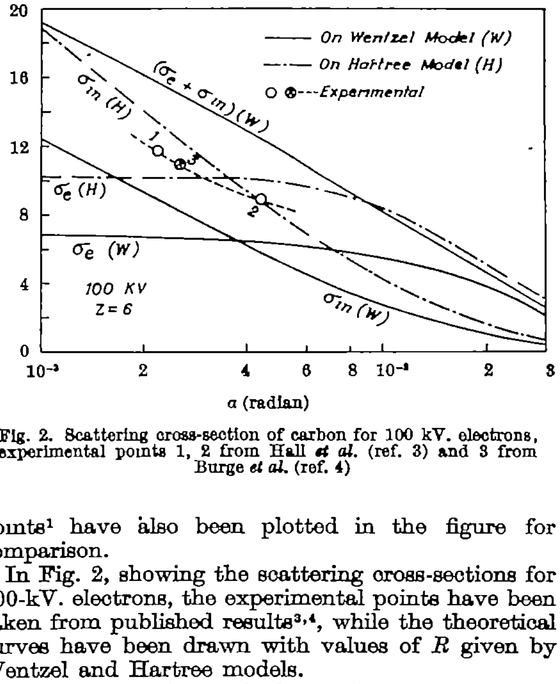

Fig. 2. Scattering cross-section of carbon for 100 kV. electrons, experimental points 1, 2 from Hall *et al.* (ref. 3) and 3 from Burge *et al.* (ref. 4)

With these two values of R, the corresponding theoretical scattering cross-section curves for 50 kV. electrons are represented in Fig. 1, where σ_e and σ_{in} signify the elastic and inelastic cross-sections respectively and $\sigma_t = \sigma_e + \sigma_{in}$; my earlier experimental points[1] have also been plotted in the figure for comparison.

In Fig. 2, showing the scattering cross-sections for 100-kV. electrons, the experimental points have been taken from published results[3,4], while the theoretical curves have been drawn with values of R given by Wentzel and Hartree models.

On a comparison of the various experimental results with theoretical values it can be concluded that with none of the values of R as considered above, one can get theoretical values of scattering cross-section agreeing satisfactorily with experimental results. Obviously, a reconsideration of the scattering formula or of the appropriate atomic radius seems to be necessary for the small angle region of scattering, which angular range is of particular importance in electron microscopic work.

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BIOCHEMISTRY

Radiosensitivity of Hydrogen Bonds in Deoxyribonucleic Acid

ALEXANDER *et al.*[1] have suggested that the 'secondary' structure of deoxyribonucleic acid (DNA) is disorganized after irradiation by breaking the hydrogen bonds which link the two molecules together into the twin helix. "In regions of the DNA molecule where this occurs, the double molecule is opened up and becomes more flexible." The molecule can coil up then into a smaller volume in solution and the viscosity of this solution will decrease. A further decrease in the viscosity is observed when the molecule is degraded due to breaks in the polynucleotide chain. As the loss of the viscosity is a result of different radiation effects, it is of interest to study the effect of the irradiation on the hydrogen bonds with other methods. The work of Sarkar and Dounce[2] provides a basis for such studies. According to these authors the formaldehyde reaction of Fraenkel-Conrat[3] can be observed only when an appreciable disruption of the interstrand hydrogen bonds occurs. This formaldehyde reaction may be more sensitive than viscosity measurements for detecting a low degree of denaturation of DNA. While Sarkar and Dounce[2] studied the denaturation after heating, I compared their results with the denaturation by X-ray irradiation.

Purified DNA (Worthington) is dissolved in 0·01 *M* sodium chloride (0·03 mgm./ml.), adjusted to *p*H 5·6–6·0 and irradiated with X-rays (Siemens-Dermopan X-ray machine; 50 kV., 25 m.amp, 140 kr./min.). Immediately after irradiation 0·1 ml. of 40 per cent formaldehyde, previously adjusted to *p*H 7·0, was added to 3·0 ml. of the DNA solution and the mixture was allowed to stand at 37° C for 1 hr. The absorption spectrum of the mixture was then determined (in terms of extinction) between 210 and 300 mμ with the recording spectrometer 'Spectronic 505' of Bausch and Lomb. As 'controls' unirradiated DNA-solutions before and after heating (10 min. at 90°) were used. A typical experiment is shown in Fig. 1.

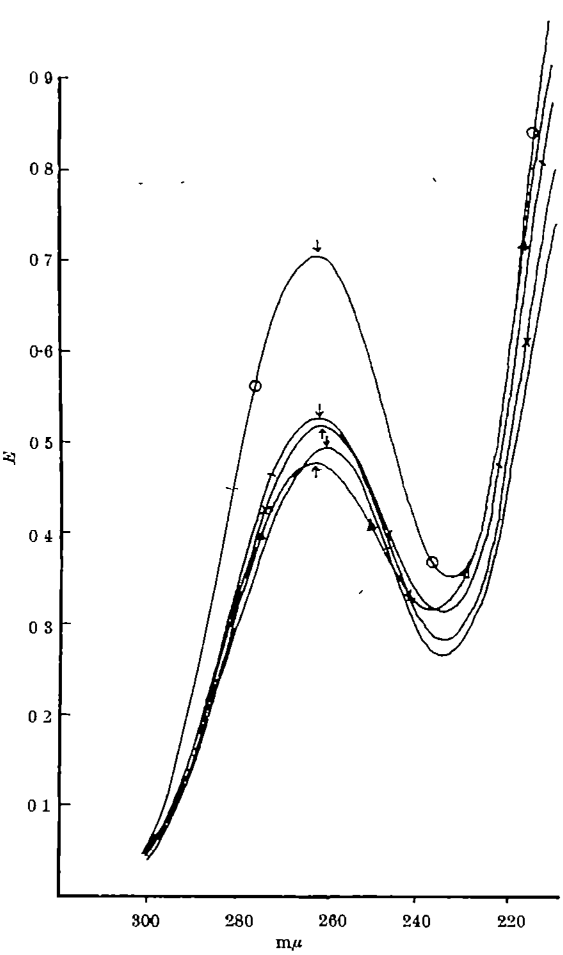

Fig. 1. DNA (Worthington), 0·03 mgm./ml. in 0·01 *M* sodium chloride. Absorption spectrum between 200 and 300 mμ after adding 0·1 ml. formaldehyde to 3·0 ml DNA solution. ———, DNA, unirradiated, —×—×—, DNA, immediately after 70 kr., —I—I—, DNA, immediately after 140 kr ; –Δ–Δ–, DNA, immediately after 210 kr.; —○—○—, DNA, 10 min. heating at 90° C. The arrows indicate the position of the maximum

The results of Sarkar and Dounce[2] were confirmed; after heating there was an increase of the extinction and a spectral shift in the position of the maximum by 2–3 mμ. A shift of this maximum is observed also after irradiation with doses of 70–210 kr. Higher doses do not produce more changes in the position of the maximum. However, the increase of the extinction after irradiation is much lower than after heating and is not dependent on the dose of X-rays used. Without formaldehyde there is an increase of the extinction after 70 and 140 kr.; higher doses render a decrease. The irradiation effect on the formaldehyde reaction and the decrease of the viscosity of the DNA-solution were compared. The shift in the position of the maximum toward unirradiated DNA has been measured after irradiation with 70, 140, 210 and 420 kr. In Table 1 the average values of the shift (6 experiments each) are compared with the intrinsic viscosity. The determination of the viscosity is described in an earlier publication[4].

Corresponding with the results of Sarkar and Dounce[2], there is no decrease in the viscosity after heating, whereas a considerable shift of the maximum can be observed. As in the heating experiments of Sarkar and Dounce, it was also found in the irradiation experiments that the formaldehyde reaction is a more sensitive method than the measurement of the viscosity. Whereas after 210 kr. the formaldehyde reaction is nearly complete, the viscosity of the solution decreases only to values of 66 per cent of the control. This loss of viscosity is in fairly good agreement with the results of Lett and Alexander[5].

Table 1. FORMALDEHYDE REACTION AND VISCOSITY AFTER IRRADIATION IN DNA SOLUTIONS

Dose (kr.)	Shift of the position of the maximum at 260 mμ (in mμ)	Intrinsic viscosity [η]
0	0	23·4
70	1·0 ± 0·15	20·4
140	2·0 ± 0·10	18·0
210	2·95 ± 0·20	15·5
420	3·20 ± 0·25	12·8
10 min 90° C.	2·50 ± 0·30	24·2

These experiments suggest that the hydrogen bonds in the DNA molecule are broken by low doses of X-rays. The molecule becomes more flexible and loses only little of its viscosity. Breaks of the nucleotide chain leading to a further decrease of the viscosity seem to occur mostly after higher doses. DNA, extracted from the thymus 3–6 hr. after 1,000-r. whole-body irradiation, shows a considerable change in viscosity decreasing to 50 per cent of the control[4]. This degraded DNA, prepared from the thymus of irradiated animals according to Kirby[6], does not, however, show any formaldehyde reaction at all. There is no difference in the absorption spectrum before and after incubation with formaldehyde, whereas after heating, a shift of the maximum and an increase of the extinction can be observed. Here, after irradiation *in vivo*, the decrease in viscosity is a much more sensitive method than the formaldehyde reaction. This supports the idea[4] that ionizing radiation destroys the DNA *in vivo* by quite different mechanisms than *in vitro*.

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Accelerated Collagen Formation and Histamine

IT has recently been demonstrated that reparatively growing tissues in skin wounds in the rat produce histamine at a high rate as a result of increased activity of histidine decarboxylase[1]. Further, the rate of repair, as measured by the tensile strength of the healing wound *in situ*, can be augmented or retarded, respectively, by artificial increase or inhibition of the histamine-forming capacity of the growing tissues. In these experiments the histamine-forming capacity was increased by injections of the histamine liberator 48/80, which depletes the skin of its histamine content and concomitantly raises the skin level of histidine decarboxylase. Extracellular histamine, derived from injected 'long-acting histamine', had no detectable effect on growth in healing. It was concluded that only intracellularly formed 'nascent' histamine is active in promoting certain types of growth[2].

Because collagen is a principal component of reparatively growing tissues in skin wounds, and the tensile strength in particular depends on collagen[3], it seemed interesting to determine the rate of collagen formation under conditions of increased histamine-forming capacity effected by injections of 48/80.

Samples of granulation tissue were obtained for study from polyvinyl sponges ('Ivalon') implanted subcutaneously in rats of both sexes from a closed colony bred at our Institute and weighing 200–300 gm. Under ether anæsthesia an incision was made in the mid-line of the back and two sponges were placed under the skin of one side of the back. On the fifth day after the implantation the sponges were removed and collagen content assessed by measuring the constituent amino-acid hydroxyproline[4]. These samples served as controls. About two weeks later each rat received 48/80 (1 mgm./kgm./day) intraperitoneally on three successive days. The activation of histidine decarboxylase resulting from this treatment was discovered by Schayer *et al.*[5], and fully confirmed in this laboratory[1]. On the next day two polyvinyl sponges were again implanted subcutaneously on the opposite side of the back and were removed for examination five days later.

In experiments on 15 rats the formation of collagen was enhanced by the pretreatment with 48/80, as judged from the hydroxyproline content. The granulation tissue formed contained on the average 55 per cent more collagen than the controls, mean ± standard error of mean, 55 ± 9·9 per cent.

These results provide further support of a connexion between certain types of rapid growth and histidine decarboxylase[6,7].

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Virginiamycin as an Antibiotic for Poultry Feeds

ANTIBIOTICS are widely used at low levels in commercial feeds to improve the early growth-rate of chickens and turkeys. The amount of response obtained from these drugs appears to be related to the nature and density of the microflora present in the feed and environment[1].

Antibiotics now used in manufactured feeds inhibit both Gram-positive and Gram-negative organisms ('broad spectrum') or, more specifically, the common Gram-positive pathogens ('narrow spectrum'). The new antibiotic, virginiamycin[2–4], is mainly active against Gram-positive organisms, but actually consists of three components having different spectra of activity. To evaluate this antibiotic for use in feed, it was compared with terramycin and zinc bacitracin, which have 'broad' and 'narrow' spectra, respectively, and are typical of the antibiotics used currently in commercial feeds.

(1) Virginiamycin, terramycin and zinc bacitracin were incorporated in the feed at low and high levels and fed to day-old, Cobb's strain, White Rock chicks reared in batteries. Four replicate pens of ten cockerels each were used for all treatments. Chicks were weighed individually at the beginning of the experiment and distributed equally by weight between the various pens. A practical starter-grower feed typical of commercial formulations which more than satisfied the N.R.C. nutritive requirements for chicks[5] was used as the basal ration. Pens were equipped with electrically heated brooders, raised wire floors and fluorescent lights.

At both four and eight weeks, all lots of birds which received antibiotics weighed more than the control lot (Table 1). These differences were significant ($P < 0{\cdot}01$), except for the high levels of terramycin and zinc bacitracin for both periods and the low level of virginiamycin at four weeks.

Table 1. EXPERIMENTAL DESIGN AND RESULTS* ON GROWTH AND FEED EFFICIENCY (F.E.) OBTAINED IN THE THREE EXPERIMENTS

Lot No.	Supplements to basal ration (Amt./ton)	4 weeks Weight (gm.)	4 weeks F.E. (Feed/gain)	8 weeks Weight (gm.)	8 weeks F.E. (Feed/gain)	Mortality (per cent)
	Exp. 1 (Chicks in battery brooders)					
1	None	494^{C}	1·73	$1,254^{C}$	2·20	7·5
2	Virginiamycin					
	4 gm.	$530^{A,B,C}$	1·64	$1,332^{B}$	2·12	2·5
3	9 gm.	559^{A}	1·56	$1,349^{A,B}$	2·00	2·5
4	100 gm.	554^{A}	1·62	$1,422^{A}$	2·04	2·5
5	Terramycin					
	9 gm.	$542^{A,B}$	1·77	$1,369^{A,B}$	2·18	7·5
6	100 gm.	$537^{A,B,C}$	1·74	$1,301^{B,C}$	2·21	10·0
7	Zinc bacitracin					
	4 gm	551^{A}	1·66	$1,341^{B}$	2·17	5·0
8	100 gm.	$515^{A,B,C}$	1·72	$1,312^{B,C}$	2·08	0·0
	Exp. 2 (Chicks on litter in floor pens)					
9	None	463	1·64	$1,317^{b,c}$	2·57	5·0
10	Virginiamycin					
	4 gm.	491	1·54	$1,421^{a,b}$	2·35	8·0
11	9 gm.	525	1·39	$1,462^{a}$	2·36	5·0
	Exp. 3 (Poults in battery brooders)					
		4·5 weeks		7 weeks		
12	None	589	1·97	$1,335^{b}$	2·04	18·8
13	Virginiamycin					
	10 gm.	660	1·79	$1,416^{a}$	1·88	3·1

* Any two means not having the same capital letter are significantly different; any two means having the same capital letter are not significantly different at the 0·01-level of probability. Small letters likewise indicate significance at the 0·05-level.

Higher levels of terramycin or zinc bacitracin were of not greater value than the lower levels at either interval. Virginiamycin, however, at the 100-gm. per ton-level, was significantly more beneficial ($P < 0{\cdot}01$) than at the 4-gm.-level at eight weeks.

Efficiency of feed utilization was consistently improved by all levels of virginiamycin, and to a lesser degree by the two levels of terramycin and zinc bacitracin. Since the measurements of feed intake were on a pen basis and thus data were limited, too much significance cannot be attached to these differences.

(2) This experiment was carried out using chicks in floor pens which had wood shavings for litter. The basal ration of Exp. 1, modified by adding a coccidiostat ('Nicarbazin', 0·0125 per cent), was used for all treatments. Day-old chicks (25 males and 25 females) were placed in each pen (5 × 9 ft.). Heat for brooding was supplied by infra-red lamps.

Duplicate pens of chicks, allocated as in the previous experiment, received each of the experimental treatments shown in Table 1. Beginning at four weeks of age, a finisher ration containing 18·5 per cent protein was fed. This ration differed from the starter ration only in that 6 per cent of corn replaced an equal amount of soybean oil meal.

At 8 weeks of age the average weights of the birds which received the nine grams per ton level of virginiamycin were greater ($P < 0{\cdot}05$) than those of the control birds. The increased weights of the birds which received four grams per ton were not significantly different from those of the control birds. Birds receiving virginiamycin also had an improved feed utilization; this improvement was about equal for both levels of the antibiotic. Since feed consumption was obtained by pen, it would require more data to be sure of the significance of these findings. It is noteworthy, however, that virginiamycin at all levels in both battery and floor experiments resulted in improved feed efficiency over the controls.

(3) Straight-run Beltsville White and Broad Breasted Bronze turkey poults brooded in batteries were used in this experiment. Duplicate pens of each breed (8 poults per pen) were used for each treatment. The experimental outline and results obtained are shown in Table 1. The addition of ten grams of virginiamycin per ton to a typical practical turkey starter feed (28 per cent protein) elicited a significant improvement ($P < 0{\cdot}05$) in the rate of growth. Livability was also apparently improved.

Summarizing, the battery birds fed virginiamycin at 4, 9 and 100 gm. per ton of feed were significantly ($P < 0{\cdot}01$) heavier at 8 weeks of age than birds fed the control ration, and best results were obtained at the highest level. The floor birds fed virginiamycin at 9 gm. per ton of feed were heavier ($P < 0{\cdot}05$) at 8 weeks than the controls, but not heavier than those which received the 4-gm.-level.

Virginiamycin was comparable to terramycin and zinc bacitracin in its effectiveness on growth, feed utilization and livability. It elicited improvements in rate of growth as it was increased from 4 gm. to 100 gm. per ton of feed. Higher levels of terramycin and zinc bacitracin (100 gm. per ton), however, were not so beneficial as the 4-gm. and 9-gm.-levels, respectively. Feed efficiency was also improved with virginiamycin, with the best utilization at the 9 gm. per ton-level. Since these data were obtained on a pen basis and therefore limited, more repetition would be required to demonstrate the reality of the improvements in feed efficiency observed in these tests.

The results of a preliminary experiment with turkey poults were in agreement with the growth and feed efficiency results obtained with chicks.

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Proportion of Bromsulphthalein Protein-bound within Incubated Rat Liver Slices

THE behaviour of rat liver slices incubated in media containing bromsulphthalein (BSP) and other dye substances, and the high *in vitro* binding of BSP to plasma proteins and various liver fractions, led Brauer and Pessotti[1] to suggest that uptake of BSP was related to intracellular protein binding.

It is therefore of interest to consider the results of incubation experiments in relation to an equation derived from the Langmuir isotherm[2]:

$$\beta = \frac{\text{bound substance}}{\text{total substance}} = \frac{1}{1 + \dfrac{k}{np} + \dfrac{(D)}{np}} \qquad (1)$$

where k is the dissociation constant, np the receptor concentration and (D) the concentration of unbound substance.

This equation indicates that the proportion bound at equilibrium (β) varies directly with the receptor concentration and inversely with the dissociation constant and the concentration of unbound substance. However, when (D) is small in relation to np the term $\frac{(D)}{np}$ may become negligible, in which case (D) will not affect β significantly.

If adsorption is the only mechanism of uptake by liver slices it follows that experimental values for β should conform to those predictable from equation (1).

The data available from incubation experiments (that is, slice weight and volume of medium, with the quantity of dye in each) is insufficient for direct calculation of β. But if it is assumed that a proportion of the slice volume (F) is available to unbound dye at the same concentration as the medium, then it can be shown that:

$$\beta = \frac{\text{bound dye within the slice}}{\text{total dye within the slice}} = \frac{\text{slice concn.} - F\,(\text{bath concn.})}{\text{slice concn.}} \qquad (2)$$

The value of 0·7 has been used for F. The reasons for this and for the overall assumption will be discussed elsewhere.

Standard incubation experiments were carried out using washed rat liver slices of approximately 0·7 gm. weight and 0·5 mm. thickness, in 3 ml. Krebs–Ringer-phosphate (pH 7·4) containing a known concentration of BSP and maintained at 37° C. Each incubation lasted 30 min., stirring being effected throughout by a vigorous stream of oxygen. Under these conditions no further uptake of dye occurred after 10–15 min. BSP was estimated by diluting a suitable aliquot with 0·5 N ammonium hydroxide and reading against the relevant blank in an absorptiometer.

Alteration of BSP concentration at equilibrium was effected by different initial concentrations. Alteration of the receptor concentration was attempted by: (a) heat denaturation (80° C. for 5 min. prior to incubation), a process believed to reveal further adsorption sites previously concealed within the molecule[3]; (b) the addition of sodium iodipamide (biligrafin) to the medium (2·24 per cent). Biligrafin reduces biliary excretion of BSP in the intact rat, presumably by competing for the same excretory mechanism, and might therefore be expected to occupy sites otherwise available for BSP adsorption.

It was found that initial dye concentrations within the range 25–400 gm./l. did not significantly alter the value calculated for β. Higher BSP concentrations, denaturation and the use of biligrafin all produced changes in the direction expected from equation (1), as shown in Table 1.

Table 1. MEAN VALUE OF β OBTAINED WITH DIFFERENT METHODS OF PROCEDURE

Procedure	Initial BSP concentration (gm./l.)	No. of experiments	$\beta \frac{\text{(Bound BSP)}}{\text{(Total BSP)}}$
Denaturation	0·05	22	0·96 ± 0·016
Standard	0·05	71	0·92 ± 0·025
Biligrafin	0·05	14	0·82 ± 0·056
Denaturation	2·0	10	0·90 ± 0·018
Standard	2·0	25	0·72 ± 0·063
Biligrafin	2·0	10	0·66 ± 0·039

While the application of a simple adsorption isotherm to the system described is a hazardous procedure, the results obtained are not incompatible with the view that adsorption is the main mechanism of BSP uptake in the incubated rat liver slice, and indicate that under favourable conditions liver is able to accept passively a large fraction of the dye presented.

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Immunoelectrophoresis with Cellulose Acetate

Previous results with immunoelectrophoresis, using human serum and its equine antiserum, have shown 25 serum constituents[1]. This communication shows that more than 35 precipitin lines may be obtained with an improved method.

The microelectrophoresis apparatus was described previously[2]. A 5-µl. drop of serum is placed at the centre of the cellulose acetate membrane, 22 mm. wide; the buffer used is veronal, pH 8·6, 0·075 ionic strength; the voltage applied is about 8 V./cm. for 120 min. After electrophoresis, a strip of Whatman No. 3 filter paper, 3 mm. wide and 7 cm. long, is placed at each outer edge of the cellulose acetate membrane. To each filter paper strip, 50 µl. of Pasteur Institute equine antihuman serum (lot No. 13,411) are applied evenly. The preparation is left in the apparatus (which is now utilized as a moist chamber) at room temperature, undisturbed for about 16 hr. or

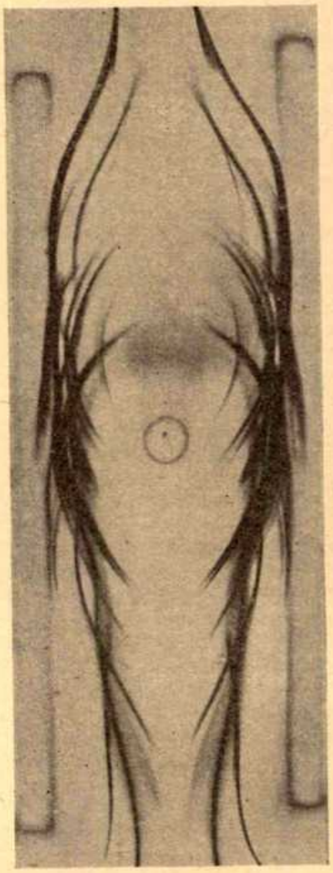

Fig. 1

longer. At the end of the diffusion time the membrane is removed and washed in several changes of physiological saline, finally rinsed in tap water and dried quickly at 110° C. The precipitin lines are stained with ponceau *S* and the background stain removed with 5 per cent acetic acid.

Fig. 1 shows that at least 35 precipitin lines can develop after a 60-hr. immunodiffusion; as many as 28 lines are seen after only 16 hr. Beyond 60 hr. there is no apparent increase in the number of precipitin lines obtained. Reproducibility is good and is mainly dependent on the total amount of serum and antiserum applied, their ratio and the distance between them.

Immunoelectrophoresis is not widely used as an experimental tool mainly because of technical difficulties. This report shows that a very simple method which requires a minimum of technical skill can produce outstanding results.

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Occurrence of Phenylacetylglutamine in Cow's Milk

PHENYLACETYLGLUTAMINE is a normal constituent of human urine[1], but other mammals including the dog, cat, rat, monkey, sheep and horse do not excrete this compound[2]. Phenylacetic acid administered orally to mammals results in the excretion of phenylacetylglycine. The synthesis of phenylacetylglutamine by human tissue has been described by Moldave and Meister[3].

During the course of an investigation on the non-protein-nitrogen fraction of cow's milk by ion-exchange chromatography[4], a fraction was obtained in small quantities which was identified as phenylacetylglutamine. The occurrence of this compound in the milk of any mammal has not to our knowledge been reported previously. Evidence to substantiate the natural existence in cow's milk of phenylacetylglutamine is given here with some characteristics of the compound.

Authentic phenylacetylglutamine was prepared by the method of Thierfelder and Sherwin[5]. Paper chromatography was conducted on 8-in.^{2} sheets of Whatman No. 1 paper using the ascending technique. Desalting of the fractions from the Moore–Stein column was accomplished following the method of Dreze *et al.*[6]. The unknown was isolated from a very small peak falling between urea and aspartic acid in the area normally occupied by methionine sulphoxide. The desalted unknown and authentic phenylacetylglutamine displayed the following properties: ninhydrin-negative on paper; failed to form an *N*-chloro derivative with chlorine gas[7] or with *tert.*-butylhypochlorite[8]; reacted readily with ammoniacal silver nitrate on papergrams; migrated identically in six different solvent systems (the six solvent systems were: $MeOH/H_2O$/pyridine (80 : 20 : 4); *tert.*-$BuOH/H_2O/HCOOH$ (69·5 : 29·5 : 1); 80 per cent EtOH; $EtOAc/MeOH/H_2O$ (50 : 25 : 25); 80 per cent *n*-PrOH; 2-butanone/*tert.*-BuOH/H_2O : diethylamine (40 : 40 : 20 : 4)); in the solvent system 2-butanone/*tert.*-butanol/water/diethylamine (40 : 40 : 20 : 4), two spots were obtained both browning and showing high fluorescence when the paper was heated in an autoclave. Attempts to hydrolyse both samples in 5·7 *N* hydrochloric acid at 110° for 10 days gave no detectable glutamic acid. Partial hydrolysis was effected in 6 *N* sulphuric acid at 180° C. for three days, the glutamic acid being isolated by adsorption on 'Amberlite *IR*-120 (H^+)'.

Isolation of crude phenylacetylglutamine from cow's milk was also accomplished as follows: Six litres of fresh, raw whole milk were concentrated to about 2 litres *in vacuo*, treated with an equal volume of 20 per cent trichloroacetic acid and filtered after 1 hr. The clear filtrate was extracted continuously for 40 hr. with ethyl ether. The serum was adjusted to *p*H 3·1, then extracted continuously for 72 hr. with ethyl acetate. The latter was evaporated *in vacuo*, the residue taken up in 10 ml. water and shaken for 1 hr. with 0·5 gm. charcoal prepared by the Partridge method[9]. The charcoal was filtered, washed with water and the filtrate passed over 'Amberlite *IR*-120 (H^+)'. Elution with 0·1 *N* hydrochloric acid gave crude phenylacetylglutamine having properties identical with the synthetic sample and with the fraction isolated from the Moore–Stein column. On the basis of the material isolated in this manner, it is estimated that milk contains at least 0·1 mgm. phenylacetylglutamine per litre.

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PHYSIOLOGY

Formation of Iodinated Protein in Saliva

AFTER intravenous injection of tracer amounts of iodine-131 in the dog, the concentration of iodine in the parotid saliva is 5–20 times that in the plasma. A variable proportion (1–95 per cent, but commonly 10–30 per cent) of this iodine is non-dialysable and is precipitated by common protein precipitants, such as 5 per cent trichloroacetic acid, zinc hydroxide, tungstic acid, and 66 per cent acetone or ethanol. None of the activity in the precipitate is extractable by *n*-butanol, unlike the protein-bound radioactivity of plasma. After hydrolysis of the protein with pancreatin, paper chromatography in a butanol/acetic acid/water system (4 : 1 : 1) revealed radioactivity almost entirely in monoiodotyrosine with only a small amount in diiodotyrosine; there was no sign of labelled iodothyronines. Evidently the organic iodide in saliva is in the form of iodotyrosines linked by peptide bonds into proteins. Formation of protein-bound iodine was completely and immediately prevented by injection of thiouracil or mercaptazole in doses of 5 mgm./kgm. or more, but was not modified by total thyroidectomy.

In vitro formation of protein-bound iodine was readily demonstrated by incubation of freshly secreted saliva with phosphate buffer (*p*H 7·0) containing a tracer amount of iodine-131. Iodine binding was demonstrable within a minute and reached its maximum after 10–30 min. The extent of binding varied between samples obtained at different secretion-rates and in different animals, but was always demonstrable. Binding by the *in vitro* system could be inhibited 90 per cent or more by mercaptazole $2·5 \times 10^{-6}$ *M*, and sulphanilamide, cysteine, mercuric chloride and ferrocyanide 10^{-3} *M*. The considerable capacity of the system was indicated by the lack of depression in the percentage organically bound until the iodide concentration exceeded 10^{-4} *M*. The addition of bovine serum albumin increased the percentage of protein bound iodine by as much as twofold.

In other isolated iodination systems[1] peroxide has been implicated as the oxidant of iodide to an active form. The formation of free peroxide in the saliva system was demonstrated by the formation of purpurogallin on the addition of pyrogallol and horseradish peroxidase. Organic incorporation of iodine was prevented by the addition of 0·5 mgm./ml. of catalase. Attempts to increase incorporation by providing exogenous substrate for the specific oxidases for glucose, xanthine, alanine, tyrosine and tyramine had no appreciable effect. Tyrosine depressed protein-binding probably by acting itself as an iodine acceptor.

Organic iodine was not found in submaxillary saliva of the dog nor in parotid or mixed saliva of man. On the other hand, considerable amounts of organically bound iodine were found in dog gastric juice, bile and succus entericus.

The properties of the *in vitro* saliva system correspond closely to those of homogenates of thyroid and salivary glands of rats[2]. The presence of protein-bound activity mainly in the form of mono-iodotyrosine is similar to the binding in the milk of some species[3]. The biological significance of protein incorporation of iodine in intestinal secretions is obscure. It has been reported[4] that in rats diiodotyrosine is found in the plasma and tissues in undiminished amounts after thyroidectomy and that even some thyroxine is formed. It is possible that formation of organic iodine in the glands of the alimentary canal is of some importance in this process; at least in the dog a considerable proportion of the 'internal' circulation of iodine through the alimentary canal is via organic iodine compounds rather than free iodide.

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Effect of Heat on the Kinetic Constants of Myosin–Adenosine Triphosphatase

Nanninga and Mommaerts used the firefly technique for detection of adenosine triphosphate (ATP) in order to establish the binding of ATP to myosin[1]. The addition of creatine phosphate and creatine phosphate transphosphorylase is necessary to counteract the hydrolysis of ATP by the myosin. In this way a maximal binding of 1 mole ATP to 1 mole myosin was established while it was also possible to determine k_1, the kinetic constant of the formation of the ATP–myosin complex, from the initial speed of disappearance of the free ATP as measured from the decrease of the luminescence[2]. The k_1 determination can be done in absence of the rephosphorylating system. The effect of heat inactivation of the myosin on both the k_1 and on the bound ATP was studied. The myosin was 1·31 per cent, dissolved in 0·3 *M* potassium sulphate, 0·01 *M* *tris*-buffer of *p*H 7·0 and was heated for 90 min. at 37° C. Then it was added to the luciferin–luciferase solution at different ATP concentrations. It was found that both k_1 and the bound ATP decrease about sevenfold (Table 1).

Table 1. INACTIVATION OF MYOSIN BY HEATING FOR 90 MIN. AT 37°. FINAL MYOSIN CONCENTRATION, $3·28 \times 10^{-6}$ *M*

	Apparent k_1	ATP bound at $2·0 \times 10^{-6}$ *M* ATP free
Before	$2·32 \times 10^{4}$ sec.$^{-1}$ mol.$^{-1}$	$2·55 \times 10^{-6}$ *M*
After	$0·30 \times 10^{4}$ sec.$^{-1}$ mol.$^{-1}$	$0·35 \times 10^{-6}$ *M*

This result shows that the inactivation is due to a decreased enzyme concentration. Obviously six out of seven myosin molecules lose the power to form a complex with ATP, presumably by oxidation of the SH-group which is involved, as is known from the inactivation by parachloromercuribenzoate, copper and other SH-reagents. The apparent k_1, derived from the initial speed of decrease of the ATP-level[2], decreases seven times, and for the same reason the enzyme substrate complex decreases seven times. The true k_1, however, remains constant.

In separate experiments k_3, the kinetic constant of decomposition of the complex, and K_m, the Michaelis constant, were measured by means of the same technique in the same milieu but with a ten-times lower myosin concentration (compare ref. 2). The constant k_3 follows from the maximal activity, divided by the myosin concentration. After inactivation it appears that K_m is nearly unchanged while k_3 is much smaller. In one series the apparent k_1 was, after 70 min. at 37°, 0·265 times the apparent k_1 before inactivation while the apparent k_3 was 0·233 times the apparent k_3 before inactivation, and K_m 0·92 times K_m before inactivation. The parallel behaviour of k_1 and k_3 must be due to a reduction in the enzyme concentration so that the true k_1 and k_3 remain the same while the enzyme concentration decreases. The alternative possibility of an unchanged formation of enzyme substrate complex with a considerable reduction in its speed of decomposition is therefore ruled out. When k_1' (k_1 after inactivation) divided by k_1 is plotted versus time of incubation, an exponential curve is obtained as $\log k_1'/k_1$ is directly proportional to time of incubation. Since $k_1'/k_1 = M/\Sigma M$ (active myosin divided by total myosin), it follows that $M = \Sigma M \times e^{-kt}$ and $dM/dt = -kM$. In this first-order reaction k is $3·6 \times 10^{-4}$ sec.$^{-1}$ at 37°.

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Hypoglycæmia induced by Manganese

PLANT extracts have long been used as home remedies for diabetes mellitus[1-3], and have been investigated for their hypoglycæmic effect[4,5]. However, there has been little experimental or clinical proof of their efficacy[6].

Recently it has come to our notice that infusions of lucerne (alfalfa, *Medicago sativa*) are being used in the treatment of this disease by certain diabetics in South Africa. We have had the opportunity of studying a young male diabetic who gives a marked hypoglycæmic response following the oral administration of an aqueous extract of this plant (Fig. 1). The patient is twenty-one years of age, having been a diabetic for the past seven years. A diabetic glucose tolerance curve has been demonstrated on repeated occasions with fasting blood sugar-levels varying from 150 mgm./100 ml. to 250 mgm./100 ml. He has been treated intermittently with insulin, requiring large doses (100–200 units daily) for even moderately satisfactory control. He himself claimed that lucerne infusions gave better control. He often exhibits the feature of marked hyperglycæmia even when under treatment with insulin with little increase in plasma or urinary ketones. Routine investigations failed to reveal evidence of thyrotoxicosis, Cushing's disease, phæochromocytoma or chronic pancreatitis.

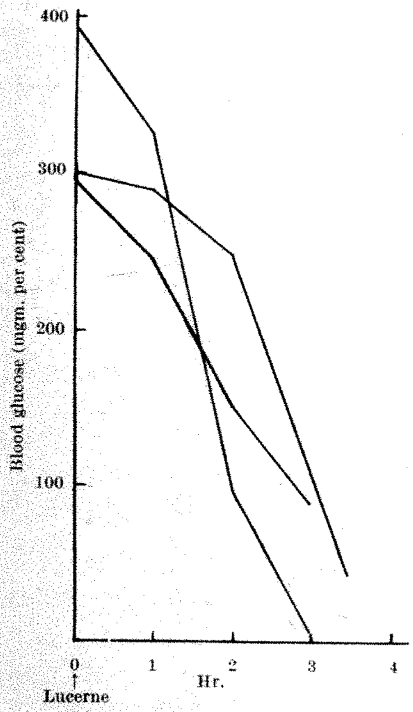

Fig. 1. Blood sugar-levels following the oral administration of lucerne extract

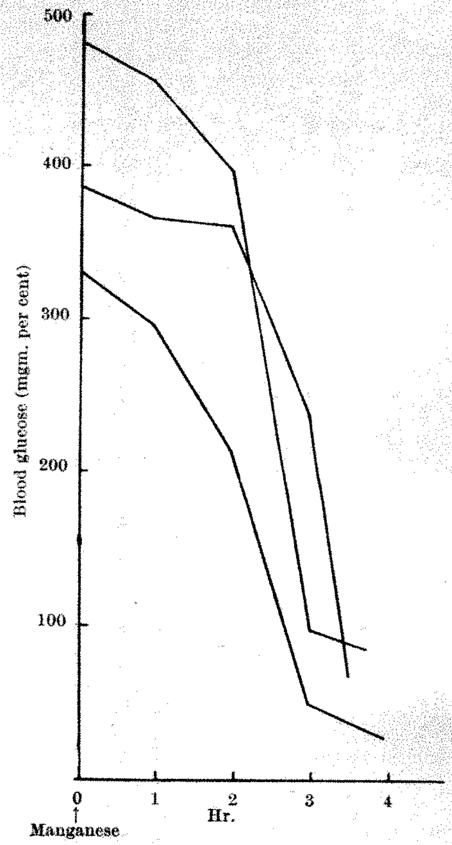

Fig. 2. Blood sugar-levels following the oral administration of 5 mgm. manganese (as manganese chloride)

There was no hypoglycæmic response to the administration of the amino-acid *l*-leucine[7,8]. Parents and sibling have normal glucose-tolerance curves.

Lucerne was noted to contain relatively large concentrations of manganese[9]. Accordingly, manganese as manganese chloride was administered many times orally in doses of 3–5 mgm. and intravenously on one occasion in a dose of 20 mcgm. to the patient. The effect on the blood glucose-level was a consistent fall often to severely hypoglycæmic levels (Fig. 2). Both lucerne and manganese when given orally modified the glucose tolerance curve (Fig. 3).

The oral administration of manganese to four middle-aged diabetics, three juvenile diabetics and one case of diabetes resulting from chronic pancreatitis did not affect the blood sugar-level.

Although manganese is well known to be a component and co-factor in certain enzyme systems including some concerned in the intermediary metabolism of carbohydrates[10], a hypoglycæmic effect of manganese has not previously been reported. The time-interval between administration of manganese and its effect on the blood glucose-level is consistent with the possibility of enzyme activation[10]. Further work on the metabolism of manganese is being done.

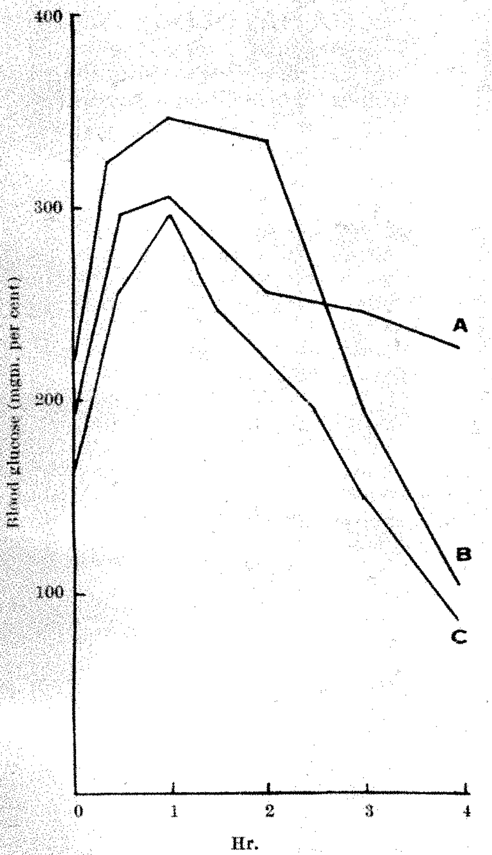

Fig. 3. Glucose tolerance curves: *A*, after 100 gm. glucose orally; *B*, after 100 gm. glucose and lucerne; *C*, after 100 gm. glucose + 5 mgm. manganese (as manganese chloride) orally

We thank Prof. B. J. P. Becker and Prof. H. B. Stein for their assistance and interest.

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In vitro Calcification of Rat Aorta in Serum

Although hydroxyapatite has been identified as a major component of aortic plaques[1], the events leading to its deposition in this tissue are little understood. Recently, we have found that substantial quantities of calcium and phosphate are deposited on to rat aortas during the incubation of this tissue in serum. This finding has provided a useful system for the study of aortic calcification *in vitro*.

Serum and aortas were obtained from mature male Sprague–Dawley rats. Penicillin (5 mgm.), streptomycin sulphate (50 mgm.) and 2 ml. of a carrier-free ^{45}CaCl solution containing approximately 1,200,000 c.p.m. were added to 100 ml. of serum. Each aorta was incubated with 1 ml. of this solution at *p*H 7·4° and 37° C. At various times during the incubation the aortas were removed, washed in several litres of distilled water, air dried, weighed and then placed in 5 ml. of 0·12 *M* hydrochloric acid. Aliquots of this solution were taken for calcium[2], phosphorus[3] and calcium-45 assay.

Following a lag period of approximately 24 hr., there was a marked increase of calcium, phosphate and calcium-45 in the aortas (Fig. 1). Hydroxyapatite was detected by X-ray diffraction in aortas incubated for 72 hr. but not in tissues incubated for 1 hr. About 15 per cent of the calcium initially in the serum was fixed on to the aortas during 72 hr. of incubation. Since incorporation of calcium-45 paralleled the deposition of calcium and phosphate on to the tissues, it was used as a measure of calcification in some experiments.

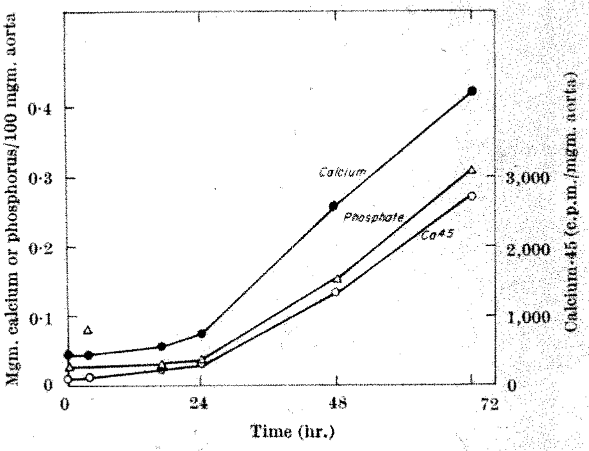

Fig. 1. Uptake of calcium, phosphate and calcium-45 by aorta from serum

Mineralization was accelerated by ageing of serum at 37° prior to incubation or by the addition of alkaline phosphatase (0·5 mgm./ml.) to the serum. These findings suggest the presence in rat serum of labile inhibitors of calcification such as those described by Fleisch and Neuman[4].

In order to identify components in the aorta essential for calcification, aortas were treated with various enzymes prior to incubation. Enzyme digestions were carried out in saline at *p*H 7·4 at 37° for 24 hr. The activities of the enzymes were assayed with 'Azocoll'[5], orcein-impregnated elastin[6] and rat tail tendon. Trypsin, a crude bacterial protease (Nutritional Biochemical Corp.), pronase (*Streptomyces griseus* protease obtained from the California Corporation for Biochemical Research) and three-times crystallized elastase (Worthington Biochemicals Corp.) rapidly digested 'Azocoll'. Only elastase and pronase digested orcein-impregnated elastin. Purified collagenase (supplied by Dr. P. Gallop) digested rat tail tendon.

Treatment of the aortas with collagenase, trypsin, protease and hyaluronidase did not reduce the uptake of calcium-45 by aorta (Table 1). Trypsin and protease increased the c.p.m./mgm. of aorta probably by reducing the amounts of non-essential components. The actual uptake of calcium-45 per aorta in these samples was similar to the 72-hr. control. Elastase and pronase treatment prevented mineralization as indicated by a negligible uptake of calcium-45. These findings suggest that elastin or some other component of the aorta susceptible to both elastase and pronase, but not the other enzymes, was required for mineralization.

Table 1. EFFECT OF ENZYMATIC DIGESTIONS ON THE UPTAKE OF CALCIUM-45 BY RAT AORTAS

Pretreatment	Incubation period (hr.)	Weight (percentage of control)	Calcium-45* (c.p.m./mgm.)
None	1	103	34 ± 0·1
None	72	100	2,865 ± 178
Elastase	72	43	53 ± 7·7
Pronase	72	58·5	308 ± 4·9
Collagenase	72	60·7	2,756 ± 128
Protease	72	65·1	4,100 ± 320
Trypsin	72	62·4	4,750 ± 321
Hyaluronidase	72	102	2,460 ± 73

* Five aortas in each group.

It is evident from these results that the *in vitro* calcification of the aorta is dependent both on the integrity of certain components of the matrix and on alterations in fresh serum. Since aortic mineralization does not ordinarily occur in the rat *in vivo* in the presence of sufficient concentrations of calcium and phosphate to produce *in vitro* calcification and, since calcification is accelerated in aged serum, it seems reasonable to postulate the existence in serum of a potent but labile mechanism for preventing the deposition of mineral in the aorta. It is conceivable that this system is impaired in those conditions where calcified plaques occur *in vivo*. In addition, it is of interest to the general problem of biological calcification that elastin rather than collagen seems to be the component of this matrix essential for mineralization to occur. Other workers[7,8] have implicated collagen in other systems. While the importance of these findings in the formation of normally mineralizing tissues is at present unknown, they do warrant further investigations on a possible role for non-collagenous constituents in initiating mineral formation in the hard tissues.

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HÆMATOLOGY

Possible Cause of Heinz Bodies in Congenital Heinz-body Anæmia

EXAMINATION of the blood of a ten-year-old girl with congenital Heinz-body anæmia has provided evidence for the presence in the subject's red cells of an unstable hæmoglobin fraction.

The subject, splenectomized eight years previously, yielded the following hæmatological data at the time of examination : hæmoglobin, 8·3 gm. per 100 ml.; packed cell volume, 30·5 per cent; mean corpuscular hæmoglobin concentration, 27·5 per cent; leucocytes, 7,000 mm.[3]; reticulocytes, 30 per cent. Almost every red cell contained one Heinz body or more.

In order to produce Heinz bodies, either *in vivo* or *in vitro*, in red cells previously free of them, it is necessary to overcome the protective action on hæmoglobin of reduced glutathione (GSH). The red-cell GSH concentration normally is maintained through the action of the hexose monophosphate (HMP) pathway, and where this pathway is defective, as in 'primaquine-sensitive' individuals, relatively mild stress on red cells yields Heinz bodies. When the HMP pathway functions normally so that the GSH concentration is maintained, the stress required to produce Heinz bodies is greater.

Table 1

	Normal subjects	Heinz-body anæmia
Reticulocytes (per cent)	0·2–2·0	30
Reduced glutathione (µmoles-SH/ml. RBC)	1·92–3·43	1·90
Oxidized glutathione (percentage total GSH + GSSG)	2·8 –11·2	6·9
Glutathione stability test (percentage of fall)	0–27	22
Glucose-6-phosphate dehydrogenase (ΔOD/min./ml. RBC)	1·40–2·87	3·56
6-Phosphogluconic dehydrogenase (ΔOD/min./ml. RBC)	1·35–2·34	2·91

GSSG, oxidized glutathione; OD, optical density.

In this work the red-cell HMP pathway appeared to function adequately (Table 1) because the concentration of GSH and oxidized glutathione was normal, while glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activities had the high values associated with a young red-cell population[1]. When the cells were stressed by incubation at 37° for 2 hr. with acetylphenylhydrazine according to the GSH stability test of Beutler[2], they showed only the slight fall in GSH that occurs with normal red cells. It appeared, therefore, that the Heinz bodies were not formed through a defect in that portion of the HMP pathway concerned with maintaining an adequate level of GSH.

Erythrocyte catalase has been reported low in a single case of congenital Heinz-body anæmia[3]. Such a defect would permit a plausible explanation for Heinz-body formation which, it is known, may occur through the action of hydrogen peroxide on hæmoglobin. However, in two other cases reported by Schmid *et al.*[4], red-cell catalase was normal. In addition, no Heinz-body formation is noted in the red cells of subjects with acatalasæmia (Takahara, S., personal communication). In the patient, the subject of the present work, the red-cell catalase activity was found to be normal.

The sole anomaly which has been demonstrated in the present work is the formation of a red-brown precipitate when a stroma-free, Heinz-body free hæmolysate of congenital Heinz-body anæmia blood was heated for some minutes at 50° C. Such a precipitate was obtained also when samples of purified patient's hæmoglobin were heated at 50° C. in isotonic phosphate-buffered saline (*p*H 6·5). No such precipitate could be obtained from hæmolysates of normal blood or cord blood, or from blood containing a large number of reticulocytes. Similarly, when purified hæmoglobin from normal blood was heated at 50° C. for the same length of time, no precipitate was obtained. The rate of appearance of the precipitate varied according to the hæmoglobin concentration of the solution. However, the bulk of the precipitate appeared over a short period and further heating produced only small amounts of precipitate such as could be obtained from normal hæmoglobin samples.

Both normal hæmoglobin solutions and those of the subject under study formed some methæmoglobin as a result of the heating procedure. It was found that if all the hæmoglobin in the solutions was converted by $K_3Fe(CN)_6$ into methæmoglobin, then the precipitate was formed more readily on heating at 50° C. and even solutions of normal hæmoglobin under these conditions yielded small amounts of precipitate. On the other hand, the presence of sodium dithionite, which reduced any methæmoglobin to hæmoglobin, suppressed formation of the precipitate. It appeared that in order to produce the precipitate it was necessary for methæmoglobin to be present in the heated solution.

Examination of the precipitate gave evidence that it was soluble in alkaline solution, and at *p*H 10·7 it gave a visible absorption spectrum indistinguishable from that of alkaline methæmoglobin at this *p*H value[5]. In amount, the precipitate accounted for approximately 20 per cent of the total hæmoglobin present. Measurement of the ratio of hæm to globin suggested that the precipitate contained less hæm to globin than does normal unheated hæmoglobin.

It is concluded tentatively that in congenital Heinz-body anæmia the formation of Heinz bodies *in vivo* is the end-result of the presence in the red cells of an unstable hæmoglobin fraction, the instability of which can be demonstrated *in vitro* by heat treatment of solutions of the hæmoglobin.

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Myoglobin in the Human Fœtus

Evidence for the presence of a fœtal form of myoglobin in human beings, analogous to fœtal hæmoglobin, has consisted of reported electrophoretic and spectral differences between material obtained from skeletal muscles of infants and that obtained from adults. Electrophoretically, so-called fœtal myoglobin appeared to behave somewhat like fœtal hæmoglobin; however, its absorption spectrum in the visible range showed, unlike either fœtal hæmoglobin or myoglobin from the adult, a "characteristic plateau between the wave-lengths 490–530 mμ at *p*H 8·6" (ref. 1).

Investigations reported here, however, suggest that myoglobin from the fœtus and adult are not distinguishable. Autopsy material of adults and infants, the latter being either premature or full-term, but in no case older than 24 hr., provided the sources of myoglobin. Samples to be compared were prepared simultaneously and by the same technique. Several different methods were used, including that in which the foregoing observations were made[1–4]. The most convenient and reliable technique proved to be a slight modification of that of Theorell and de Duve[2], in which an aqueous extract of muscle was treated with saturated lead subacetate, the supernatant, brought to *p*H 7, was dialysed against 3 *M* phosphate buffer, *p*H 6·6, and the resulting supernatant dialysed against increasingly saturated solutions of ammonium sulphate. Individual cases provided the myoglobin in the adult, whereas in the infant both pooled and individual preparations were studied. These were compared with each other and also with hæmoglobins A and F by means of analytic ultracentrifugation, electrophoresis on various media and at different *p*H's, quantitative amino-acid analysis, spectrophotometry and two-dimensional peptide analysis.

From both infants and adults, myoglobin was obtained which exhibited a single peak on ultracentrifugation with an S_{20} of 2, consistent with its molecular weight. In all cases this myoglobin, whether fœtal or adult in origin, showed the same properties on electrophoresis in the *p*H-range of 6·3–8·6 on paper, starch block and agar gel when care was taken prior to each run to dialyse the preparations against the buffer employed. Total amino-acid composition of myoglobin from the fœtus and adult, determined on a Moore-Stein chromatographic column, and calculated as residues per mole (mol. wt. 16,700) revealed no differences beyond the range of experimental error (Table 1). The absorption spectrum was also identical in the range thought to distinguish two myoglobins. Furthermore, the 'characteristic plateau' could be demonstrated in myoglobin from either source simply by dilution (Fig. 1). Two-dimensional peptide patterns produced by trypsin and trypsin plus chymotrypsin digestion were indistinguishable within the

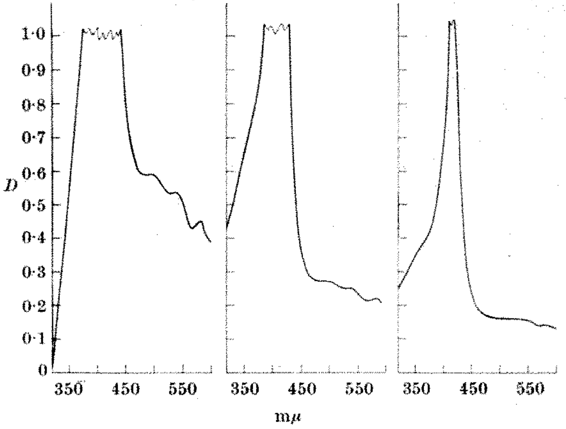

Fig. 1. Absorption spectra at *p*H 8·6 of metmyoglobin from fœtal or adult source at various dilutions: (left) 120 mgm. per cent; (centre) 60 mgm. per cent; (right) 30 mgm. per cent. The latter has been described as 'characteristic' for myoglobin from the fœtus only

Table 1. Amino-acid Composition of Human Myoglobin (No. residues/molecule)

Amino-acid	Adult	Fœtus	Amino-acid	Adult	Fœtus
Alanine	14	14	Serine	6	6
Glycine	17	17	Methionine	2	2
Valine	9	9	Arginine	2	2
Leucine	17	17	Histidine	8	8
Isoleucine	7	7	Lysine	15	15
Proline	6	6	Aspartic acid	13	12
Phenylalanine	7	6	Glutamic acid	18	16
Tyrosine	2	2	Threonine	5	5

Results of 17-hr. hydrolysis of myoglobin from the adult and fœtus. Tryptophan was not determined. Cf. Rossi-Fanelli *et al.* (ref. 5).

limits of the technique used[6] (Fig. 2). It is worth noting that crystals of myoglobin obtained from adult and fœtal sources also appear to be identical[2,3].

These results suggest that myoglobin from the human fœtus is indistinguishable from that of the adult, and that previous observations may have been due both to its presence in small amounts in the fœtus and the difficulty in separating it from fœtal hæmoglobin.

I thank Drs. C. B. Anfinsen, R. W. Canfield and H. Metzger, along with Mrs. M. Thoren, for advice and help in this work, and Dr. J. Bolveri for providing fœtal muscle.

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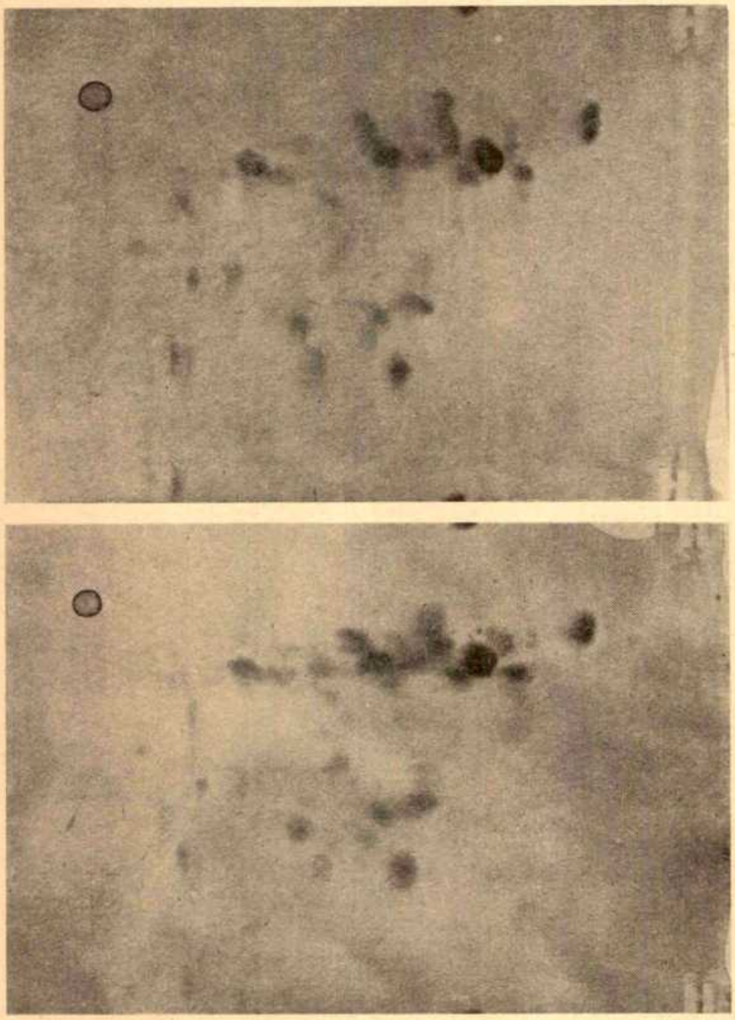

Fig. 2. Two-dimensional peptide patterns of myoglobin from the adult (above) and from the fœtus (below). Heat denaturation was followed by incubation at 37° C. for 24 hr. with 4 per cent trypsin, followed by incubation for 2 hr. with 4 per cent chymotrypsin. escending chromatography (downward direction) was followed by electrophoresis (toward right). A small resistant 'core' is present at both origins

A Rapid Screening Dye Test for the Detection of Glucose-6-Phosphate Dehydrogenase Deficiency in Red Cells

Motulsky *et al.*[1] have reported the adaptation of the spectrophotometric glucose-6-phosphate (G6P) dehydrogenase estimation[2] to a visual qualitative test; the rate of decolorization of incorporated brilliant cresyl blue, acting as an electron acceptor, enabled red cells with normal and deficient enzyme activity to be distinguished (see Vella[3] for description). Some brands of brilliant cresyl blue were found to be far more effective as electron acceptors than others, requiring standardization of the decolorization time for each particular dye to assess normal enzyme activity[4].

The development of a screening test, adapted from a general colorimetric assay for dehydrogenases[5], revealed certain advantages. In the conversion of glucose-6-phosphate, in the presence of triphosphopyridine nucleotide (TPN), by glucose-6-phosphate dehydrogenase, to 6-phosphogluconate and TPNH, phenazine methosulphate (PMS) is used as the electron carrier between TPNH and 2,6-dichlorophenol-indophenol (DPIP) or 2,6-dichlorophenol-indo-*o*-cresol (DPIC):

$$\text{TPNH} + \text{PMS} \longrightarrow \text{TPN} + \text{reduced PMS}$$

$$\text{Reduced PMS} + \underset{\text{(blue)}}{\text{DPIP}} \longrightarrow \text{PMS} + \underset{\text{(leucobase)}}{\text{reduced DPIP}}$$

The rate of decolorization of DPIP is thus a measure of the erythrocytic glucose-6-phosphate dehydrogenase activity.

Water, 1 ml., was placed in 75 × 10 mm. test tubes, and 0·02 ml. well-mixed blood with a packed cell volume of 40–50 per cent added from a hæmoglobin pipette (with suitable refrigeration, oxalated or heparinized samples were tested satisfactorily 4–5 days after sampling, while blood preserved in acid citrate dextrose showed little increase of time after 10–14 days). The tube was well mixed to hæmolyse the red cells. The dye reagent, 0·5 ml., was added and mixed. The dye solution consisted of 0·74 M *tris* buffer, pH 8·0, containing 2,6-dichlorophenol-indophenol, 0·5 mM, that is, 14·5 mgm. per cent (the optimal pH for red cell glucose-6-phosphate dehydrogenase activity showed a broad peak between 7·5–8·4); TPN (2·3 mgm./ml., about 3 mM, stored in deep-freeze); G6P (15·2 mgm./ml. anhydrous or 17·9 mgm./ml. Boehringer, 50 mM, stored in deep-freeze); and PMS (2 mgm. per cent, fresh weekly and stored refrigerated in the dark)—in the proportion of 8 : 0·5 : 0·5 : 1, prepared fresh before use. The mixture was overlaid with mineral oil and stood at room temperature or incubated at 37° C. The time was noted for decolorization to a red-yellow end-point (two absorption bands of oxyhæmoglobin); afterwards, the colour changed to red-purple (single absorption band due to reduced hæmoglobin).

The substrate and co-enzyme concentrations have been adapted from optimal amounts used in the quantitative enzyme assay[6]; however, since TPN was regenerated in the system, the quantity used could be reduced. While increasing or decreasing the dye content altered the decolorization times proportionately, the amount of DPIP used was best suited to the appearance of the colour change. As the decolorization time was only slightly less at 37° C. than at room temperature, the latter method could be most usefully adopted in small laboratories or in the field. The use of phenazine methosulphate to mediate electron transfer between TPNH and DPIP obviates the use of diaphorase required in colorimetric assays of dehydrogenases. Phenazine methosulphate is an oxidation-reduction indicator (E = + 0·080), but could not be used by itself, because of its pale yellow colour, as a visual indicator. On the other hand, omission of PMS resulted in the DPIP being decolorized very slowly, namely, 4–6 hr., indicating that PMS acted as a highly active non-enzymatic agent for electron transfer.

Using the above system, decolorization times ranged from 5 to 20 min. for 280 subjects shown to have normal glucose-6-phosphate dehydrogenase activity by the quantitative Glock and McLean assay[6], and 1½–3½ hr. for 40 samples with deficient red cell enzyme activity. Comparative tests with the most active brands of brilliant cresyl blue, using similar concentrations of substrate, co-enzyme and dye, gave end-points of 30–75 min. in red cells with normal enzyme activity and 2–8 hr. in cases of deficient activity on incubation at 37° C. and considerably longer times when tested at room temperature. A difficulty encountered with the brilliant cresyl blue test has been to assess the enzyme status of samples decolorizing in 75–120 min. The phenazine methosulphate test gave a clearer separation between red cells with normal enzyme activity and cases with inherited or acquired glucose-6-phosphate dehydrogenase deficiency; further, the phenazine methosulphate test was more successful in detecting the heterozygous female with an intermediate enzyme activity in the sex-linked inherited glucose-6-phosphate dehydrogenase deficiency[7].

To date, no false results have occurred in more than 500 samples checked in parallel with the quantitative glucose-6-phosphate dehydrogenase assay[6], methæmoglobin reduction test[8], and reactions (Heinz body production[9], glutathione instability[10]) that are associated with erythrocytic glucose-6-phosphate dehydrogenase deficiency. Where there is marked anæmia, an appropriately greater volume of blood should be used to avoid the possibility of a false positive. Caution in interpretation may be required in blood specimens showing a reticulocytosis, where the higher enzyme content of young red cells[11] may mask a virtual deficiency; and in conditions of leucocytosis and leukæmia—here packed red cells should be tested after removal of the buffy coat.

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PATHOLOGY

Incorporation of Host Nuclear Materials into Transplanted Tumour Cells in Surgical Wounds

AREAS of acute inflammation contain extracellular deoxyribonucleic acid (DNA) derived principally from dead leucocytes[1–3]. It has been suggested that components of this DNA may be re-utilized by proliferating cells; but direct evidence for this concept has not been reported[3,4].

Evidence that DNA components can be re-utilized following death of a cell was obtained in the work reported here by showing that proliferating tumour cells incorporated tritiated thymidine into their nuclei following transplantation into an animal which had been given this labelled nucleoside 24 hr. previously. The experimental design was based on the following considerations: (1) tritiated thymidine is incorporated exclusively into the DNA of proliferating cells and remains within the cell until its death or division when distribution of label to daughter cells occurs[5]; (2) tritiated thymidine is available for utilization for only a short period (1–2 hr.) following its administration to an animal, being either incorporated into DNA or converted into non-utilizable end products which are excreted[6]; (3) the tritium label in the thymidine base is stable and, because it is a low-energy β-emitter, permits localization at the cellular level by autoradiography[5]; (4) transplanted tumour tissue can be distinguished from host tissue in histological preparations.

Each of 21 *DBA* mice received two intraperitoneal injections 12 hr. apart of 25 μc. each of tritiated thymidine. Twenty-four hours after the last injection a square of viable sarcoma 180 tissue, measuring approximately 3 mm. in diameter, was transplanted into a fresh surgical wound in each mouse. An incision was made in the abdomen down to, but not including, peritoneum and the transplant was placed in this incision. The wound was closed with two interrupted silk sutures. These procedures were carried out under light ether anæsthesia. Following this, three mice were killed at intervals of 24, 48, 72, 96 and 120 hr. Sections of the wound and tumour were taken for routine histological and autoradiographic examinations. Tissues were fixed in 10 per cent buffered formalin. Autoradiographs were prepared with '*AR* 10' stripping film and were exposed for four weeks prior to development.

After 24 hr. a marked acute inflammatory reaction was seen at the site of implantation. Roughly 20 per cent of the infiltrating neutrophiles and mononuclear cells were labelled. Areas of karyorhexis were present and showed extracellular radioactive material. Tumour cells did not contain any label at this time. A few fibroblasts were labelled.

Sections of tissue taken after 48 and 72 hr. also showed marked acute inflammation with an abundance of labelled leucocytes; there was more karyorhexis and considerable extracellular label over nuclear debris. Most important, a small number of tumour cells showed 5–10 grains over their nuclei. After 96 and 120 hr. fairly large nodules of tumour tissue were seen with focal inflammation and adjacent areas of fibroplasia. The percentage of labelled leucocytes was less than that seen earlier. After 120 hr. the greatest degree of labelling in tumour cells was seen, although only a minority of tumour cells were labelled even at this time; most of the labelled tumour cells were of a less bizarre type and were scattered throughout the tumour (Fig. 1). A large number of proliferating fibroblasts showed moderate labelling.

This work indicates that some constituent of DNA is liberated from dead leucocytes in an area of surgically produced inflammation which is then incorporated into proliferating tumour cells and probably into fibroblasts. The precise nature of the component of DNA thus liberated and the extent to which it is re-utilized cannot be determined on the basis of the present observations. In view of recent investigations concerning the incorporation of tritium-labelled DNA into HeLa cells in tissue culture, the possibility exists that relatively large fragments of the DNA molecule can be utilized by a growing cell population[7].

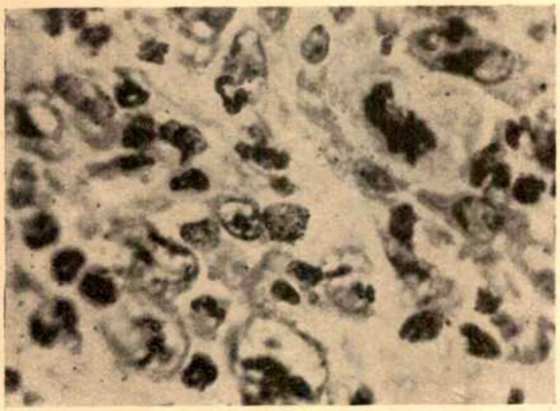

Fig. 1

Previous observations made in connexion with studies of normal wound healing have led to the speculation that availability of DNA or its breakdown products from areas of inflammation may influence the growth-rate of tumours. DNA components from leucocyte nuclear debris may be regarded as constituting a temporary metabolic pool. This work has shown that, locally, transplanted tumour cells and probably proliferating fibroblasts can incorporate the DNA components from this pool.

Previous work on the incorporation of tritiated thymidine by fibroblasts, ascites tumour cells, and osteoblasts has been reported; but in all these studies thymidine was injected simultaneously or after the wounding or tumour transplantation and therefore the probability of primary or direct incorporation of the labelled material by the fibroblasts or tumour cells makes any conclusions about re-utilization of DNA unfeasible[8-10]. Attempts at demonstrating re-utilization of labelled adenine by sarcoma 180 cells transplanted into mice by a trochar have met with limited success and have led others to the view that re-utilization does not occur "to any great extent"[11]. However, results supporting this conclusion were obtained from analysis of tissue homogenate and is difficult to evaluate on a cellular basis.

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Intracellular Phase of Existence of Lymphocytes during Remission of Acute Lymphatic Leukæmia

THAT lymphocytes may be found inside other larger cells is now well established[1,2]. In these laboratories this phenomenon has been observed in material examined by tissue culture methods from many patients suffering from Hodgkin's disease and the other malignant reticuloses. Further work[3] has shown that in certain cases of acute leukæmia, the leukæmic cells may be difficult to maintain alive by themselves but can be maintained for long periods in association with established living cell sheets, for example, thyroid cells or embryonic tissues.

A feature of acute leukæmia in children is the occurrence of remissions during which it may be impossible to detect any sign of the disease process by conventional diagnostic methods. In one such case, a boy aged four, in remission from acute lymphatic leukæmia, bone marrow was examined by the 'slide-cell'[4] technique as well as by conventional stained films. The films showed merely a very cellular marrow with an excess of nucleated red cells (Fig. 1). The 'slide-cell' preparation after 24-hr. incubation confirmed this finding, but after 48 hr. when the dying cells had been washed away on replacing the culture medium many large reticulo-endothelial macrophages were found each containing many small active lymphocytes (Fig. 2). It is possible that an intracellular phase of the leukæmic cells may therefore occur during the remissions of acute leukæmia.

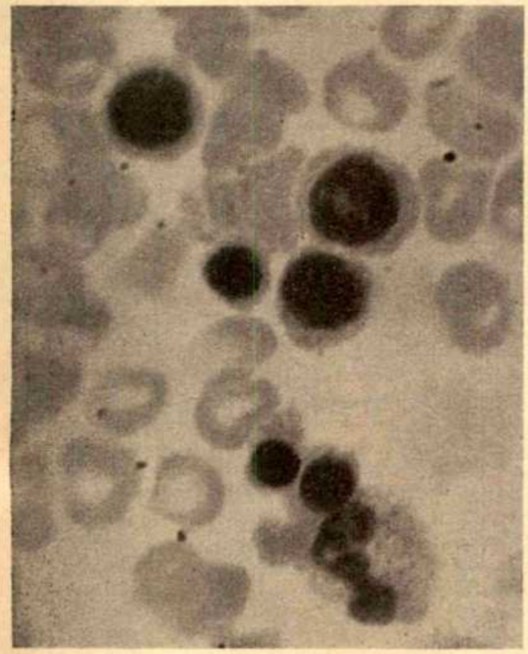

Fig. 1. Bone marrow film. (× 1,250)

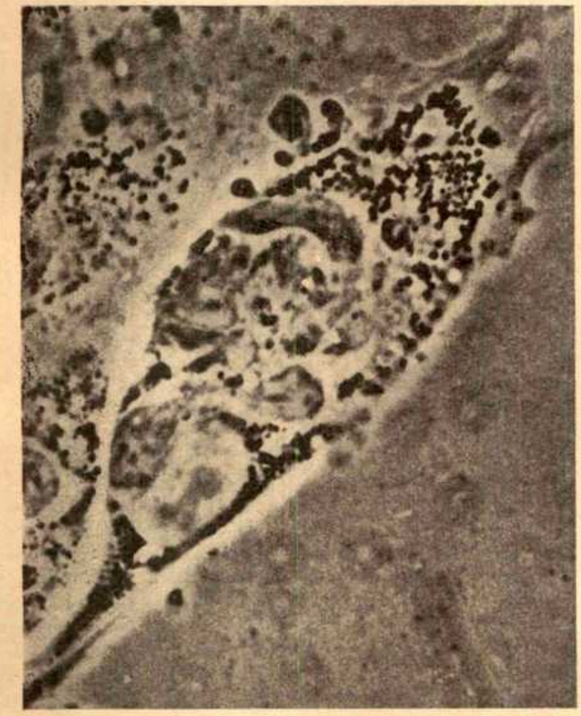

Fig. 2. Slide cell culture. Giant macrophage containing four lymphocytes. (× c. 1,300)

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Glucose-6-Phosphatase, Glucokinase and Selenium

Rats fed a diet containing yeast as the sole source of protein—the 'necrogenic yeast diet' of Lindan and Himsworth[1]—develop, after a latent period of varying length, a fatal liver necrosis. While the phenomenon itself is well known and has been extensively studied, there is little information available concerning the changes which take place during this period of latency, and which culminate in the rather sudden onset of liver necrosis. It is, therefore, of interest to see that Schwarz, who has extensively studied the problem of dietary liver necrosis, considers that there exists, in the animal fed the necrogenic yeast diet, a "lack of glyconeogenesis"[2].

In recent years it has been shown[3-5] that selenium, added in trace amounts to the necrogenic yeast diet, prevents the onset of liver necrosis, probably by relieving a true deficiency[6]. If, therefore, the defect in gluconeogenesis in the rat liver is in any way causally associated with the phenomenon of liver necrosis, it is reasonable to assume that the necrosis-preventing effect of selenium would be reflected in, or paralleled by, changes in gluconeogenesis. Since it has become apparent that alterations in the rate of gluconeogenesis by the liver are often associated with corresponding alterations in the activity of glucose-6-phosphatase[7], we considered it of interest to study the effect of supplements of selenium on the activity of this enzyme in the liver of rats fed the necrogenic yeast diet, and to combine this investigation with a simultaneous study of the liver glucokinase of these experimental animals. Although little work has as yet been carried out on changes in the activity of the latter enzyme, due to the difficulties associated with its assay, it might be expected that alterations in the activity of glucokinase would be associated with gluconeogenesis, in the sense that an increase in gluconeogenesis would be associated with a decrease in glucokinase activity. Di Pietro and Weinhouse[8] have, for example, shown that the activity of liver glucokinase was decreased in the starved rat, and in rats made diabetic by alloxan. These considerations, therefore, prompted us to undertake the following experiment.

Male Wistar rats, weaned on the twenty-first day of life, the first day of the experiment, were fed the necrogenic yeast diet of Lindan and Himsworth[1]. On the eighteenth day of the experiment the animals were divided into two groups each. One group was given the necrogenic yeast diet as before, but their litter-mates were now fed the necrogenic diet supplemented with sodium selenite at a level of 0·00002 per cent. All animals were killed six days later by a blow on the head. The livers were rapidly removed, weighed, minced, and aliquot portions were taken for determinations of total nitrogen, glucose-6-phosphatase activity and hexokinase activity. Total nitrogen was determined by digestion according to Kjeldahl, followed by Nesslerization. The glucose-6-phosphatase was assayed by measuring the release of inorganic phosphate on incubation of glucose-6-phosphate and of the liver homogenate at a *p*H of 6·5, at a temperature of 37° C. Glucokinase was assayed by a method, similar in principle to the method of Di Pietro and Weinhouse[8], the details of which will be published at a later date[9]. It is sufficient to say that a homogenate of liver was incubated for 30 min. at 37° C. to decrease endogenous glucose-6-phosphate, and then centrifuged to remove coarse particles. The supernatant was incubated at 37° C. with a mixture of *tris* buffer (*p*H of 7·4), magnesium chloride, glucose, adenosine triphosphate, a large excess of glucose-6-phosphate dehydrogenase and triphosphopyridine nucleotide (TPN). The glucokinase present in the liver supernatant catalyses the production of glucose-6-phosphate which, under these conditions, is immediately oxidized by the TPN. The reduction of TPN was followed by means of a spectrophotometer and the rate of this reaction was taken as a measure of the glucokinase activity. In the present method for the assay of glucokinase, the necessity of high-speed centrifugation of the liver homogenate, recommended by Di Pietro and Weinhouse[8], was obviated by the presence of large amounts of glucose-6-phosphate dehydrogenase. The comparatively small amounts of glucose-6-phosphatase present in the liver supernatants could be shown to produce only negligible errors in the determinations of glucokinase.

Table 1. EFFECT OF SUPPLEMENTS OF SELENIUM ON THE ACTIVITY OF GLUCOSE-6-PHOSPHATASE AND GLUCOKINASE IN THE LIVER OF RATS

Necrogenic yeast diet	Selenium	No selenium
No. of rats	8	9
Weight of rat (gm.)	44·4 ± 1·7	41·5 ± 1·1
Weight of liver (gm.)	2·47 ± 0·10	2·14 ± 0·07
Percentage of nitrogen in liver	2·53 ± 0·15	2·47 ± 0·07
Glucose-6-phosphatase μM./hr./mgm. liver nitrogen	20·5 ± 2·2	21·7 ± 1·3
Glucokinase μM./hr./mgm. liver nitrogen	2·72 ± 0·21	3·17 ± 0·14

The results of this experiment are presented in Table I. It can be seen from these data that the administration of selenium over a period of six days to rats previously fed the deficient diet for 17 days had little effect on the body-weight, the weight of the liver or the level of nitrogen, glucose-6-phosphatase or glucokinase of these livers. A lower level of glucokinase was found in the rats treated with selenium, but this difference is not significant at the 0·05 level. Similarly, no significant differences are found when the enzymatic activities are expressed per mgm. of liver (wet weight), or per 100 gm. of body-weight. It could be argued, of course, that both the period of depletion of, and the period of supplementation with, selenium may not have been sufficiently long to demonstrate clearly any possible effects. Since, however, the average survival-time of weanling rats on this necrogenic diet is only approximately 30 days, it is questionable whether a prolongation of either of these periods would have demonstrated an additional effect. In the light of the results presented here we, therefore, conclude that selenium, under the experimental conditions described, has no effect on the activity of glucose-6-phosphatase and glucokinase. While we cannot exclude the defect in gluconeogenesis stated by Schwarz to develop during the latent interval before the onset of liver necrosis, we have not been able to demonstrate this defect by the indirect means used here. The failure of selenium, however, to produce any changes in the enzymatic activities studied here suggests that its main effect in the prevention of liver necrosis probably will have to be looked for in other directions.

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IMMUNOLOGY

Congenital Syphilis and the Timing of Immunogenesis in the Human Fœtus

RESULTS obtained in the study of immunological tolerance have lent credence to the generalization that the mammalian fœtus is incapable throughout gestation of responding specifically to an antigenic stimulus. Data are accumulating, however, which suggest that in at least some mammalian species the fœtus may be able to mount an immunological response. Schinckel and Ferguson[1] demonstrated that the fœtal lamb is capable of rejecting adult skin homografts in a manner comparable to that of the adult sheep. Fennestad and Borg-Petersen[2] reported that fœtal calves infected *in utero* with *Leptospira* could be shown at birth to have a higher anti-*Leptospira* titre than their mothers. Uhr[3] has observed that fœtal guinea pigs are capable of an active production of delayed hypersensitivity against protein antigens. We have observed[4], following injection of antigenic stimuli into the fœtal lamb, the production of γ-globulin and of antibody by the normally agamma-globulinæmic fœtus. Finally, the older literature[5] contains a number of references to the occurrence in congenital syphilis of Wassermann titres higher in the new-born's serum than in that of the mother, and to positive Wassermann titres in the child's serum in the presence of negative maternal serology. We may anticipate that future investigations will establish the earliest time of appearance of immunological differentiation in some of these species.

Data on the timing of immunogenesis in man will be more difficult to attain. It has generally been considered that the new-born human reacts poorly, if at all, to the common pædiatric immunizations, although this has been open to question[6]. On the morphological level the absence of plasma cell development in the human fœtus[7] and in the normal new-born[8] has been cited as evidence for immunological immaturity at these age periods. Bridges *et al.*, in an examination of the normal offspring of an agamma-globulinæmic mother, have shown that only after the second month of life did the new-born produce antibodies and γ-globulin, with the concurrent appearance of plasma cells. I would make the point, however, that a functionally and anatomically immature lymphoid system and the absence of plasma cells in the human fœtus may represent the high state of protection from antigenic stimulus conferred on the fœtus by the placenta, and may reflect the time required to mobilize a response to the first stimuli rather than being indicative of the existence of the fœtus in an immunological 'null' period.

Lukes and I have recently had the opportunity[9] to re-examine the histopathology of congenital syphilis in the human stillborn fœtus, and observed a rather consistent pattern of mature plasma cells and plasmacytoid cells associated with the inflammatory lesions in the fœtus. The youngest case in which we had an opportunity to observe good plasma cell development was in a 1,210-gm. stillborn. Based on recent concepts of the immunological role of the plasma cell in response to antigenic stimulus, it seems not unreasonable to conclude that the stimulus provided by the *Treponema pallidum* in these fœtuses has induced the morphological analogue of an active immunological response.

The morphological response of the human fœtus to stimulus during congenital syphilis infection may not only furnish an indication of fœtal ability to respond immunologically but may also provide a clue to the timing of immunogenesis in the fœtus. Such data will not be obtainable experimentally, and will have both practical and theoretical interest.

It is generally agreed that congenital syphilis does not occur much before the sixth month of gestation. Table 1 indicates the age incidence of stillbirth from diagnosable congenital syphilis. It has been considered that this age distribution is attributable to the development of the ability of the placenta to transmit the organism from mother to fœtus. Such transplacental transmission has been assumed to be impossible during the earlier stage of gestation. The incidence of congenital syphilis with gestational age may be indicative, however, of the timing of development of fœtal ability to respond to stimuli. A fœtus younger than 5 or 6 months might not yet have matured its ability to respond to a stimulus with the production of antibody, of γ-globulin, or with the corresponding morphological analogue of a mature reticulo-endothelial system able to mount a chronic inflammatory response, and able to differentiate plasma cells. In the absence of its ability to respond to the *Treponema*, early infection might not induce sufficient embarrassment to cause death and expulsion of the fœtus. (It is conceivable that a heavy infestation of the tissues by *T. pallidum* during early fœtal development may result in an interference with normal parenchymal differentiation without inflammation, which may account for the interstitial fibrosis of the liver, for example (Smetana, H. F., personal communication). Only after the genesis of fœtal ability to respond to the treponemal stimulus would one see the inflammatory response, as an attempt to end the previously unmolested existence of the invading pathogen within its tissues. Stowens[10], in fact, cites the rare instance of very young fœtuses showing positive stains for *Treponemae* in the absence of inflammatory response—a very suggestive observation.

Table 1. AGE INCIDENCE OF STILLBIRTH WITH CONGENITAL SYPHILIS *

Gestational age (month)	Incidence (per cent)
5	1·6
6	7
7	20
8	24
9	22
10	19
Term	5·3

* Data taken from Rietschel (ref. 11).

Concomitant with the response of the fœtus there might develop active lesions suggestive of a mechanism for host embarrassment and death. The appearance of plasma cells in association with the inflammatory response of the fœtus suggests a relationship between the timing of specific immunogenesis and the maturation of other less specific participating reticuloendothelial elements. We may, therefore, consider the possibility that the earliest occurrence of inflammatory response and plasmacytosis in congenital syphilitic fœtuses does not represent the timing of initial infection of the fœtus, that is, of the placental transmission of the organism. Rather, this may be an indication of the timing of maturation of fœtal ability to respond to the stimulus with differentiation of immunologically active cells.

From the available data we may tentatively assign approximately the sixth month of gestation as the time of observable maturation of host response and of immunogenesis. I would emphasize two points: first, that at least the final stages of immunological differentiation may proceed only in response to an exogenous stimulus, and secondly, that stimuli may plan a 'non-specific' part in the induction of a more mature lymphoid tissue able to provide a more fertile ground and a greater supply of undifferentiated stem cells on which the specific antigenic stimulus may then operate.

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Antibody Production *in vitro* by Means of a New Perfusion Chamber

THE *in vitro* production of antibodies by explanted tissues or cells has been demonstrated by numerous workers. With a few exceptions, most of the reports have utilized qualitative or semi-quantitative methods to assay the antibody-levels. In those studies in which the investigators were able to measure the amount of antibody produced, either by isotopic techniques[1–3], or quantitative precipitin assay[1,4], the production of antibodies *in vitro* was of relatively short duration.

This communication describes a perfusion apparatus encompassing Trowell's principle of maintaining the tissues near the surface of the medium[5] as well as certain aspects of Steiner and Anker's chamber[4], in

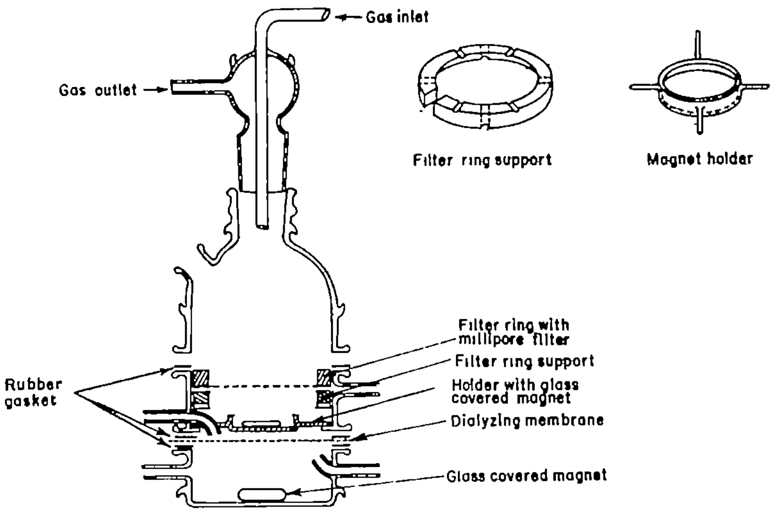

Fig. 1. Cross-section of the perfusion chamber and inserts

which the dialysable metabolic products were constantly removed.

The perfusion apparatus (Fig. 1) is divided into three chambers: (1) Upper chamber: various environmental gas phases can be maintained and the gas mixtures can be regulated to flow at any desired rate through the chamber. (2) Middle chamber: this contains a well consisting of a plastic ring to which is cemented a 'Millipore' filter. Tissues are explanted on the filter within the well. The pore size of the filter can be regulated so that the cells are contained but the fluids can pass freely to and from the culture. The well is supported by a glass ring which maintains the culture near the surface of the medium. Medium can be pumped through the chamber at any desired rate and mixed continuously by means of a glass-enclosed magnet. (3) Lower chamber: the lower chamber is separated from the middle chamber by a dialysing membrane. Medium of the same composition flows through this chamber at an independently regulated rate and is mixed by a stirring magnet. The apparatus can be autoclaved without the Millipore filter portion, which is sterilized by ultra-violet and inserted aseptically.

Advantages of this apparatus are that a relatively large amount of cells or tissues can be maintained in a functioning state for long periods of time. The culture can be supported near the surface of the medium; dialysable and non-dialysable metabolic products removed at any desired rate; and a given concentration of nutrients maintained within the chamber. Moreover, it offers a dynamic system for the study of physical and chemical influences on cellular metabolism and growth.

Table 1. Composition of Medium

1-Amino-acids	Mgm./l.	Carbohydrates	Mgm./l.
Arginine	168	Glucose	4,500
Cystine	96		
Glutamine	293	Salts	
Histidine	84	NaCl	6,800
Isoleucine	210	KCl	400
Leucine	210	$CaCl_2$	200
Lysine	292	$MgSO_4.7H_2O$	200
Methionine	60	$Fe(NO_3)_3$	0.1
Phenylalanin	182	$NaH_2PO_4.H_2O$	138
Threonine	190	$NaHCO_3$	3,700
Tryptophan	32		
Tyrosine	145	Miscellaneous	
Valine	187	Penicillin	0 5
Vitamins		Phenol red	20
Choline chloride	4	Insulin	1 82
Nicotinamide	4	Homologous rabbit serum (28 per cent)	
Calcium pantothenate	4		
Pyridoxal hydrochloride	4		
Riboflavin	0·4		
Thiamine	4		
-Inositol	7		
Folic acid	4		

The use of this apparatus is illustrated in the following experiment in which antibody was produced in sufficient concentration so that quantitative precipitin tests could be used for antibody estimation.

An adult rabbit was immunized with hæmocyanin (*KLH*) obtained from the giant keyhole limpet. The animal was immunized by intravenous injections spaced two months apart. After an additional rest period of three weeks, the rabbit was given 10 mgm. *KLH* intravenously on two consecutive days and was killed two days after the last injection. The spleen was dispersed in 20 ml. of a modified Eagle's medium (Table 1) using a stainless steel screen, centrifuged at 1,000*g* for 3 min., and resuspended in the modified Eagle's medium; 0·9 ml. of packed spleen cells were explanted in the middle chamber, which contained 40 ml. of the medium. Incubation was at 37° C. in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. Changes of fluid were made at daily intervals (except the ninth day when two samples were taken) by removing 10 ml. of medium and immediately adding 10 ml. of fresh medium. Approximately 50–75 ml. of medium flowed through the bottom chamber each day.

Microquantitative precipitin tests were carried out using a modification of the method described by Lanni and Campbell[6]. The precipitin tests were made at *p*H 6·5 using a cacodylate buffered saline ($\mu = 0{\cdot}15$) as diluent[7].

The calculation of net cumulative amount of antibody was made using the equation:

$$AB = V_t C_n = \sum_{i=1}^{n-1} V_{si} C_i$$

AB, net cumulative amount of antibody; V_t, total volume in middle chamber; C_n, concentration of antibody on day '*n*'; V_s, volume of sample removed.

The results (Fig. 2) demonstrate a rapid rise in the amount of antibody in the chamber during the first four days, a levelling off during the next four days, and then a decrease in both the antibody concentration in the chamber and the net cumulative amount of antibody. Precipitating antibodies were demonstrable for 13 days. The net total production of antibody approximated 4·5 mgm. of protein per ml. of packed spleen cells. The possibility that fluctuations of the antibody-levels are the result of degradation or inactivation of the antibody produced is under investigation. Further work is also in progress endeavouring to establish optimal conditions for antibody production utilizing the perfusion chamber.

This investigation was supported in part by the National Institutes of Health, U.S. Public Health Service.

I thank Dr. Dan H. Campbell, in whose laboratory the apparatus was devised. I also thank Drs. J. S.

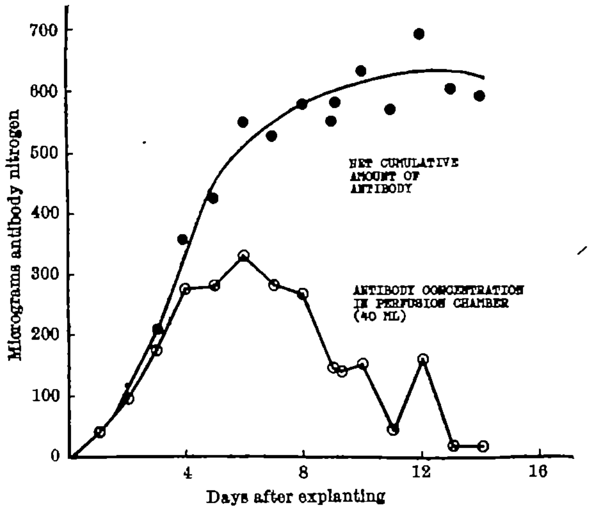

Fig. 2. Plots of the net cumulative amount of antibody and the antibody contained in the perfusion chamber at various time-intervals after explanting spleen from rabbit immunized with keyhole limpet hæmocyanin

Garvey, I. L. Trapani, and R. T. Jordan for the discussions during this work.

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Use of a Non-persisting Dye for demonstrating Local Anaphylaxis

The introduction by Ramsdell[1] of the use of vital dyes for demonstrating increase in capillary permeability in local anaphylactic reaction opened up a whole new field of investigation of this phenomenon in experimental animals[2,3]. The characteristics required in dyes for such purpose are low toxicity, retention within the blood vessels for a reasonable period and the ability to diffuse out with the blood plasma when a local increase in capillary permeability permits escape of the latter into the tissues.

Emphasis heretofore in local anaphylactic studies has been on dyes which persisted in the animal body for considerable periods of time. Such dyes, however, diffuse through the capillary walls in a matter of hours to produce an undesirable, persisting coloration of the entire skin of the animal.

We have now investigated the use of a non-persisting dye, 'Coomassie Blue', in cutaneous and pinnal[4,5] anaphylaxis. This dye does not produce generalized skin coloration, and we have found it to present distinct advantages over persisting dyes for certain types of investigations. At the present time we wish to report the characteristics of the dye and make available injection schedules for mice and guinea pigs.

The dye we have found suitable for injection is 'Coomassie Blue Medical' (Imperial Chemical Industries, Ltd.). This we have made up in saline as a 1·75 per cent solution for use in guinea pigs, the dosage being 0·1 ml. per 100 gm. body-weight intravenously, or 2·0 ml. per 350–400 gm. animal intraperitoneally. For mice we have found a 0·5 per cent solution of coomassie blue in saline more practical. For an 18–25-gm. mouse 0·15 ml. is used for intravenous injection, or 0·25 ml. for intraperitoneal injection.

Intravenous injections are given simultaneously with the challenging antigen, where sensitization has been passive and local. Where sensitization is systemic, either passive or active, dye is administered 15 min. after challenge when given intravenously, or 15 min. (guinea pigs) or 30 min. (mice) before challenge when given intraperitoneally.

When used in this way 'Coomassie Blue' produces a readily discernible blue-mauve coloration at the sites of local anaphylactic reaction. These locally coloured areas begin to fade within 30–60 min. after appearing and within 3–6 hr. of administration the dye has completely disappeared from the animal—both systemically and from the local sites of plasma-dye extravasation.

Thus, with 'Coomassie Blue' there is no persistence of the locally coloured sites and no gradual generalized coloration of the skin, such as is the case with persisting dyes. Consequently, the same animal may be used repeatedly for investigations where a sequence of anaphylactic events is under observation.

We are applying 'Coomassie Blue' to such investigations as the rate of development of anaphylactic sensitization, the influence of a prior challenge on the anaphylactic reactivity of a sensitized site and the better visualization of both immediate and delayed local anaphylactic reactions in the same animal.

We thank Dr. G. E. Davies, of Imperial Chemical Industries, Ltd., for supplying us with the dye and for useful background information on it.

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RADIOBIOLOGY

Spontaneous Agglutination and Hæmolysis in Acidified Serum of Erythrocytes from Irradiated Mice

During experiments concerning another problem, we have repeatedly observed that suspensions of washed erythrocytes from irradiated mice are unstable and agglutinate spontaneously. Since this defect in the stability of erythrocytes in suspension might be one of the causes of post-irradiation anæmia, we examined this characteristic further.

Thirty mice of strain *H* males with an average weight of 25·8 gm. were totally X-irradiated with a dose of 550 r. at the rate of 60 r./min. (180 kV., 15 m.amp., filters 1 mm. aluminium and 0·5 mm. copper, target distance 45 cm.). Blood was drawn from the tail vein into a 2 per cent sodium citrate-saline solution 24 hr. prior to irradiation and then at 2–3 day intervals. The erythrocytes were washed four times in saline solution buffered with 0·01 *M* phosphate to *p*H 7·0, which was used throughout. 0·1 ml. of 2 per cent suspension of washed erythrocytes in saline was added to 0·1 ml. of the saline solution. Each sample of erythrocytes was incubated in three test tubes for 2 hr. at 37° C. and in another three tubes for 4 hr. at 4° C. The degree of agglutination was evaluated with the naked eye in the following manner: the fine clumps of erythrocytes coating the whole hemisphere of the test-tube bottom were marked with three crosses, and the clumps coating only a small area of the bottom with a compact central button-shaped sediment with one cross. The button-shaped sediment without any coating of the test-tube bottom means that agglutination did not take place. The agglutination was, at the same time, examined microscopically. The results are expressed, on one hand, as a mean degree of agglutination (Fig. 1), and on the other hand, as a percentage of mice (from the total number of mice) with a minimum agglutination of one cross (Fig. 2).

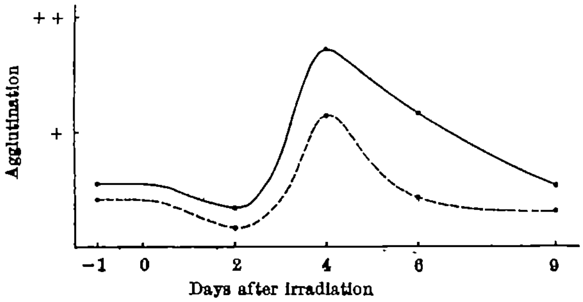

Fig. 1. Mean degree of agglutination of erythrocytes in mice prior to irradiation and at different intervals after irradiation with 550 r. (—, 37° C.; – – –, 4° C.)

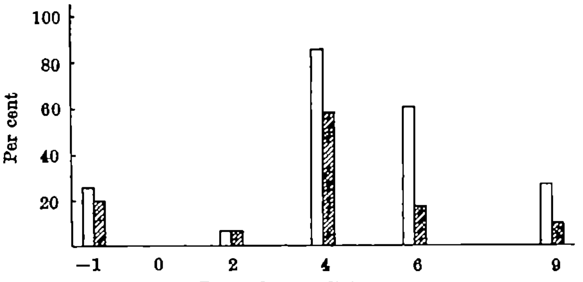

Fig. 2. Frequency of spontaneous agglutination of erythrocytes in mice prior to irradiation and at different intervals after irradiation with 550 r., expressed as a percentage of mice (from the total number of mice) with a minimum agglutination of one cross. White, 37° C.; shaded, 4° C.

The tendency of erythrocytes to agglutinate spontaneously in a saline medium reached its maximum on the fourth day after irradiation. In this series of mice, agglutination was more intense and frequent at 37° C. than at 4° C., both prior to irradiation and at all following intervals. However, in the next series of mice irradiated with 700 r., agglutination was more intense and frequent at 4° C. Hence it follows that spontaneous agglutination of erythrocytes after irradiation is not dependent on temperature within the range of 4°–37° C.

When erythrocytes from irradiated or non-irradiated mice are incubated under similar conditions in normal mouse serum, no agglutination occurs at serum dilutions lower than 1 : 1,024. Consequently, spontaneous agglutination of erythrocytes is blocked by the protein medium or by some inhibitor in the normal serum. The presence of incomplete auto-antibodies on the surface of erythrocytes was excluded by means of a direct Coombs's test. Antiglobulin serum was obtained by immunizing a rabbit with the whole mouse serum. The direct Coombs's test with erythrocytes from both irradiated and non-irradiated mice was completely negative in all cases tested.

Apart from the agglutinability of erythrocytes from irradiated mice, we have also established their marked sensitivity to the hæmolytic effect of normal mouse serum acidified with 10 per cent by volume of 0·2 *N* hydrochloric acid. Titration was performed by doubling the serum dilutions up to 1 : 16,384 using normal saline as diluent; one volume of test cells was then added and incubated for 2 hr. at room temperature (22° C.). The erythrocytes of mice irradiated with 550 r. 14 days previously became regularly hæmolysed in dilutions from 1 : 256 upwards in serum from both irradiated and non-irradiated mice. On the other hand, hæmolysis of erythrocytes from non-irradiated mice occurred sporadically in the highest serum dilutions.

From these experiments it follows that some changes occur on the surface of erythrocytes after irradiation. Consequently, these changes are the cause of their spontaneous agglutination in saline solution and of their hæmolysis in acidified normal serum. The abnormal erythrocyte surface might result in intravascular hæmolysis, the occurrence of which has been already assumed by Davis *et al.*[1] and especially by Stohlman *et al.*[2]. In my opinion, apart from the cessation of erythropoiesis and loss of erythrocytes by hæmorrhage, a significant role is to be attributed to hæmolysis of erythrocytes in the pathogenesis of post-irradiation anæmia.

I thank Dr. Z. Hill, of the District Blood Transfusion Centre at Třebíč, for the preparation of antiglobulin serum and for his valuable discussion comments.

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Relative Availability of Strontium in Cereals and Milk

McCance and Widdowson[1], and later Anderson and Hoff-Jørgensen[2], have shown that the phytic acid of certain cereals may reduce the absorption of calcium from the gastrointestinal tract by the formation of insoluble calcium phytate. If strontium behaves like calcium in this respect, the absorption of strontium-90 from cereal sources in the diet may be less than that from milk which does not contain phytic acid. An experiment has been carried out to test the relative availability of strontium from wholemeal flour and milk using normal subjects from this Unit.

Three male adults (aged 38–56) took a diet of constant composition for 28–32 days. The diet (about

Table 1. Composition of Diet

	Mean consumption per day	Strontium-90 ($\mu\mu$c.)	Strontium-85 (μc.)
Milk	1,100 ml.	5·7	0·5
Bread	440 gm.	66	nil
Butter	60 ,,	0·7	,,
Jam or honey	100 ,,		,,
Bacon	60 ,,		,,
Tinned fruit, pears, pineapple, peaches	180 ,,		,,
Orange juice*	300 ,,		,,
Beer*	570 ml.		,,

* Optional extras + tea and/or coffee infusions with distilled water.

3,000 calories) is shown in Table 1. The wholemeal bread was made from grain which contained about 10 times as much strontium-90 as current British flour. It was in fact grown on soil which has been deliberately contaminated for experimental purposes. The milk was obtained from a cow which, a short time before milking, had been given an intravenous injection of strontium-85, a 65-day γ-emitting isotope of strontium. The strontium-90 was assayed on an anti-coincidence β-particle-counter and the strontium-85 on a well-type γ-scintillation-counter. The interference of the radiation from one isotope on the radioactive assay of the other was negligible. The relative absorption of strontium from the milk and flour could thus be measured by determining the ratio:

$$\left(\frac{\text{strontium-90}}{\text{strontium-85}}\right) \text{urine} \Big/ \left(\frac{\text{strontium-90}}{\text{strontium-85}}\right) \text{diet}$$

The strontium-85 activity (about 0·5 μc. per day) was derived entirely from the milk. On the other hand, the strontium-90 in the diet was not wholly derived from the special bread. The total daily intake of strontium-90 was about 70 $\mu\mu$c., some 10 per cent of which came from other components of the diet, chiefly the milk.

Table 2. Comparison of Availability of Strontium from Bread and Milk

Week	(Strontium-90/Strontium-85) urine/(Strontium-90/Strontium-85) diet J. F. L.	G. E. H.	T. E. F. C.
1	0·88	0·98	0·74
2	0·75	1·20	0·69
3	0·83	0·95	0·72
4	1·26	1·10	0·75
5	0·84	1·10	0·68
Mean	0·91	1·06	0·71

Overall mean and standard error 0·90 ± 0·05.

The results are shown in Table 2. Urine was bulked over seven-day periods. During the first four weeks the diet was as in Table 1. During the fifth week the diet was of the same composition except that the milk contained no strontium-85 and the bread much less strontium-90 since it was baked from a commercial British flour of the same calcium content as the more active grain.

It will be seen that the mean ratio of:

$$\left(\frac{\text{strontium-90}}{\text{strontium-85}}\right) \text{urine} \Big/ \left(\frac{\text{strontium-90}}{\text{strontium-85}}\right) \text{diet}$$

ranged from 0·71 to 1·06 for the different subjects while the overall mean and standard error is 0·90 ± 0·05. Even although about 10 per cent of the strontium-90 in the diet came from non-cereal components, it may be concluded that the strontium in wheat and milk in human diet is approximately equally available.

We thank the Director and staff of the Radiobiological Laboratory, Agricultural Research Council, Wantage, for the supply of the grain used in this experiment and the Director and staff of the Field Station, Agricultural Research Council, Compton, for the supply of the radioactive milk.

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BIOLOGY

A Late-Glacial and Post-Glacial Climatic Correlation between East Africa and Europe

The classical work on the Quaternary chronology in Africa has been carried out in East Africa by geologists, palæontologists and archæologists. The general scheme of a sequence of pluvial and non-pluvial periods which had been developed was later on widely adopted in all parts of Africa. Revaluation of the factual evidence and warning against uncritical acceptance of the East African pluvial chronology, especially from geological quarters[1–3], led to the conclusion that there existed in East Africa evidence for at least one major pluvial, the Upper-Pleistocene Gamblian pluvial.

Botanical evidence has so far not been forthcoming from the East African area, but some palynologists are at present engaged in the analyses of a number of pollen containing cores from Ruanda-Urundi, Uganda, Kenya and south Tanganyika. I am carrying out my work mainly in the last two areas and have made several borings in Lake Naivasha, Lake Narasha, and in swamps and tarns on the Cherangani Hills and Mount Kenya. The preliminary results of a boring at Kaisungor in the Cherangani Hills open a new perspective for the study of the climatic chronology in East Africa and are described shortly. A more detailed description will be published at a later stage[4].

The Cherangani Hills form part of the western rim of the Rift Valley in north-west Kenya. The boring was made in a swamp at Kaisungor situated in a valley head at ± 1° N. lat. and 35° 28′ E. long. at 9,600 ft. (292 m.) altitude.

The highest point in the Kaisungor area is 10,000 ft. (3,048 m.). The part around the swamp is at present covered by montane conifer forest. The tree line in the Cherangani Mountains is found at 10,500 ft. (3,200 m.). Under natural conditions the summits would probably be covered by bamboo forest mixed with trees of the highest forest. Under undisturbed conditions a zone of high shrubs of *Stoebe kilimandscharica* occurs just above the forest on several of the East African mountains. Tall Compositae shrubs are often found in great numbers in the lower part of the ericaceous belt above the forest. On the eastern mountains in East Africa the ericaceous belt usually occurs between 9,842 ft. (3,000 m.) and 11,811 ft. (3,600 m.), but sometimes goes up as far as 13,123 ft. (4,000 m.) (ref. 5). The lowest level of this belt in the Cherangani Mountains is the tree line at 10,500 ft. (3,200 m.). The highest vegetation belt or Alpine

Table 1. TENTATIVE COMPARISON OF LATE- AND POST-GLACIAL CHRONOLOGY OF EAST AFRICA AND EUROPE

Age calculated according to sedimentation (B.C.)	Zones (see van der Hammen and Gonzalez, refs. 6 and 7)	Vegetation at Kaisungor	Climate at Kaisungor 2,926 m. altitude	Europe
700	VIII	Tree-line coming down	Wet, becoming colder	Subatlanticum
3,010	VII	Forest maxima	Warmer Colder	Subboreal
5,790	VI	Forest closed Tree-line rising	Warmer and wet Temperature rising	Atlanticum
6,675	V	Tree-line around the swamp	Warmer and relatively wetter	Boreal
8,040	IV	Open vegetation. Stoebe maximum Tree-line below swamp	More humid rising temperature	Preboreal
8,840	III	Tree-line low, grass maximum	Cold and dry	Younger Dryas time
	II	Compositae maximum	Warmer and dry	Allerød
	I	?	?	Older Dryas time
		?	?	Bølling
(*GRN* 8,048) 10,690 ± 100		Maxima of grasses and ericaceous belt	Cold and dry	Oldest Dryas time
		Alpine vegetation	Very cold and dry	

belt, which is often rich in tussock-grassland, does not occur in the Kaisungor.

At the site of the boring the swamp was 3·70 m. deep. The following pollen types have been included in the pollen sum: the forest trees, *Stoebe*, Compositae (which may include some swamp plants), the shrubs of the ericaceous belt (*Protea*, *Myrsine*, *Alchemilla*, etc.) and Gramineae.

As in the case of the Eastern Cordillera of Colombia (van der Hammen and Gonzalez[6,7]) a high percentage of Gramineae can be taken as an indication of a lower temperature. The grass pollen is principally produced by the open tussock-grassland of the ericaceous and Alpine belts.

The general trend of the pollen diagram can be interpreted as follows. The high percentage of grass pollen at the bottom of the diagram and the maximum of other plants of the ericaceous and Alpine belts above that indicate cold conditions as could occur at an altitude of at least 3,400 m. The maximum of Compositae between −2·70 and 2·45 m. indicate the approach of the tree line.

Another cold period between −2·45 and 2·30 m. is suggested by a grass pollen maximum. The forest types then show a definite rise which is in the beginning marked by a maximum of Stoebe. Optimal conditions of temperature and rainfall for the conifer forest of the higher altitudes seem to have existed between −1·70 and 1·15 m. Above −1·15 m. the forest gradually opens up. The climate apparently becomes less favourable.

The age of a sample from level −2·85–3·00 m., as measured at the Radiocarbon Station, Groningen, was found to be 12,650 ± 100 B.P. (*GRN* 3,048) (10,690 ± 100 B.C.): the Suess effect was taken into account in this determination. More radiocarbon dates will be obtained later. A provisional absolute time-scale has been worked out on the assumption that the annual growth of the peat deposits was approximately the same throughout the profile, namely, 0·231 mm. per annum. It then appears that the changes in temperature which occurred are in general contemporaneous with the climatic chronology of the Late- and Post-Glacial of Europe (Table 1).

The dated horizon (−2·85–3·00 m.) falls in the Oldest Dryas time of Europe and represents also a cold period in the Cherangani Mountains.

The Allerõd is well represented between the levels −2·49 and 2·72 m. (according to sedimentation 8,840–9,840 B.C.).

A more detailed analysis supported by more radiocarbon dates will be necessary to prove whether there existed an equivalent to the Bõlling Interstadial in East Africa.

In general Table 1 resembles the results obtained by van der Hammen and Gonzalez in the Andean mountains of Colombia and suggests very strongly that the climatic chronology of the Late- and Post-Glacial of Europe also applies to equatorial East Africa.

Conservatively estimated the tree line, during the Oldest Dryas time, was 500–600 m. lower than at present, which accounts for a drop in temperature of at least 3°–4° C. During the coldest phase of the Weichsel Glacial the temperature would have been

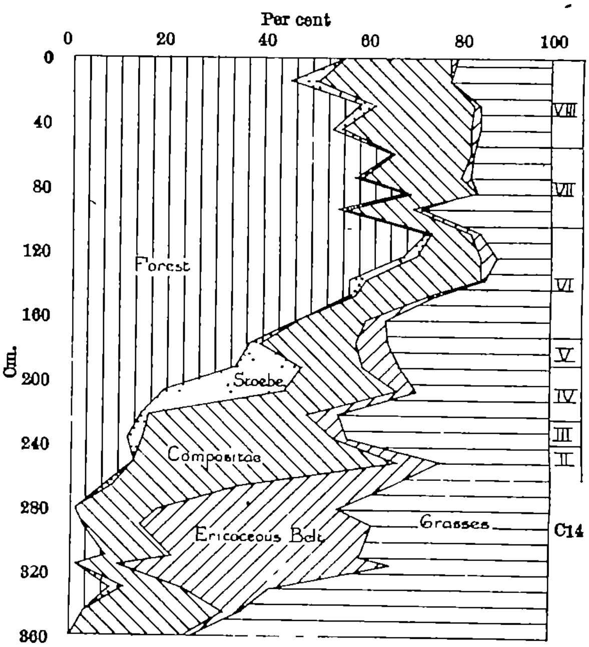

Fig. 1. Pollen diagram of Kaisungor. The Roman figures indicate the periods described by van der Hammen and Gonzalez (refs. 6 and 7)

appreciably lower. These changes in temperature occurred apparently on a world-wide scale (Flint and Brandtner[8], Emiliani[9,10], van der Hammen and Gonzalez[6,7]) and were contemporaneous. The implications on humidity, geological events, the life of man, animals and plants, etc., will have to be studied in great detail in order to obtain a better understanding of the Quaternary in Africa and of its so-called Pluvial periods.

I thank the South African Council for Scientific and Industrial Research for its continuous support of this research, and Prof. H. de Waard, of the Radiocarbon Station, at Groningen, for having the sample *GRN* 3048 dated.

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A Specific Stimulatory Action of Indolyl-3-Acetic Acid on Potassium Uptake by Plant Cells, with Concomitant Inhibition of Ammonium Uptake

THE evidence collected so far for an effect of growth-promoting concentrations of auxins on cation uptake by plant cells gives a somewhat confused picture. Auxins have been variously reported as promotive, inhibitory, or without effect, according to the conditions of the investigation[1-4]. I wish to report here experiments yielding clear evidence for a marked effect of indolyl-3-acetic acid (IAA) on potassium and ammonium uptake by segments of sunflower hypocotyl. In the case of potassium the effect is stimulatory, in that of ammonium, inhibitory.

Table 1. EFFECT OF 10^{-4} *M* INDOLYL-3-ACETIC ACID ON THE UPTAKE OF AMMONIUM AND POTASSIUM IONS BY SEGMENTS OF SUNFLOWER HYPOCOTYL

Treatment	Water uptake (percentage initial fresh weight)		Cation uptake (μequiv./gm. initial fresh weight) K^+		Cation uptake (μequiv./gm. initial fresh weight) NH_4^+	
− IAA	10·8	9 8	2·1	3 4	13·9	13 9
	9·4		3 6		13 1	
	9·3		4 5		14·6	
+ IAA	48 7	47 2	14·2	14·3	10 6	10·5
	46·4		14·2		10·5	
	46 5		14·5		10·3	

35 segments (approximately 1 gm.) in 5 ml. medium buffered at $pH = 6·5$ with K_2HPO_4, KH_2PO_4, $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$. Final concentrations: NH_4^+ and K^+ each $6 3 \times 10^{-3}$ *M*; phosphate 10^{-2} *M*. Uptake measured after 15·5 hr.

The experiments were carried out with etiolated, 5–9-day-old seedlings of *Helianthus annuus* var. Jupiter. 1-cm. segments were removed at a distance of 1 cm. below the crook and were washed for 6–8 hr. in running water, then rinsed in distilled water, blotted, weighed and transferred to Petri dishes containing 3–5 ml. solution buffered with phosphate. The cations under investigation were added to the solution as indicated in Tables 1, 2 and 3. Uptake was determined by analysis of the external solution, by flame photometry in the case of potassium, sodium and lithium, and by the Conway method in the case of ammonium. The IAA concentration used was such as to produce maximum stimulation of water uptake.

Table 1 gives details of a typical experiment where the uptake of K^+ and NH_4^+ was measured simultaneously. It will be seen that IAA brought about a large increase in K^+ uptake, while NH_4^+ uptake was depressed. Both effects were significant at the $P = 0·01$ level.

In order to ascertain whether the stimulatory effect was specific for potassium, or whether the uptake of other monovalent cations was similarly affected, further experiments were carried out comparing potassium uptake with that of sodium and lithium. The results of three such experiments, each performed in duplicate, are given in Table 2. The figures in Table 2 are for net uptake, as it may be assumed that, simultaneously with uptake, loss of cations from the tissue into the solution took place. It will be noted that in several cases loss was greater than uptake. Table 2 shows that the difference between control and IAA treatment was considerably bigger in the case of potassium than in that of lithium or sodium. An analysis of variance made on the three experiments treated together indicated that the stimulation of K^+ uptake was significant at the $P = 0·001$ level, the effect on Li^+ at $P = 0·01$, while the effect on Na^+ uptake was not significant even at $P = 0·2$.

Reinhold[6] observed a release of ammonium ions by IAA-treated tissue, an effect probably closely related to the inhibition of ammonium uptake reported here. She also mentioned, without quantitative data, a reduction in the loss of potassium to the solution in the presence of IAA. The influence of IAA on cation efflux into a solution initially free of the cation under consideration was therefore investigated. No significant effect of IAA on the loss of sodium could be detected. The previously reported

Table 2. EFFECT OF INDOLYL-3-ACETIC ACID ON THE UPTAKE OF POTASSIUM, SODIUM AND LITHIUM BY SEGMENTS OF SUNFLOWER HYPOCOTYL

		K^+ treatment			Li^+ treatment			Na^+ treatment		
Exp	IAA (concentration in *M*)	H_2O* uptake	K^+† uptake	Difference† between K^+ uptake of control and that of IAA treated	H_2O* uptake	Li^+† uptake	Difference† between Li^+ uptake of control and that of IAA treated	H_2O* uptake	Na^+† uptake	Difference† between Na^+ uptake of control and that of IAA treated
I	0	5·2	−4·9	9 4	5·8	4·1	2·5	6·0	−1 9	2·8
	5×10^{-5}	41·9	4·5		40·3	6·6		38·0	0·9	
II	0	3·7	−2 1	8 6	5·0	1·7	1·0	4·2	−3·1	0 7
	10^{-4}	37·1	6·5		32 7	2 7		36 8	−2·4	
III	0	5 1	−0·4	8 3	5 6	2·1	0 5	4·0	1·3	0 9
	10^{-4}	28·3	7 9		26·5	2·6		25 6	2·2	

* Initial fresh weight, per cent.

† μequiv./gm. initial fresh weight.

35 segments (approx. 1 gm.) in 4 ml. medium buffered at $pH = 6·5$. $5 \times 10^{-3}M$ ammonium phosphate buffer present in all cases , in addition KCl, LiCl or NaCl at a concentration of 5×10^{-3} *M*. Uptake measured after 18 hr. The figures are the mean of duplicates.

Table 3. EFFECT OF 10^{-4} *M* INDOLYL-3-ACETIC ACID ON THE RELEASE OF POTASSIUM BY SEGMENTS OF SUNFLOWER HYPOCOTYL INTO AN INITIALLY POTASSIUM-FREE MEDIUM

IAA concentration	0		10^{-4} *M*	
K+ released into solution (μequiv./gm. initial fresh weight)	1·4 1·5 1·2 1·3	1·4	0·3 0·3 0·3 0·2	0·3

50 segments (approximately 1·4 gm.) in 4 ml. medium. No buffer present. Potassium in external solution measured after 16 hr.

stimulation of ammonium release was confirmed. Table 3 gives results of a typical experiment in which the release of potassium was measured. The influence of IAA in diminishing potassium loss was significant at the $P = 0·001$ level.

In the present investigation, therefore, IAA had no significant effect on the passage of sodium either into or out of the cell; it had an inhibitory influence on the entry of ammonium and a stimulatory effect on its release, while the auxin had the opposite effect on the movement of potassium, that is, a stimulation of uptake and a reduction in release. The results thus reveal a relatively high degree of specificity in the action of IAA on cation movement. A hint of similar differential effects may perhaps also be seen in the results of Pickles and Sutcliffe[7], who noted a reduction in K^+ efflux from IAA treated beet disks under conditions where Na^+ uptake was depressed.

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Effects of Indolyl-3-Acetic Acid on the Movement of Nutrients within Plants

IT has been suggested that apical dominance and the 'correlative inhibition' of lateral buds may involve the diversion of nutrients towards actively growing regions, and that the role of auxin in correlative inhibition may be to stimulate the movement of nutrients towards meristematic regions[1], which are known to be centres of high auxin production. Investigations of the movement of metabolites within the plant using isotopically 'labelled' compounds have, indeed, frequently indicated that such substances tend to move out of mature leaves towards actively growing regions such as young developing leaves and fruits[2,3]; but no attempt has apparently been made to determine whether such movement is affected by the auxin content of the developing organs.

Radioactive compounds afford a ready means of investigating this problem and preliminary experiments with this object were carried out by two of us (A. B., J. M.) in the University of Manchester several years ago[4,5]. In one of these experiments, young plants of *Solanum andigena* were divided into three series (each of 10 plants) as follows: (1) untreated, intact plants; (2) plants decapitated in the first fully extended internode and the cut surface covered with lanolin; (3) plants decapitated and the cut surface covered with lanolin containing 200 p.p.m. indolyl-3-acetic acid. A drop of aqueous solution containing 0·2μ of 2 per cent ^{14}C-sucrose was applied to the upper surface of the 3rd or 4th mature leaf of each plant. After 6 hr. the plants were cut up to separate the apical region of the shoot of the intact plants, and the stumps of the uppermost internodes of the decapitated plants of series (2) and (3). These tissues were then dried and powdered. Solid samples (of equal weights) were prepared from the powdered material and placed on planchettes 2 cm.^{2} in area. These were counted at a distance of 2 cm. from the end-wall of a Geiger–Muller tube. The results are given in Table 1.

Table 1. EFFECT OF DECAPITATION, WITH AND WITHOUT APPLIED INDOLYL-3-ACETIC ACID, ON ACCUMULATION OF CARBON-14 IN THE TOP INTERNODE OF PLANTS OF *Solanum andigena*

Treatment	Activity (counts/min.) with *S.D.* *
(1) Intact plant	45·0 ± 2·6
(2) Decapitated plus lanolin	33·7 ± 2·3
(3) Decapitated plus IAA in lanolin	61·8 ± 2·9

* Standard deviation calculated according to Comar (ref. 6). (In this experiment, the parts from the 10 plants of each treatment were combined.)

It is seen that very much less radioactivity occurred in the stumps of the decapitated plants supplied only with lanolin than in the intact plants. When, however, indolyl-3-acetic acid was applied to the stumps after decapitation, the accumulation of radioactivity was greatly increased, although the period of the experiment extended over only 6 hr. These results were confirmed by other experiments involving autoradiography of whole plants. Similar results were obtained by Moorby[5] with phosphorus-32 applied to Meteor pea, using both autoradiography and direct counting of activity.

A further series of experiments has now been carried out by one of us (C. R. D.) which fully confirms the earlier experiments. Plants of Meteor pea bearing 4–5 expanded leaves were selected for uniformity and divided into three series, each of 10 plants. Two series of plants were decapitated, and 12 hr. after decapitation the cut stumps were covered with either (*a*) lanolin alone or (*b*) lanolin containing 1,000 p.p.m. indolyl-3-acetic acid. After 3 hr. a solution containing 1·0 μc./0·05 ml. of ^{14}C-sucrose was applied to one of the mature leaves of each of the decapitated plants and of the third series of intact plants. After a further 8 hr., the decapitated stumps and the corresponding internodes of the intact plants were removed, weighed and pulverized in liquid air. The tissue samples were extracted with 80 per cent aqueous methanol, and the filtered extracts were reduced to a small volume and spread evenly over the surface of planchettes. After drying, the radioactivity was counted with a proportional counter converter in association with a 'Panax' scaling unit. The activities are shown in Table 2, expressed as counts/min./100 mgm. fresh weight of tissue, after correction for 'background'.

Table 2. ACCUMULATION OF METHANOL-SOLUBLE CARBON-14 IN TOP INTERNODES OF INDOLYL-3-ACETIC ACID TREATED AND DECAPITATED PLANTS OF *Pisum sativum* VAR. METEOR

Treatment	Mean activity (c.p.m.) of 10 internode samples ± *S.E.* of mean
Intact plant	129·4 ± 27·7
Decapitated plus lanolin	47·7 ± 10·4
Decapitated plus IAA (1,000 p.p.m.) in lanolin	116·6 ± 25·7

In this experiment the samples from each plant were counted separately.

It is seen that the application of indolyl-3-acetic acid to the decapitated stump has resulted in a significantly greater accumulation of radioactivity than in the plants to which only lanolin was applied.

The foregoing results seem clearly to indicate that externally applied indolyl-3-acetic acid in some way stimulates the movement of substances towards the point of application. This conclusion is supported by the results of a variety of experiments involving Scots pine, birch and pea seedlings, in which autoradiography was mainly used[5]. Moreover, it was found that ^{14}C-sucrose moves not only into decapitated internodes, but also into mature leaves to which auxin has been applied externally.

It is possible that the effect of indolyl-3-acetic acid on the transport of substances within the plant is an indirect one, resulting from the stimulation of growth in the tissues to which it is applied; it is well known that nutrients tend to move towards actively growing regions in the normal plant, possibly because synthetic processes are proceeding actively in such regions, which thus constitute metabolic 'sinks'. It is also known that high concentrations of exogenously applied indolyl-3-acetic acid will stimulate the renewal of growth in differentiated tissues, such as the decapitated internodes used in the present experiments. The renewal of growth in differentiated tissue is not immediate, however, and it seems very unlikely that over the short period of the experiments (6–11 hr.) any appreciable growth had yet commenced, especially in the experiments in which indolyl-3-acetic acid was applied to fully mature leaves. Moreover, in further experiments (not described here), autoradiographs of extracts of the stumps of decapitated plants treated with indolyl-3-acetic acid, partitioned by paper chromatography, indicated that no new labelled compounds were present as compared with those found in the leaf to which the ^{14}C-sucrose was applied. Thus, it would seem that the effect of indolyl-3-acetic acid on transport of metabolites is not primarily to create a metabolic 'sink' by stimulating synthesis, but may be rather to stimulate active uptake and accumulation by the tissues to which it is applied. Zimmermann[3] has argued that the exchange of sugars between phloem and surrounding tissues may be analogous to the active secretion of sugars by nectaries, which is known to be stimulated by auxin[7].

This effect of auxin on the movement of substances within the plant is paralleled by the effect of kinetin on the movement and accumulation of amino-acids, sugars and inorganic ions when applied to differentiated leaf tissue, as observed by Mothes and his co-workers[8,9]. In the experiments with these latter substances and with kinetin, however, observations were made on movement within a single leaf, whereas in the present experiments with indolyl-3-acetic acid phloem transport over considerable distances was evidently involved. It would seem that auxin-directed transport may be important in the re-distribution of nutrient reserves from storage organs and older tissues to young growing regions, and may also play a part in apical dominance and the correlative inhibition of buds. It is possible that the phenomena reported here are also related to the effects of auxins on leaf-senescence, reported by Osborne[10].

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Role of the Shikimic Acid Pathway in the Formation of Tryptophan in Higher Plants: Evidence for an Alternative Pathway in the Bean

IT has been assumed generally, without adequate supporting evidence, that tryptophan and other indole compounds are generated via the shikimic acid pathway in higher plants. Evidence for this biosynthetic pathway has been derived mainly from microbial studies and from theories based on the unity of biochemistry[1-3]. Proof that shikimic acid is a precursor to the aromatic amino-acids, phenylalanine and tyrosine, has been clearly obtained by McCalla and Neish[4]. It has also been demonstrated that higher plants possess a mechanism to convert quinic acid to shikimic acid and phenylalanine and tyrosine[5-7]. The latter aromatic acids are assumed to be derived from prephenic acid, which is transformed to phenylpyruvic and *p*-hydroxyphenylpyruvic acids, and afterwards to phenylalanine and tyrosine, respectively[1]. Since anthranilic acid, a precursor to tryptophan, may be derived from a separate branch of the aromatic pathway in micro-organisms[8], one might expect a similar situation to exist in higher plants as well. Recently, Wightman *et al.*[9] have indeed reported that shikimate-U-^{14}C gives rise to tryptophan and gramine in young barley seedlings in the light. Similar investigations on the conversion of quinate-U-^{14}C and shikimate-U-^{14}C to tryptophan in bean seedlings in the light and dark have been under investigation in this laboratory.

Young trifoliate leaves of the bean (*Phaseolus vulgaris* L.) plants were supplied through cut petioles with 6 mgm. of shikimate- or quinate-U-^{14}C (specific activity approximately 35,000 c.p.m. per μmole) in 5 ml. of 0·01 *M* phosphate buffer, *p*H 6·75. After absorption of the labelled compounds and small aliquots of water, the leaves were placed in water in small chambers in the dark or under fluorescent lights. The leaves receiving light were supplied with about

250 foot-candles (2,690 lux). Air free of carbon dioxide was continuously passed through the chambers and carbon dioxide evolved from the tissues was trapped in alkali. The leaves were gathered after 24 hr. and extracted.

Quinate and shikimate were separated from the tissue extracts by gradient elution with acetic acid from 'Dowex 1-X8' (acetate-form)[4]. Bound amino-acids were liberated by hydrolysis with barium hydroxide[10], and both free and bound amino-acids were separated by column chromatography[11]. Tryptophan was identified by paper chromatography and by a modified Salkowski reaction as follows: to 50 ml. of 35 per cent perchloric acid, 1 ml. of 0·367 ferric chloride was added. Two ml. of sample in citrate buffer, *p*H 5·28 (ref. 11), was combined with 4 ml. of reagent. The reaction mixture was heated for 15 min. in a water bath at 70° C. and then cooled to room temperature. The absorbancy of the yellow colour, which was stable for more than 96 hr., was determined in a spectrophotometer at 455 mμ.

Tyrosine and phenylalanine were identified by their elution pattern from the column, followed by paper chromatography and autoradiography. Identification of quinate and shikimate involved elution patterns from columns, paper chromatography, autoradiography, and reactions on chromatograms with potassium periodate–piperazine–nitroprusside[12] and the thiobarbituric acid reagent of Saslaw and Waravdekar[13].

Table 1. CONVERSION OF QUINATE-U-^{14}C TO SHIKIMATE-U-^{14}C, THEIR CONVERSION TO AROMATIC AMINO-ACIDS, AND $^{14}CO_2$ EVOLVED IN BEAN LEAVES

Treatment, bean leaves	C.p.m./μmole				$^{14}CO_2$ evolved as percentage of total carbon-14 supplied
	Shikimate	Quinate	Phenylalanine + tyrosine *	Tryptophan *	
Shikimate-light	18,560	0	4,620	16	1·7
Quinate-light	17,240	22,170	2,880	10	1·1
Shikimate-dark	21,910	0	4,175	8	5·6
Quinate-dark	32,510	20,850	1,785	3	6·9

* Free and bound.

Table 1 shows results from a typical experiment. Similar experiments with quinate-U-^{14}C were carried out at three other times.

Little carbon-14 was incorporated into free or bound tryptophan, although there was a slightly greater incorporation in the light than in the dark. Phenylalanine and tyrosine, however, were preferentially labelled when either quinate- or shikimate-U-^{14}C was supplied in the light or dark. Shikimate was a more efficient precursor than quinate. There was no evidence of conversion of shikimate to quinate in the light or dark. Quinate, on the other hand, was readily converted to shikimate, as has been shown previously[5–7].

Although based on information of a negative nature, it seems very unlikely that the shikimate pathway is responsible for tryptophan synthesis in the bean. It is, of course, possible that the pathway to tryptophan in the bean constitutes an exception to the expected pathway.

Since more radioactivity was found in tryptophan in the light than in the dark, it is possible that this resulted from photosynthetic fixation of respiratory $^{14}CO_2$ into other compounds, such as serine, which leads to the tryptophan side-chain. Experiments carried out under light intensities higher than that reported here might result in greater adventitious labelling of tryptophan in this manner. Table 1 shows that dark-treated tissues liberated about 5 times more $^{14}CO_2$ than did light-treated tissues. The difference may have been due to photosynthetic fixation of evolved $^{14}CO_2$.

The irreversibility of the quinate→shikimate conversion leads one to suggest that this pathway in bean may not be directed through the intermediate compounds 5-dehydroquinate and 5-dehydroshikimate.

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Premature Bursting of Sultana Buds

OF the two buds formed in the axil of vine leaves in spring, one usually develops into a lateral shoot; the other (hereafter termed 'bud') becomes dormant, and does not burst until the following spring.

Huglin[1], working with varieties of wine grapes, found that up to three terminal buds could be induced to burst without first undergoing winter dormancy. Bud-burst was induced by simultaneous removal of lateral shoots and growing points, provided that the leaves subtending the buds were not more than six weeks old.

Bernstein and Klein[2] reported that, in parts of Palestine, shoots of the variety Chasselas Doré, pruned to eight buds after the normal harvest, could produce a second crop following bud-burst in the terminal positions. These buds are up to six months old at the time of bursting.

This communication reports bud-burst without winter rest in sultana vines having growing points still intact. The vines were grown in aerated solution culture at this Station, during the summers of 1959 and 1960.

During the 1959–60 season, laterals on young sultana rootlings in solution culture were pinched out as they appeared (October to end of December) to confine growth to single shoots. Nutrient solutions were completely replaced on December 8, and monthly thereafter. The experiment ended in February. Vines made good growth and by late December shoot-lengths ranged from 100 to 200 cm., and nodes numbered 30–40. Bud-burst commenced on December 22 and continued during January and February. Fig. 1 illustrates a typical premature bud-burst

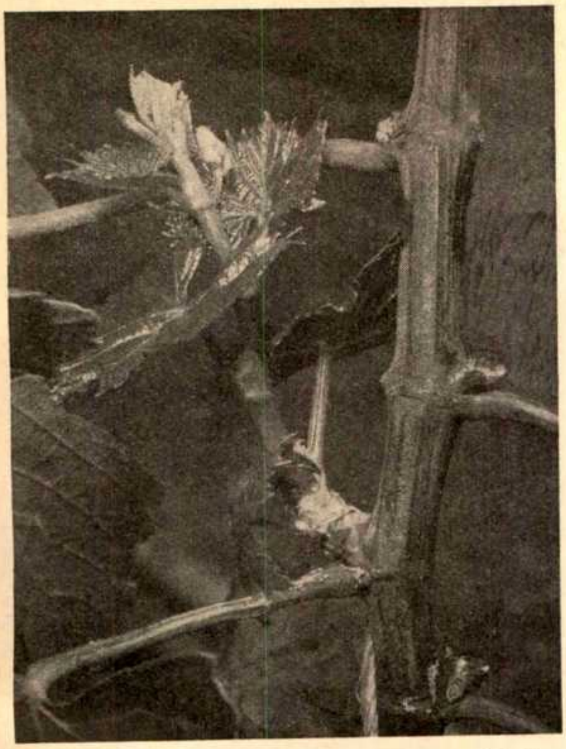

Fig. 1. Premature bursting of sultana bud; note scars left after removal of laterals

By February, at least one of the basal 25 buds had burst on 55 of the 72 vines; on 16 vines, more than three buds had burst. Of 164 buds which burst, 46 were on the basal third of the shoots. The number of buds bursting prematurely ranged up to 12 per vine.

In another experiment, two young sultana rootlings with immature shoots were transferred from solid media to solution culture in January. The shoots were cut back to 12 buds (1/3 of shoot-length) and all laterals removed but primary leaves were left intact. At the time of transfer basal leaves were 16 weeks, and leaves at node 12 were 12 weeks old. After 12 days in solution culture, 11 buds on one vine and 6 on the other had burst. Buds burst basipetally.

To examine the abnormal bud-burst occurring in solution culture, five treatments (varying numbers of both young leaves and untipped lateral shoots) were applied to young sultana rootlings (11–15 nodes) in October 1960. Contrary to the previous season, nutrient solutions were not changed but nutrients were added from time to time to maintain active growth. Treatment details and data on buds bursting by December 30 are given in Table 1.

Table 1. TREATMENTS APPLIED TO LATERAL SHOOTS, AND PREMATURE BUD-BURST IN SULTANA VINES GROWN IN SOLUTION CULTURE AS AT DECEMBER 30

Treatment of laterals	No. of buds bursting prematurely	No. of vines affected	Mean No. of primary nodes per vine
1 Removed on appearance	12	5	41
2 Removed when 4 nodes long	1	1	38
3 Tipped when 4 nodes long*	0	0	37
4 Tipped when 8 nodes long*	0	0	36
5 Removed progressively when distal two were 4 nodes long	1	1	30

* Further lateral growths were removed on appearance

Of the 14 buds which burst, 4 (3 from one vine of treatment 1, and 1 from treatment 5) were associated with loss of the shoot tip due to wind. A further 3 cases (from two vines of treatment 1) were associated with wind damage to the shoot tip without loss of the growing point; damage occurred within 10–20 days of the first appearance of the leaf subtending the bud which burst. These 7 buds were in the terminal region of the shoot at the time of damage, and burst 2–3 weeks later.

In 4 other cases (from two vines of treatment 1) the subtending leaf was at least 10 weeks old at the time of bud-burst. These buds were in the basal portion of the shoot, and there was no damage to shoot tips. In all cases of premature bursting, affected vines had active growing points on either the main shoot or lateral extension at the time of burst.

No bud-burst occurred on 7 of the 18 vines in treatments 2, 3 and 4 although growing points on main shoots were lost; after short periods these were replaced by laterals.

These observations are in accordance with Huglin's hypothesis—of the production of bud-burst inhibitor in growing points and young leaves. But in our experience, buds older than 6 weeks, which he found dormant, burst on numerous occasions. Contrary to the findings of both Huglin[1] and Bernstein and Klein[2], bud-burst was not confined to the terminal positions. This suggests that the onset of dormancy is delayed in solution culture, or that leaching of roots can interfere with bud-burst inhibition. Evidence suggests that even a temporary depression (7 days) of terminal growth retards the development of this inhibitor.

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Gradient Analysis in Swamp Forests

DURING 1960 and 1961 we studied periodically flooded wetlands dominated by the trees *Larix laricina*, *Thuja occidentalis*, and *Ulmus americana* along transects from lake margins to the upland in the Rideau Lakes Region of Ontario. This vegetation shows distinct belts of the dominants from the air which have often been described as separate plant communities and were interpreted as stages in a hydrosere[1,2].

Relative elevations along transects were determined with a hand level and stations measured at 50-m. intervals. At each station we measured the water-level repeatedly, determined *p*H and Eh in the field for a hummock and a hollow both at 5 and 30 cm. depth, and collected the four corresponding soil samples. By spinning a cross we determined four directions. The closest ¼ sq.m. of hummock in each direction was sampled for herb- and ground-layer. The procedure was repeated for the hollows. In a 10 × 10 m. quadrat density and basal area in the tree layer and density and cover in the shrub layer

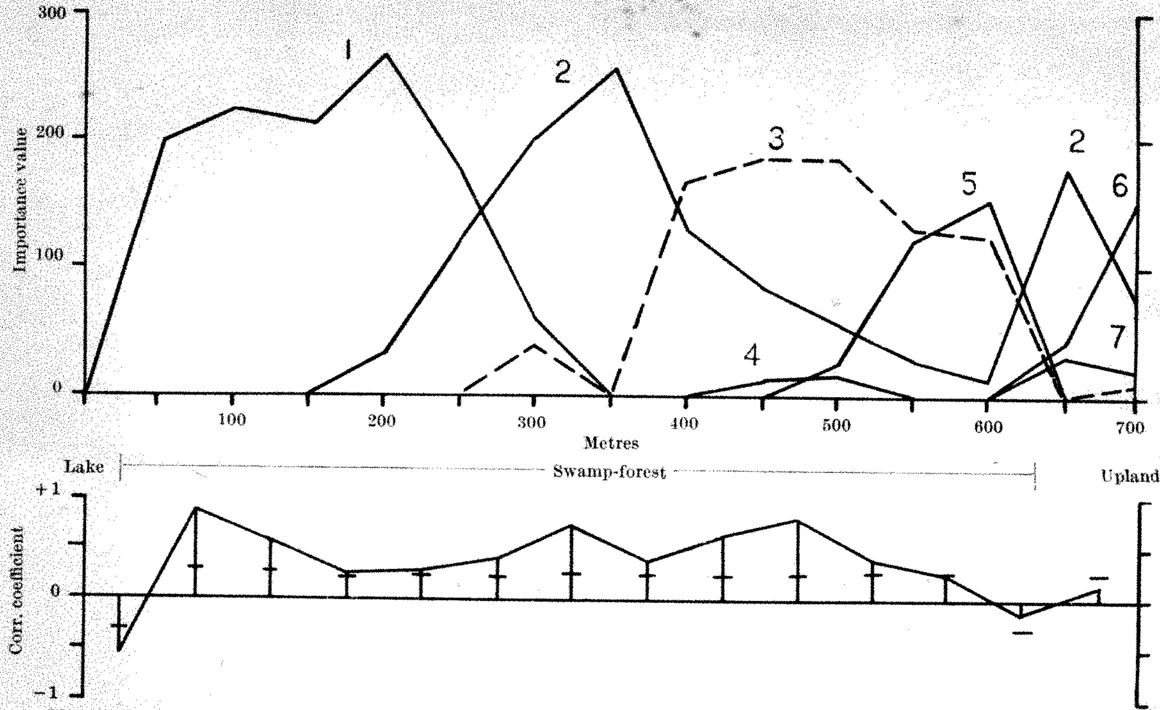

Fig. 1. Transect of a swamp forest near Portland, Ontario. Above, Importance values for the sampled stations of (1) *Larix laricina*, (2) *Thuja occidentalis*, (3) *Ulmus americana*, (4) *Fraxinus nigra*, (5) *Acer saccharinum*, (6) *Acer saccharum*, (7) *Fraxinus americana*. Below, correlation coefficients of the vegetation between adjoining stations. Probability at the $P = 0{\cdot}05$ level is indicated by –

were noted. From six points, selected close to randomness within 10 m. radius of each station, we sampled trees using the quarter method[3]. The limits of the quadrants were again found by spinning a cross.

The analysis of the gradient may be shown on one transect. About 30 species were noted per station. The whole transect yielded a total of 161 species of tracheophytes, bryophytes and ground-lichens. Sampling at a station comprises less than the commonly used minimum areas; but an increase of the sampled area rectilinear to the transect raised the species number only slightly and demanded a much longer sampling time. Due to micro-environmental differences a larger sampling area mixes also elements narrowly restricted to sections of the gradient and would give a false picture of higher continuity and homogeneity.

When the importance values[4] of the trees are plotted for each station, as at the top of Fig. 1, a gradual replacement of one species by another is seen. Only at the upland margin do the values change rapidly. Combined frequency and cover values for plants in the lower layers give very similar patterns. However, these do not coincide with the ranges of the dominant trees and show a wide variety of width and position of the ranges. Most patterns resemble normal curves. A large number of curves is difficult to assess by inspection alone. Trends of ranges to coincide and coinciding modalities of curves may be hidden. These trends could give indications of limits of communities or seral stages. Therefore we calculated various community coefficients[5] and Pearson's r for the changes between adjoining stations. For the transect shown we calculated r also for every station with each other station.

The degree of correlation depends on the way the data are adjusted, which is a matter of choice. We tried to simplify the procedure of tabulating. Further, we wanted to avoid high correlations resulting from excessive loading through the truism that rare and small plants are so throughout and that the number of species in the top two layers is always lower than in the two other layers. We hope to improve adjustments with the aid of a computer. For the tree layer we used importance values divided by three. Cover percentages were listed for the shrub layer. The added cover percentages of the eight quadrats were divided by two for the herb layer. The same was done for the ground layer. Seedlings and saplings were counted as separate items in their respective layers. The layers are thus counted in the ratio 1 : 1 : 4 : 4 and the values on one station could add up theoretically to 1,000. The number of items occurring in the station pairs compared is taken as n, which fluctuates from 39 to 63.

The r-values of adjoining stations are shown in the figure with a transverse bar for a probability of significance of $P = 0{\cdot}05$ derived from the t-test. All neighbouring stations within the swamp forest except the last pair are significantly correlated. Contrarily, the lake shore and the upland limit appear as sharp breaks. If the distance of the two stations compared is increased linearly, r drops geometrically, decelerating into significant negative correlations. Other comparisons based either on cover or only on species shared and not shared between stations give similar patterns. The sign test[6] proved very useful for the latter comparison as it permitted also an assessment of probability. Slight fluctuations of the lines connecting values of similarity for adjoining stations are not paralleled by the various methods. Different adjustment of the data varies the fluctuations as well.

The connecting line of the r-values permits an assessment of the steepness of the vegetation gradient for the distance of the compared stations. A dip over short distances into negative correlations indicates an ecotone. Peaks and plateaux of significant positive correlation show high homogeneity. The mean level of this line changes with varying sampling intensity at a station and with the distances between stations. Standardized sampling allows comparison of the steepness of the vegetation gradient in different localities and correlation of the changes of environmental factors with the overall changes of the vegetation.

We did not find a sufficiently sharp break along the transects within the swamp forests to warrant a separation into different communities despite the extreme dissimilarity of the vegetation several stations apart.

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A Possible Link between Root Initiation and Anthocyanin Formation

IN an examination of the rooting of cuttings from *Acer rubrum* L. and *Eucalyptus camaldulensis* Dehn. seedlings the unexpected observation was made that the rooting response of *Acer rubrum* cuttings appeared to be related more closely to the amount of anthocyanin formed in the leaves during the experimental period than to the treatments under investigation.

Acer rubrum and *Eucalyptus camaldulensis* seedlings were grown in controlled environment rooms at 23° C. Cuttings were made from 4–5-week old *Acer* seedlings by cutting the seedlings immediately above the cotyledons. 10-cm. tip cuttings were taken from *Eucalyptus* seedlings about 13 weeks old. Rooting of the cuttings was tested in continuous light at 26° C. The bases of the cuttings were immersed in a darkened rooting solution of 1·0 mgm./l. indolyl butyric acid (IBA), an auxin and concentration previously determined to be optimal.

The cuttings were used in investigations of some of the physiological factors affecting root formation. Though some of the treatments tested yielded significant overall effects there was considerable variation in the number of roots formed on replicate cuttings within any one treatment. In the *Acer rubrum* cuttings this variation in number of roots formed appeared to be correlated with the redness of the leaves on the cutting. The red coloration developed during the experimental period, very little or no redness being present in the leaves at the start of the experiments. A typical relation between mean roots/cutting and leaf colour found in a preliminary experiment is shown in Fig. 1. Another experiment then was examined quantitatively. The anthocyanin present in the two leaves retained on each cutting was extracted by treatment with cold 0·1 *N* hydrochloric acid for 24 hr. The amount of anthocyanin present in the leaves was estimated, spectrophotometrically, by measuring the absorption of the extract at 510 mμ. A highly significant correlation ($r = 0{\cdot}677$, $P < 0{\cdot}01$) was observed between the total amount of anthocyanin present in the leaves of each cutting and the number of roots formed on those cuttings (Fig. 2). The correlation between anthocyanin concentration (anthocyanin units/gm. fresh weight of leaves) and number of roots was also highly significant ($r = 0{\cdot}608$, $P < 0{\cdot}01$).

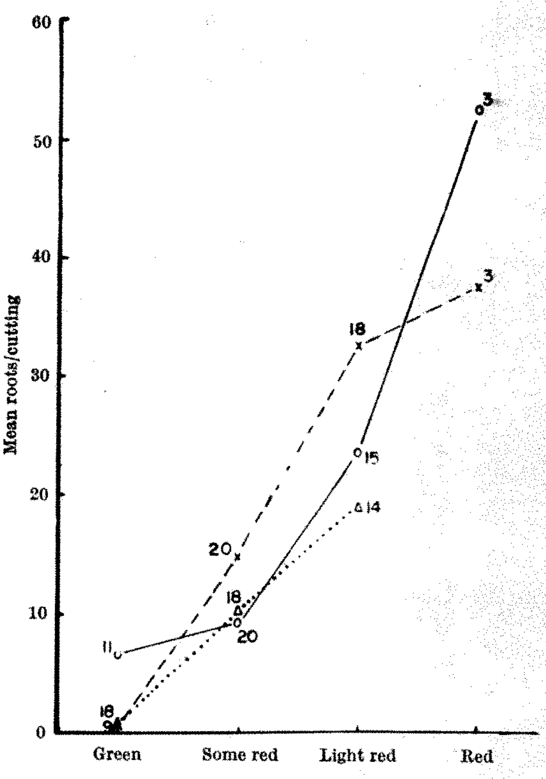

Fig. 1. Relation between leaf colour and mean roots/cutting of *Acer rubrum*, three weeks after treatment with indolyl butyric acid. Cutting from seedlings grown under: ○, continuous light; ×, 16-hr. light period; Δ, 8-hr. light period. Numbers indicate number of plants averaged for each experimental point

No correlation between anthocyanin content of the leaves and root formation was observed in cuttings rooted in distilled water alone. No difference could be detected between the total amount of anthocyanin in the leaves of cuttings rooted in distilled water and in the leaves of the cuttings rooted in 1·0 mgm./l. IBA. This suggests that anthocyanin formation in the leaves of the cuttings was not dependent on the number of roots formed. In terms of concentration of anthocyanin (anthocyanin units/gm. fresh weight of leaves), however, IBA did significantly increase ($P < 0{\cdot}05$) the amount of anthocyanin formed.

The anthocyanins in the leaves of *Acer rubrum* cuttings have been identified, by paper chromatography techniques, as cyanidin glycosides in agreement with Lawrence *et al.*[1]. The correlation between leaf anthocyanin content and root number on *Acer rubrum* cuttings indicates that some of the factors involved in anthocyanin biosynthesis may also be involved in root formation. Sucrose[2,3] and purines and pyrimidines[4] have been shown to promote anthocyanin biosynthesis in some tissues. Thimann and Radner have suggested that riboflavin may play a

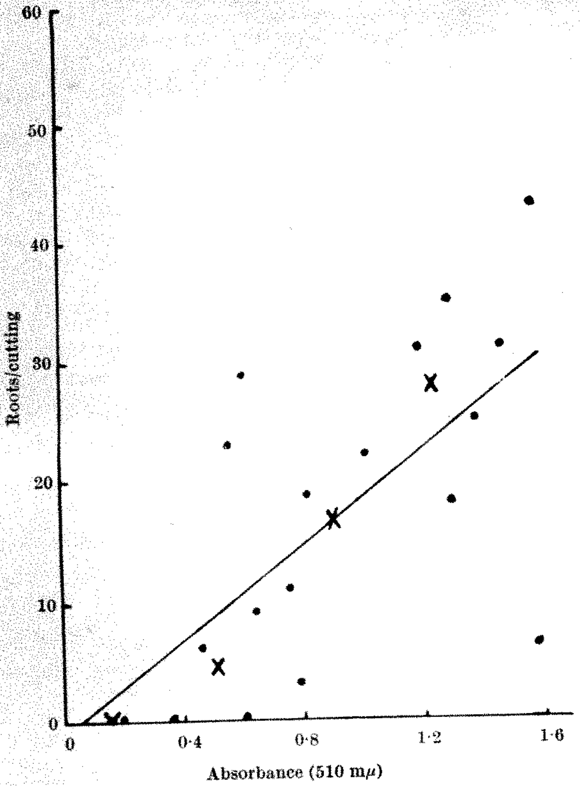

Fig. 2. Relation between anthocyanin content of the leaves (estimated from the absorbance of 510 mμ of a 0·1 *N* hydrochloric acid extract) and number of roots/cutting of *Acer rubrum*. The line drawn is the least squares estimate. The leaves of the cuttings were classified subjectively into four colour classes, as in Fig. 1, before extraction of the anthocyanin. *X* denotes the mean absorbance of extracts of the leaves in each colour class plotted against the mean root number on cuttings in each class. Cuttings from seedlings grown under continuous light. $r = 0{\cdot}677$ ($P < 0{\cdot}01$)

main part in anthocyanin formation[5]. A number of investigations[6–8] have indicated that sucrose and nitrogenous compounds, both organic and inorganic, affect the rooting of cuttings.

Basal pretreatment of *Acer rubrum* cuttings with either sucrose (4 per cent) or with riboflavin (0·1–10·0 mgm./l.) for 24 hr. prior to placing the cuttings in the rooting solution of 1·0 mgm./l. IBA did not enhance rooting of these cuttings. However, some preliminary experiments indicated that both riboflavin and sucrose may promote rooting of *Eucalyptus camaldulensis* tip cuttings. An experiment to test this possibility further yielded the data summarized in Table 1. Sucrose by itself but not riboflavin alone promoted the rooting of *E. camaldulensis* tip cuttings in this experiment. The interactions sucrose × light conditions, riboflavin × light conditions, and riboflavin × sucrose were all significant ($P < 0{\cdot}05$).

Table 1. EFFECT OF RIBOFLAVIN AND SUCROSE ON THE ROOTING OF *Eucalyptus camaldulensis* TIP CUTTINGS

Mean roots/cutting (17 days after treatment) (8–10 cuttings/treatment)

	Cuttings from seedlings grown under Continuous light		16-hr. light period	
Sucrose %	0	4	0	4
Riboflavin (mgm./l.)				
0	18·2 ± 4·30*	24·6 ± 4·88	19·4 ± 4·17	10·6 ± 3·72
0·1	26·4 ± 4·60	31·6 ± 5·32	14·4 ± 5·58	12·4 ± 2·98
1·0	12·5 ± 3·81	26·6 ± 6·02	10·3 ± 4·05	23·9 ± 5·04
10·0	19·5 ± 3·65	46·0 ± 4·92	10·0 ± 3·77	17·4 ± 3·51

* *S.E.* of the mean; *M.S.* error (whole experiment) = 183·5.

Treatment with 4 per cent sucrose + 10 mgm./l. riboflavin significantly increased ($P < 0{\cdot}01$) the number of roots formed on cuttings taken from seedlings grown under continuous light. In cuttings taken from seedlings grown under 16-hr. light period the number of roots formed after treatment with 1·0 mgm./l. riboflavin + 4 per cent sucrose was significantly greater ($P < 0{\cdot}05$) than the number of roots formed after treatment with sucrose alone.

Limited subjective evidence indicated that riboflavin may also promote anthocyanin formation in *E. camaldulensis* leaves.

These results indicate that a link between anthocyanin biosynthesis and root formation may exist. This possibility is supported by some observations from the literature. van Overbeek and Gregory[9] found that cuttings from a white-flowered variety of *Hibiscus* were much more difficult to root than cuttings from a red-flowered variety. *Spirodela* and *Lemna*, the only anthocyanin-forming genera in the Lemnaceae, are also the only genera in this family with roots[10]. Other evidence that anthocyanin-like materials may play a part in the growth of plant tissues is provided by the identification of leucoanthocyanins as one of the growth active components in coconut milk[11].

These observations combined with ours suggest that further attention should be paid to possible interrelations between anthocyanin formation and the rooting process.

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Experimental Lathyrism in Turkey Embryos

LATHYRISM was first produced experimentally more than a quarter of a century ago (Geiger *et al.*, 1933) by feeding rats seeds of the flowering sweet pea, *Lathyrus odoratus*. Since then various chemicals have been tested for their lathyrogenic activity including semicarbazide hydrochloride, the drug used in this experiment.

The lathyrogenic effect of semicarbazide given by yolk-sac inoculation has been investigated mainly in chick embryos[1–8]. This experiment deals with the

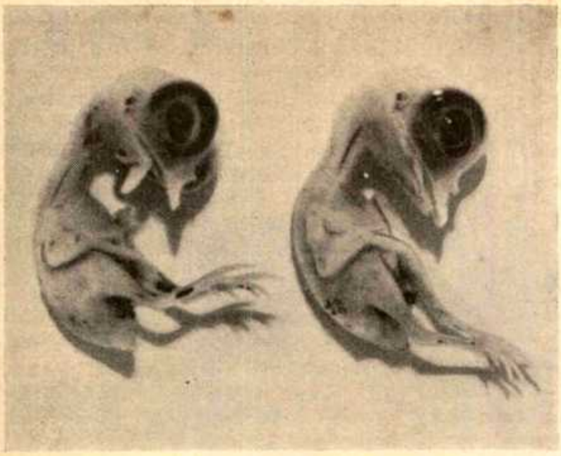

Fig. 1. Turkey embryos one week after inoculation. Note the beak deformity of the test animal

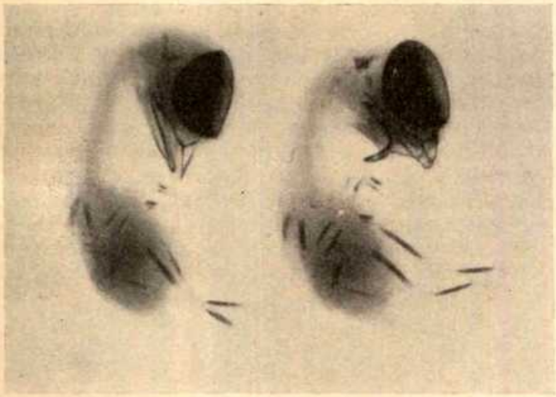

Fig. 2. Alizarin red specimen of the embryos shown in the previous photograph. The 'L'-shaped deformity of the lower beak is more evident

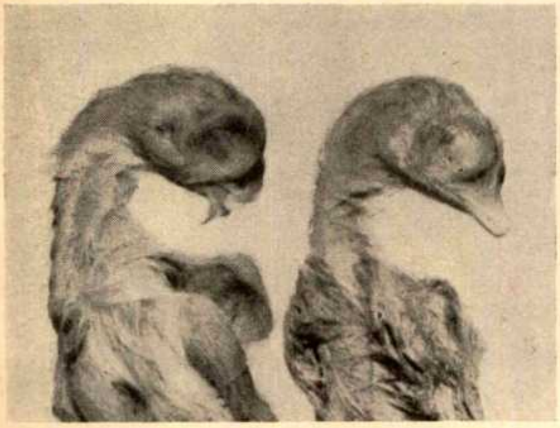

Fig. 3. Photograph of turkey embryos two weeks after yolk-sac inoculation. The beak deformities are more marked

resultant abnormalities produced by semicarbazide yolk-sac inoculation of fertile turkey eggs.

Fertile turkey eggs were placed in a Curfew electric observation incubator at 37·5° C. On the 6th day of incubation 2 mgm. of semicarbazide hydrochloride in 0·2 ml. solution was inoculated into the yolk sac. Control experiments using 0·2 ml. normal saline were also performed. On the 14th day of incubation the embryos were collected (Fig. 1). It will be seen that the only obvious abnormalities are in the beak and cervical spine. There was a slight lateral scoliosis of the cervical spine and a bilateral L-shaped deformity of the lower beak as well as an asymmetrical twisting of the upper beak. These skeletal changes are better seen in the alizarin red specimens (Fig. 2). Changes in the beak were more marked by the 21st day (Fig. 3), but no gross bending of the tibia and fibula was noted, although there was occasional dislocation of the tibio-tarsal joints. Another feature was the presence of a pseudo-umbilical hernia. No abnormalities were noted on histological examination of the skeleton or aorta.

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ENTOMOLOGY

Susceptibility to DDT of Previously Resistant Nymphs of the Sugar-cane Froghopper (*Aeneolamia varia saccharina* Distant)

It has been noted in field trials conducted over a three-year period that nymphs of the sugar-cane froghopper (*Aeneolamia varia saccharina* Distant), derived from populations of the insect which have been resistant to chlorinated hydrocarbons for the past five years, are now once again susceptible to DDT (5 per cent in ground limestone). The unusual toxicity curves, a typical example of which is presented in Fig. 1, are worth mention because they differ from the original toxicity curves for susceptible froghopper nymphs, in which DDT invariably caused a quicker initial kill of nymphs[1]. A brief description of the procedures used in the experiments will facilitate the interpretation of results.

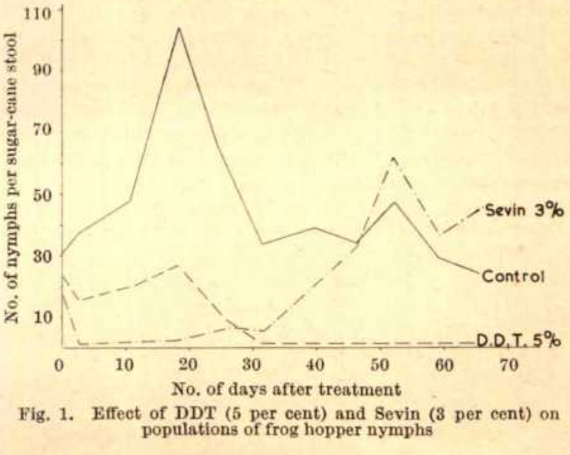

Fig. 1. Effect of DDT (5 per cent) and Sevin (3 per cent) on populations of frog hopper nymphs

Insecticides to be evaluated were formulated in ground limestone and applied as dusts to the soil surface immediately surrounding the base of sugar-cane 'stools'. It is in this area that froghopper nymphs abound. Preliminary counts of nymphs prior to application of insecticides gave an indication of the severity of infestation. The majority of nymphs present at the time that insecticides were applied were in 3rd–5th instar stages. Subsequent counts at intervals after application of insecticides provided information on 'initial' kill of nymphs as well as efficiency of control of succeeding broods. Insecticides were applied with 'Rotver' knapsack dusters at the rate of 2½ cwt. per acre of 5 per cent DDT and 3 per cent Sevin (*N*-methyl-1-naphthyl-carbamate). Randomized blocks with plots 1/40th of an acre were used with at least five sugar-cane 'stools' per plot sampled at each date of observation. Nymph populations in controls reached a peak nineteen days after the experiment was begun (Fig. 1). However, in other experiments the peaks of population densities varied depending on the time of year that the experiments were initiated. Sevin maintained a low count (less than 5 nymphs per stool) up to nineteen days after application (Fig. 1). This is significant, particularly since the population of nymphs in controls was at its height at this particular time. DDT, on the other hand, provided a slow initial destruction of nymphs. Some degree of insecticidal activity was evident especially if the numbers of nymphs in controls are compared with those in DDT-treated areas (Fig. 1). Forty and fifty-five days after application it became quite clear that DDT had controlled succeeding broods of nymphs, while this was not the case in plots which had been treated with Sevin. Nymph counts had also risen in control areas.

One may conclude that froghopper nymphs are susceptible to DDT. However, there appears to be a 'selective' tolerance of nymphs to the chlorinated hydrocarbon. Those nymphs in more advance instar stages (3rd–5th), which happened to be present when the insecticide was applied at the beginning of the experiments, were less susceptible to DDT than younger instars (1st and 2nd), which were the first stages to emerge in the successive broods. From a theoretical point of view it would be of interest to determine whether the more advanced nymphs possess a DDT-dehydrochlorinase system of the type described by Sternburg, Kearns and Moorefield[2] in the housefly. From a practical point of view it is of value to record that froghopper nymphs are again susceptible to DDT after a lapse of nearly five years, during which time other types of compounds have been used to combat the insect.

Combinations of DDT (5 per cent) with more rapidly acting compounds (such as Malathion 5 per cent or Sevin 3 per cent) should prove of value as a practical control measure, particularly in view of the effective residual action of DDT. The results also suggest that rotation of insecticides of different chemical structure might be a useful practical measure in the long-range battle against the froghopper and other insects.

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A Celloidin Impression Technique for Recording Egg Sculpturing in *Aëdes* Mosquitoes

SCULPTURE patterns of the eggs of mosquitoes have been used for identification of closely related species by Horsfall, Miles and Skoatch[1] and Craig and Horsfall[2,3]. The technique used by these authors was outlined by Craig[4]. It involved first the removal of the exochorion by abrasive action followed by the bleaching of the endochorion. The endochorionic patterns revealed by these treatments were recorded by phase contrast photography.

During my recent investigation of the egg coverings of the mosquitoes of the genus *Aëdes*, it was observed that the exochorion carried surface markings identical with those occurring on the endochorion. This is not surprising: both layers are secreted by the same follicular cells of the ovary and presumably the exochorion is moulded over the preformed endochorion. However, owing to the pigmentation of the endochorion the markings of the exochorion were not obvious in the intact egg, and it was not possible to remove a strip of this fragile layer large enough to show a complete pattern. These difficulties were overcome by the use of celloidin films, which were applied to obtain impressions of the exochorionic markings.

A selected egg with the exochorion intact is orientated on a microscope slide on the stage of a binocular dissecting microscope and a second slide is dipped in a 10 per cent solution of nitrocellulose[5]. Before the celloidin sets the slide is gently lowered

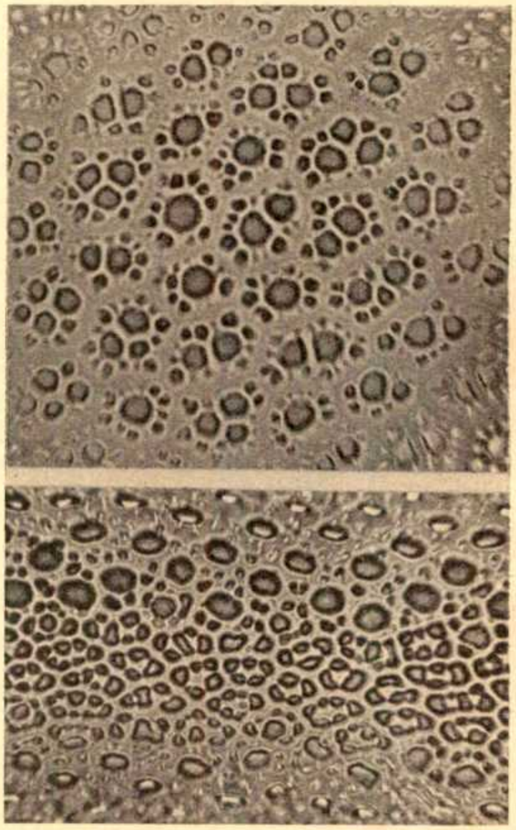

Fig. 1. Photomicrographs of celloidin impressions of exochorionic patterns. Above, *Aëdes* (*Ochlerotatus*) *alternans*, ventral (× 500); below, *Aëdes* (*Finlaya*) *alboannulatus*, ventral (× 500)

over the egg which sticks to the film. This is then hardened in 70 per cent alcohol for 1–2 min., after which the egg is removed with a fine needle. The impression left on the celloidin film remains sharply defined for 10–20 min. and can be photographed with Köhler illumination.

Observations have been made on 11 species belonging to the two subgenera *Ochlerotatus* and *Finlaya*. The sculpturing in each of the species appears to be remarkably constant, and permits accurate identification of the species. It is interesting to note that *Ochlerotatus* retains a single basic pattern over the entire surface of the egg. In *Finlaya* the sculpturing of the dorsal and ventral sides differs from those of the lateral aspects.

It is possible that the application of this technique to sculptured eggs of other insects or perhaps selected portions of their postembryonic cuticle may reveal other interspecific differences.

My thanks are due to Dr. N. Dobrotworsky, of the University of Melbourne, for identifying the adult mosquitoes used in this work.

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Infestations of *Carbula pedalis* in Northern Nigeria

As there appears to be no published account of the great nuisance caused by the invasion of houses in various parts of Africa by *Carbula pedalis* Begr. (Hemiptera, Pentatomidae), this phenomenon, as observed in Northern Nigeria, together with notes on the behaviour of the species, are presented here.

The species has a general distribution between latitudes 10° and 15° N., occurring right across Africa from Senegal to the Sudan. It is, however, by no means common over this entire area and in Nigeria only the occasional specimens are found between latitudes 10° and 11° 38′ N. Infected areas are restricted to the 20–33 in. mean annual rainfall belt of the Sudan savannah, but even within this area infestation can be extremely localized. Fig. 1 gives all localities in Nigeria in which infestation of habitations is known to occur.

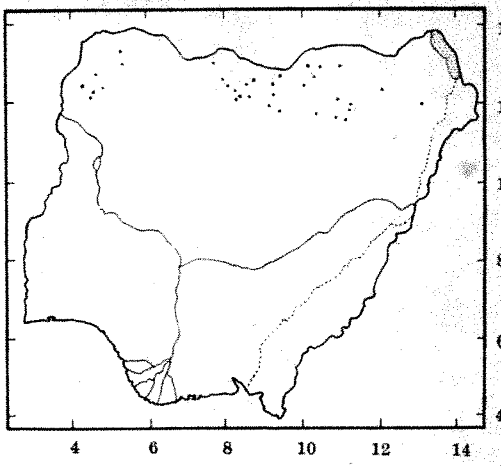

Fig. 1. Distribution of *Carbula pedalis* in Northern Nigeria

Adults start entering houses during the latter part of October or at the beginning of November at the end of the rains; the invasion period lasts for about a month. Once in a house they are strongly photophobic and tactophilic, hiding away in books, clothing, furniture, fuse boxes, under mats, among food and in nearly every conceivable niche. The smell produced by isolated individuals from the odoriferous glands is barely perceptible; but when they are present in great numbers their characteristic sickly odour is nauseating and even food becomes tainted. They can prove so troublesome that occupants of infested houses may have to vacate them at the beginning of each yearly invasion, only re-occupying them when the invasion has subsided. In some instances houses have been so badly invaded that the only remedy was to burn them down; while in one locality 12 one-gallon tinfulls were removed daily from houses during the first two weeks of the invasion period. The insects appear to show no marked preference for either thatched or non-thatched houses, and even when present in the former type they are not particularly abundant in the thatch itself. Sexing adults from various localities shows that both sexes are present in about equal numbers at both the beginning and towards the end of the indoor æstivating period (182♀, 159♂, 79♀ and 87♂). No spermatozoa were found in the spermotheca of the 40 females dissected. Fat bodies were well developed at the beginning of æstivation but diminished in size towards the end of this quiescent period.

It would appear that with the onset of the severe dry season adults seek out a more suitable environment for protection against the extreme dryness and fluctuations in temperature to which they would otherwise be subjected. Consequently houses are invaded, though more natural resting sites exist, as *C. pedalis* has been found under the barks of the trees, *Tamarindus indica* and *Ceiba pentandra*, and has been observed hanging in clusters from the fronds of the palm tree, *Borassus aethiopium*. They have also been reported sheltering in considerable numbers in hollow stumps of unidentified trees. With the onset of the rains, in April or May, they leave their æstivating sites and presumably mate and oviposit on the host plant, which is unfortunately unknown. However, the consensus of opinion among agriculturists in Northern Nigeria is that no cultivated plant is attacked by this insect.

Several methods of control have been tried in order to eradicate *C. pedalis* from houses but so far results have been disappointing. Except for one area, in which intensive residual spraying of houses has been in operation for several years, both DDT and BHC have failed to give any appreciable reductions in numbers, and this is undoubtedly due in part to the gregarious and inaccessible habits of the adults. It is, however, possible that some degree of control could be exerted by spraying with a residual insecticide prior to the invasion period, at the end of which the resident population is reduced by the application of thermal aerosols, which have the advantage of being able to penetrate into the cracks and crevices harbouring these insects.

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VIROLOGY

Purification and Serology of Tulare Apple Mosaic Virus

GILMER[1], after comparing the woody host range and sap transmission of several virus isolates which caused mosaics in apple (*Malus* spp.), designated the one which could be mechanically transmitted to herbaceous plants Tulare apple mosaic virus (TAMV). Our TAMV isolate originated from a single primary lesion on the systemic host *Nicotiana tabacum* L. var. Connecticut Havana 423 inoculated with leaf tissue taken from an apple seedling bud-inoculated with TAMV stock kindly furnished by Dr. Gilmer. This isolate originated from Yarwood[2] and retained the symptomatological, host range and stability characteristics described by him.

During experiments on *in vitro* stabilization of the virus, we found that infectivity could be retained for at least 23 hr. at 25° C. by macerating leaves in an aqueous solution of 0·01 *M* sodium diethyldithiocarbamate and 0·01 *M* cysteine hydrochloride. The method of preparation of this solution had a profound effect on its stabilizing ability. Simultaneous dissolution of a mixture of the reagents resulted in a loss of stabilizing properties. Greatest stabilization was obtained when 0·02 *M* cysteine hydrochloride was added to an equal volume of 0·02 *M* sodium diethyldithiocarbamate. A pale yellow colour developed shortly after mixing and increased in intensity for about 2 hr., but the ability to stabilize was unaltered during this time.

Virus extraction was accomplished by pulverizing frozen leaves into a coarse powder at − 22° C., covering the powder with an excess of the stabilizer and infiltrating *in vacuo* at room temperature for 1 hr. Excess liquid was decanted and the tissue macerated. The crude juice was clarified by the addition of 8·5 per cent (v/v) *n*-butanol[3]. Clarified supernatants were given 2 cycles of differential ultracentrifugation in a Spinco model *L* centrifuge at 39,000 r.p.m. for 1 hr. in a No. 40 rotor. High-speed pellets were resuspended in 0·01 *M* *p*H 4·9 acetate buffer. Final solutions, representing at least a 25-fold concentration, were water clear and a marked difference in ultraviolet absorption was evident between preparations from control and virus-infected tissue (Fig. 1). The yields varied, but were in the range of 0·2–0·4 mgm. of virus for 100 gm. fresh weight tissue.

Concentrated preparations were subjected to rate density-gradient centrifugation[4] in an *SW* 25·1 swinging-bucket rotor for 2 hr. at 23,000 r.p.m. Control tubes showed no bands and contained no infectious material whereas tubes layered with the virus preparations showed a single band which was infectious on tobacco and on the local lesion host *Phaseolus vulgarus* L. var. Bountiful. When samples were removed from rate-tubes into equilibrium-tubes and centrifuged for 5 hr., a single sharp infectious band was found in the sucrose portion of the virus tubes. A faint diffuse low-density zone was also observed from both control and virus preparations in the buffer section of the tubes.

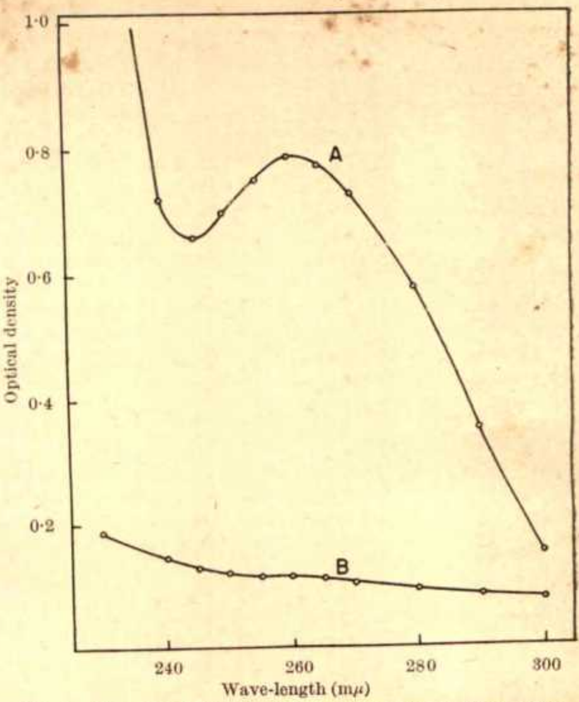

Fig. 1. The ultra-violet absorption of preparations from Tulare apple mosaic virus infected (*A*) and healthy (*B*) tobacco tissue. Curves are not corrected for light scattering

Antiserum was obtained from a previously bled rabbit injected intravenously 3 times over a 10-day period with TAMV prepared by differential ultracentrifugation and suspended in the acetate buffer. The rabbit was bled at intervals 3–16 days after the last injection and titres of 1/64–1/256 were found by the interfacial ring-precipitin test against purified virus. No reactions occurred between control preparations and antiserum or between virus and normal serum. A strong reaction occurred between antiserum and stabilized infectious tobacco sap in gel-diffusion plates, but none between the control pits (Fig. 2). Further, a faint but definite reaction

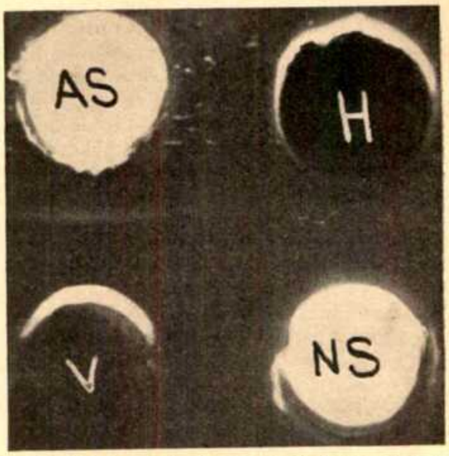

Fig. 2. Gel-diffusion plate showing precipitin reaction between antiserum (*AS*) and sap from tobacco infected with Tulare apple mosaic virus (*V*) and no reactions between control combinations. (*NS* refers to normal serum and *H* to sap from healthy tobacco)

occurred between antiserum and stabilized extracts of mosaic-infected apple leaf tissue in gel-diffusion tests.

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GENETICS

Distance and Sequence of the Loci for Protan and Deutan Defects and for Glucose-6-Phosphate Dehydrogenase Deficiency

Proof for the separate localization on the human *X*-chromosome of the protanopia-protanomaly alleles and of the deuteranopia-deuteranomaly alleles requires the demonstration of: (1) non-allelic compound hemizygous males which in conditions of equilibrium should occur once among a thousand men (one in eighty colour-blind men) or of (2) cross-over males in families combining both classes of defect.

Reviving the one-locus theory, Stern[1] asserts that protan-deutan men have not been found or are at least exceptional. However, assessment of the existence and frequency of the various compound hemizygotes depends on the kind of colour vision expected of them; some could be *sui generis*, others difficult to distinguish from deutans, protans or normals. Walls and Mathews[2] consider two brothers each combining protanopic and deuteranopic features as slightly different compounds, though Stern prefers to consider them as bearers of a new allele at a single locus. Men combining features of 'red' and 'green' blindness have also been described in the older literature (see Bell[3]) and more recently by Jaeger[4]; they are indeed familiar to careful investigators. On the other hand, some such compounds are placed among the pure protans or deutans by insufficient testing, while others may have a sufficiently balanced colour vision to pass an anomaloscope test or may be sufficiently different from the ordinary run of colour-blind people not to fail on pseudoisochromatic charts. These uncertainties make one hesitate to deny the existence of protan-deutan hemizygotes and to dismiss the two loci hypothesis.

This hypothesis has recently been considerably strengthened by the publication of a pedigree by Vanderdonck and Verriest[5] containing two or possibly three cross-overs between the deuteranopia and the protanomaly genes. The family consists of the offspring from a protanomalous wife (daughter of a deuteranopic man and a normal woman) and a normal husband (son of a normal man and a woman of unknown colour vision). This union produced two deuteranopic, one protanomalous and two normal sons as well as one protanomalous and one normal daughter. The colour vision of the members of this family has been expertly investigated and their nuclear sex shown to agree with apparent sex. The authors consider the two normal sons as recombinants from the X-chromosomes of their mother, one of which carries and shows a protanomaly gene and the other a deuteranopia gene. The normal daughter is not discussed.

Before attempting an estimate of the recombination fraction between the protan and deutan genes one may ask why in spite of prolonged attempts[6] recombination has not been previously found. Women, heterozygous for protan and deutan genes, are rare (about 1 in 250), and having either near normal[7] or atypical colour vision[3,4] they can only be discovered through having a protan and a deutan son. At least a third son is needed to show recombination, but his chance to do so is less than a half. Fewer than a handful of such families had been previously described[8], and as no recombinants had been found, it was concluded that the two colour vision loci are likely to be closely linked[9]. This conclusion is no longer warranted.

The distance between protan and deutan genes may also be inferred from linkage studies including other sex-linked genes. An estimate by Haldane and Smith[10] of a recombination frequency of 9·8 per cent between 'colour blindness' and hæmophilia, which then was not clinically subdivided, seems to be based on 16 pedigrees of deutans and only one small pedigree of protans. Pedigrees combining colour blindness with muscular dystrophy are as yet not numerous. Porter, Schulze and McKusick[11] recently estimated the recombination fraction between the genes for glucose-6-phosphate dehydrogenase deficiency and that for deutan defects as 0·05 (between 0·009 and 0·18 at 90 per cent confidence-level) and the 90 per cent probability of recombination between the glucose-6-phosphate dehydrogenase gene and a protan gene as less than 0·2, and they considered their results insufficient to decide between the two loci and one locus hypotheses. However, considering Vanderdonck and Verriest's pedigree it is at present reasonable to assume that the protan locus and the deutan locus are not very close and that perhaps the locus for glucose-6-phosphate dehydrogenase deficiency lies between them, probably nearer to the deutan locus.

The position of these three loci should be considered in the linkage studies with the sex-linked blood group antigen, promised by Mann *et al.*[12].

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An Unusual Bi-paternal Litter in Sheep from a Natural Double Mating

Although it is known that many cases of naturally occurring bi-paternal litters are probably born each year in domestic sheep flocks no well-authenticated examples seem to have been reported. Certainly none seems to have been studied in any detail when they

Table 1

Sheep No.	Follicles per mm.² $\bar{n}_{p+s}$	$\bar{n}_p$	Ratio $\bar{n}_s/\bar{n}_p$	Fibre diameter in microns (μ) $\bar{d}_p$	$\bar{d}_s$	$\bar{d}_{p+s}$	Ratio $\bar{d}_p/\bar{d}_s$	K^+	Ratio Na^+/K^+	Hb	*Tf*
Sire 1											
Soay 1 ♂ (Pure Soay)	16·8	2·7	5·2	35·2	19·7	22·1	1·79	L/—	8·3	A/A	*
Dam											
5 W. 180♀ (S. × Wilts.)	13·1	2·2	4·9	57·4	28·5	33·4	2·01	H/H	2·9	A/B	*
Progeny											
8 W. 1 ♂ (S. × S. × W.)	18·8	3·1	5·1	30·6	26·5	27·2	1·16	L/—	10·8	A/B	C/C
8 W. 2 ♂ (S. × S. × W.)	21·3	3·6	5·2	30·6	23·3	24·5	1·31	L/—	10·4	A/B	B/C
8 W. 3 ♀ (M. × S. × W.)	36·6	2·9	11·7	26·3	24·6	24·8	1·07	H/H	2·7	A/A	C/D
Sire 2											
R.R.T. 429 ♂ (Pure Merino)	66·2	2·8	22·5	15·1	16·1	16·0	0·94	H/H	2·8	A/B	B/D

* Not available June 13, 1961.

have been seen. Apart from its value as an established case of this phenomenon, the present example is unusual in the wide diversity of the genotypes involved and the small but significant array of characters it has been possible to observe in this very mixed family group.

During the night of October 21–22, 1957, a single Merino (imported in May, 1955, from the 'Valleyfield' stud in Tasmania) entered the field where a pure dark brown Soay (from the Duke of Bedford's flock at Woburn Abbey) was being mated to a small group of white Soay × Wiltshire F_1 ewes. This unwanted occurrence was reported and confirmed on the morning of October 22, and the identity of the only Soay × Wiltshire ewe then in œstrus noted. She had been clearly marked by both the Soay and the Merino ram and, when seen, was still the object of their joint attentions. Thus a normal record of the mating as an experimental mishap was made at the time in expectation of either of the two possible crosses in any ensuing progeny.

Toward the expected date of lambing the particular ewe was closely watched and, on the morning of March 17, 1958, she gave birth to triplets after a gestation period of 146 days. First, two white male lambs were born (birth weights: 7·0 and 6·5 lb.), both of an obvious Soay phenotype except in colour. These were followed by a larger white female lamb (birth weight: 8·75 lb.) whose phenotype strongly resembled a Merino. The parturition occurred in isolation in the field, was the only one recorded that day, and, being actually observed throughout, any risk of cross-mothering or of mistaken identity was absent.

As the litter grew to maturity all three remained white and all displayed the characteristic horn-type of the Soay which the dam also possessed. The two male members are remarkably similar in general appearance and in the first series of other characters observed. The female triplet differs most noticeably from her half-brother in growing a non-deciduous fleece with a short, uniform, well-crimped staple of a distinctive Merino cross-bred type but showing such a marked dorso-ventral growth gradient that the ventral body surface is almost bare, like the Wiltshire and the Soay breeds. In 1960 and 1961 her fleece weights have been 4·5 and 5·0 lb. respectively while rearing Merino back-cross progeny on each occasion. No fleeces have been recovered from the two males, which have displayed synchronous fleece shedding each year according to the Wiltshire and Soay pattern.

On September 5, 1958, all members of the litter were sampled by skin biopsy, the parental animals having been similarly examined on other occasions. On December 8, 1958, all six members of this complex family were bled for an estimation of biochemical blood characters. The skin biopsy specimens were evaluated by the methods of Carter and Clarke[1]; the hæmoglobin types by paper electrophoresis according to the technique of Harris and Warren[2]; the red blood cell electrolytes, K^+ and Na^+, by the method of Evans[3] using an EEL flame photometer. On May 1, 1961, reciprocal whole thickness skin homografts using the Carter biopsy punch (with autograft controls on each animal) were exchanged between the two male members of the litter to test the remote supposition that these may just possibly have been one-egg twins. This hypothesis was shown to be untenable by the mutual rejection of the homografts with some asymmetry of response (8 versus 14 days). More recently, on June 13, 1961, this genetic dissimilarity was confirmed by the identification of the serum beta-globulin types (symbol *Tf*, Table 1) by starch gel electrophoresis using the discontinuous buffer system of Poulik[4], hydrolysed starch from Connaught Medical Laboratories, Toronto, Canada, and the notation of Ashton[5]. Thus all three members of the litter are from three separately fertilized eggs and the segregation of characters may be interpreted accordingly.

From this brief report it is clear that what began as an experimental mishap is now well established as an authentic case of bi-paternity in sheep of a particular kind which well displays genetic segregation for a series of characters not commonly seen among contemporaries in the same family, and certainly unusual among members of a single litter. The influence of the Merino genotype is particularly well shown in the characters of the follicle population and continuity of fibre growth in the female triplet compared with those of the deciduous pelage of the two male non-Merino phenotypes differentiated and reared under similar uterine and postnatal environments. The genetic value of suitable combinations of morphological, biochemical, and immunological criteria is also clearly evident in this case. Other experimental possibilities are not difficult to visualize if a suitable method for producing such mixed litters can be established, exploiting present superovulation and transplantation techniques with fertilized ova or even by natural service under suitable conditions.

We are indebted to Dr. J. G. Hall of this Organisation for his identification of the hæmoglobin types and the serum β-globulins; and to Dr. E. J. Butler of the Moredun Institute, Edinburgh, for estimating the red blood cell electrolytes.

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FORTHCOMING EVENTS

Monday, April 16

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. L. G. C. Pugh and Mr. Barry Bishop: "Man at High Altitude".

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Eighth Annual General Meeting.

Monday, April 16—Wednesday, April 18

British Society of Soil Science (in the New Lecture Theatre, London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2)—Meetings on "Productivity and Soil Type" and "Soil Microbiology".

Tuesday, April 17

British Occupational Hygiene Society (in the Physics Lecture Theatre, Birkbeck College, Malet Street, London, W.C.1), at 9.30 a.m.—Conference on "Carbon Monoxide".

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W.C.2), at 5 30 p.m.—Mr. G. Syke and Mr. C. Burns: "The Use of Closed-Circuit Television for Inspecting Fast-Moving Surfaces".

Tuesday, April 17—Wednesday, April 18

British Computer Society, and the Northampton College of Advanced Technology (at the Northampton College of Advanced Technology, St. John Street, London, E C.1), at 9.30 a.m daily—Conference on "Automatic Programming Languages for Business and Science".

Borough Polytechnic, Division of Metal Science (at Borough Road, London, S.E 1), at 10 a.m. daily—Symposium on "Automation in Metal Finishing".

Institute of Biology (in the Fyvie Hall, The Polytechnic, 309 Regent Street, London, W.1), at 10 a m. daily—Conference on "The Training of Biologists".

Wednesday, April 18

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 2.30 p m. and 5.30 p.m.—Discussion on "Automation in the Supply Industry".

Worshipful Society of Apothecaries of London, Faculty of the History of Medicine and Pharmacy (at Apothecaries Hall, Blackfriars Lane, London, E.C.4), at 5 p.m.—Lord Cohen of Birkenhead; "James Carson, M.D., F.R S., of Liverpool"(Gideon de Laune Lecture).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer in Theoretical Physics—The Registrar, The University, Manchester 13 (April 14).

Assistant Lecturer in Philosophy—The Registrar, The University, Keele, Staffs (April 23).

Lecturer (with a special interest in teaching and research in applied mechanics) in the Department of Mechanical Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 27).

Demonstrator (graduate, with qualifications in dairying and dairy microbiology) in Dairy Science—The Registrar, University College of Wales, Aberystwyth (April 28).

Research Fellow (organic chemist with a Ph.D.) in the Department of Chemistry, for work in connexion with the development of organic dyes—The Registrar, The Manchester College of Science and Technology, Manchester 1 (April 28).

Lecturer in Mathematical Statistics—The Registrar, The University, Manchester 13 (April 30).

Lecturer in Pure Mathematics—The Secretary, The Queen's University, Belfast (April 30).

Lecturer or Assistant Lecturer (with research interests in low temperature or solid state physics) in Physics—The Registrar, The University, Manchester 13 (April 30).

Lecturer (with research or industrial experience in automatic control and/or engineering systems design, and preferably a knowledge of digital and/or analogue computing techniques) in the Department of Electrical Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 30).

Temporary Lecturer (with a higher degree in zoology and preferably some teaching experience in comparative physiology) in the Department of Zoology and Comparative Physiology—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (April 30).

Staff Tutor (with a good honours degree, preferably in one of the biological sciences, although geology will also be considered) in Science in the Department of Adult Education—The Registrar, The University, Hull (May 1).

Research Assistant (with a degree in botany, biochemistry, microbiology or genetics, and special interest in genetics, cytology, or cellular metabolism) in the Department of Botany (Prof. D. Lewis)—The Secretary, University College, Gower Street, London, W.C.1 (May 4).

Lecturer or Assistant Lecturer in Sociology in the Department of Social Studies—The Registrar, The University, Leeds 2 (May 7).

Senior Lecturer or Lecturer (medical graduate) in Anatomy at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, May 10).

Reader in Animal Husbandry and Hygiene at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 14).

Senior Lecturer or Lecturer (preferably experimental physicist interested in the development of rocket-borne equipment for upper atmosphere and space research) in Physics at the University of Adelaide—The Registrar, The University of Adelaide, Adelaide, South Australia (May 15).

Senior Lecturer or Lecturer in Physics at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, May 30).

Lecturer in Psychology at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 31).

Senior Lecturer or Lecturer in Marine Biology, to work at Portobello Marine Biological Station (University of Otago), Dunedin—The Director, Portobello Marine Biological Station, Box 8, Portobello, Dunedin, New Zealand, or The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (June 30).

Assistant Professor (with a Ph.D. degree or equivalent, with teaching specialization in European geography—U S S.R. or physical geography, especially climatology) in Geography—Prof. T R. Weir, Head of the Department of Geography, University of Manitoba, Winnipeg 19, Manitoba, Canada.

Electron Microscope Technician (female) in the Biophysics Department—The Secretary, University College, Gower Street, London, W.C.1, quoting Ref. BioPh./2.

Lecturer in Electrical Engineering (Grade II), both in power and radio subjects—The Registrar, The University, Bristol 8.

Lecturer (preferably with a good honours degree and postgraduate experience of research) in Zoology—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Research Assistants (graduates in physics, chemistry or metallurgy), for programmes in metallurgy and polymer physics—The Registrar, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Research Students in Physics, for work in the following topics: magnetic resonance of the solid state, gas discharge studies, investigations of defects in glass and other fibres, and studies on the properties of oxide cathodes—The Registrar, The University, Keele, Staffs.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Department of Scientific and Industrial Research: Building Research Station. National Building Studies—Research Paper No. 35: The Effect of Impact Loading on Prestressed and Ordinary Reinforced Concrete Beams. By Dr. S. C. C. Bate. Pp. iv+52+2 plates. (London: H.M. Stationery Office, 1961.) 4*s*. net. [101

Colonial Research 1960–61: Reports of the Committee for Colonial Agricultural, Animal Health and Forestry Research; Colonial Economic Research Committee; Colonial Fisheries Advisory Committee; Colonial Medical Research Committee/Tropical Medicine Research Board; Colonial Pesticides Research Committee; Colonial Social Science Research Council; Tsetse Fly and Trypanosomiasis Committee; Director, Anti-Locust Research Centre, and Research Matters not covered by the above Reports of the Specialist Advisory Bodies. Pp. 353. (Cmnd. 1584). (London: H.M. Stationery Office, 1961.) 18*s*. net. [161

The Institute of Physics and the Physical Society. Handbook of Scientific Instruments and Apparatus—1962 Exhibition. Pp. xv+294+80. (London: Institute of Physics and the Physical Society, 1962) [161

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 103: Catch Crops. Pp. 3. Leaflet No. 139: Breeding for Poultry Meat. Pp. 7. Leaflet No. 140: Fowl Typhoid. Pp. 2. (Belfast. Ministry of Agriculture, 1961 and 1962.) [161

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National Union of Teachers. Investment for National Survival. (The Report of an Independent Committee under the chairmanship of Sir Charles Morris on the Expenditure needed for Future Educational Development.) Pp. vi+42. (London: National Union of Teachers, 1962.) [181

National Coal Board (Medical Service). Medical Research Memorandum No. 2: Behaviour of Respiratory Apparatus. By Dr. E. A. Cooper. Pp. v+104. (London: National Coal Board (Medical Service), 1961.) 20*s*. [181

Meteorological Office. M.O. 265a: Hygrometric Tables for use with Stevenson Screen Readings in Degrees Fahrenheit, Part 1. Fifth edition. Pp. 98. 8*s*. 6*d*. net. M.O. 265b: Hygrometric Tables for use with Stevenson Screen Readings in Degrees Celsius, Part 2. Pp. 95. 10*s*. net. M.O. 265c: Hygrometric Tables for Aspirated Psychrometer Readings in Degrees Celsius, Part 3. Pp. 81. 8*s*. 6*d*. net. (London: H.M. Stationery Office, 1961.) [181

University of Cambridge: School of Agriculture. Memoir No. 33: A summary of the papers published by the members of the Staff of the

School of Agriculture and its Associated Research Organizations during the period Oct. 1st., 1960—Sept. 30th., 1961. Review Series No. 16: Agricultural Plant Pathology in Cambridge: a Historical Outline. By Dr. P. H. Lowings. Pp. 26. (Cambridge: The University School of Agriculture, 1961.) 3*s*. [181

Building Research Station Digest (Second Series). No. 18: Design of Timber Floors to Prevent Dry Rot. Pp. 4. (London: H.M. Stationery Office, 1962.) 4*d*. [181

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1042, Vol 254 (11 January 1962): Lead Isotope Abundance Studies on Mineral Occurrences in the British Isles and their Geological Significance. By S. Moorbath. Pp. 295–360. (London: Royal Society, 1962.) 21*s*. [181

The Royal Institution. By Thomas Martin. Third edition. Pp. 69. (London: The Royal Institution of Great Britain, 1961.) [191

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Department of Scientific and Industrial Research. DSIR Studentships and Fellowships, 1962. Pp. vi+26. 2*s*. net. DSIR Research Grants, 1962. Pp. vi+10 1*s*. 6*d*. net. (London: H.M. Stationery Office, 1962.) [231

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CONSERVATION OF NATURE IN BRITAIN

IN opening a debate in the House of Lords on February 28, directing attention to the recent report of the Nature Conservancy*, Lord Shackleton initiated what Lord Hailsham afterwards agreed was the first debate of any length in either House of Parliament on the Conservancy. Lord Shackleton pointed out that something like half the income of the Conservancy was spent on research, but the main emphasis of his speech was on the necessity for an ecological approach to the whole problem of conservancy and the vital importance of public understanding and co-operation if the work of the Conservancy was to be effective. He was concerned also that the resources available to the Conservancy were inadequate and that in consequence it was unable to make any really effective attack on many problems with which it was confronted or even to embark on publicity on a sufficient scale to ensure public support. He instanced in particular the lack of effort to protect the public interest in such matters as the use of toxic chemicals, provision of advice for the Department of Technical Co-operation on nature conservation in Africa, and the waste of public money in certain matters, such as the bounty given for the destruction of grey squirrels in the absence of adequate investigation.

This concern about adequate financial resources and staff and about public co-operation was freely expressed by other speakers in the debate, for example, by Lord Forbes, Lord Wilmot of Selmeston, Viscount Massereene and Ferrard. Lord Methuen strongly urged the ecological aspect, referring particularly to the international aspects of conservation, as did Lord Douglas of Barloch, Lord Chorley and the Earl of Northesk, and Lord Walston stressed the need for greater co-operation and co-ordination between the Conservancy, the Agricultural Research Council, the Council for Scientific and Industrial Research, and the Medical Research Council as well as with the universities. He also referred specifically to the question of oil pollution, and expressed the hope that the Conservancy would take a strong line at the Inter-governmental Maritime Consultative Organization's Conference on this subject on March 26, and that international agreement would be reached which would prevent this serious menace to wild-life, especially birds, through the disposal around Britain's shores of vast amounts of oil. There was general concern over the use of toxic chemicals in agriculture and also for spraying roadside verges.

Lord Hailsham, in replying as Minister for Science, pointed out that since 1955 the grant to the Nature Conservancy had increased from about £200,000 to £535,000, and in 1962–63 was likely to be £590,000, and he did not doubt that it would continue to increase until it eventually reached its optimum. Lord Hailsham fully recognized the importance of education and public relations in the work of the Conservancy, especially in conservancy, and it was because of this dependence on co-operation with the farming community, the local authorities, landowners and bodies like the Wildfowl Trust as well as with the general public that its constitution differed from that of the other Research Councils. The difference was reflected in its annual report which, Lord Hailsham claimed, was not only larger but also had a wider appeal than those of the other Research Councils.

Up to now, 95 nature reserves, covering 180,000 acres, had been declared in Great Britain, out of the 250,000 acres spread over 140 reserves in the original programme, and as this programme approached completion the Conservancy should be able to concentrate increasingly on research and advisory work, and Lord Hailsham was particularly anxious to encourage the Conservancy, within its resources, to build up its ecological research. In this connexion he stressed the long-term character of the work, instancing that on woodlands and population dynamics and coastal erosion. As regards the use of toxic chemicals in agriculture, he said that the new experimental station at Monks' Wood would be undertaking work on the effects on wild-life and the natural environment. He fully supported the recommendations of the Select Committee on Estimates for increased research grants to universities and other institutions, and the Conservancy's expenditure on such research grants had risen from £9,000 in 1954–55 to £29,000 in 1960–61, and the grants were intended both to foster ecological research and a more comprehensive teaching approach, as well as advanced training in conservation. Lord Hailsham indicated that he expected later this year the report of the Committee which, under Sir William Slater, was reviewing the present organization of research in fisheries, forestry, hydrology and other subjects, and he thought that the biological subjects were probably on the verge of great developments which might well transform them in the next fifty years as the physical sciences had been in the past half-century. We must make sure that our organization of scientific research is such as to encourage developments in Great Britain adequate in scale and quality to permit speedy growth and exploitation.

Lord Hailsham referred to the serious effects of the use of herbicides on the flora of roadside verges and claimed that the work of the Conservancy had spared us from the worst features of the destruction that had occurred in other countries. On the question of international conservation, while he gave no indication of the scale of support through the Department of Technical Co-operation or of its adequacy, he fully recognized the value of the Conservancy's contribution. The Conservancy had been prominent in planning the work of the International Union for the Conservation of Nature, particularly the African Special Project, and he thought that this international co-operation was bearing fruit in the constructive attitude of the emerging countries of Africa. If he did not altogether satisfy Lord Shackleton as to the adequacy of the financial resources at the disposal of the Conservancy, so far as international co-operation is concerned, Lord Hailsham said enough to suggest that the Department of Technical Co-operation should be able to count on his sympathy and support if it finds it necessary to ask for greater help and resources to be made available for that purpose.

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* Report of the Nature Conservancy for the year ended 30th September, 1961. Pp. vii+114+14 plates. (London. H.M.S.O., 1961.) 8s. 6d. net.

In his reply, Lord Hailsham also referred to the question of hydrological research which had been specifically mentioned by Lord Shackleton and also by Lord Walston, who was concerned that hydrological research was being held up by shortage of funds, particularly against the background of conservation policy generally. Lord Shackleton had pointed out that we now had to face the consequences of a decision taken in an earlier debate on February 8, to strike out of the Manchester Corporation Bill the part which permitted the Corporation to obtain water from Ullswater. The decision implied that water, and the demands for it, must not be treated in isolation from the other needs of the community. Lord Shackleton said that he was wholly in sympathy with the decision of the House as related to the amenity factors of Ullswater, but Britain's supplies of water were not unlimited and within the next thirty years we would almost certainly have to seek water sources other than fresh water.

The decision on Ullswater thus accentuated the need for hydrological research, to which some reference is made in the Nature Conservancy's Report. The Interdepartmental Hydrological Research Group has been disbanded and replaced since April 1961 by the Committee on Hydrological Research, but the Conservancy is already engaged in investigations in extensive watershed areas, and, in view of its specialized knowledge on biological and physiographical aspects, quite rightly holds that it would be in the interests of economy and efficiency for them to make the necessary investigations into these aspects, while, on the other hand, they are not competent to conduct studies into engineering and physical aspects, which are of equal importance. Discussions are continuing with the view of deciding how best a research programme should be drawn up and implemented, and the Conservancy has already urged at a conference last June of the British Waterworks Association that foresight and co-operation were required if competing claims to the use of water were not to lead to friction and wise policies were to be widely accepted.

The Conservancy observes in this annual report that, although some experiments are being pursued, money is needed for adequate scientific research, and it seems that the concern which Lord Shackleton and Lord Walston expressed as to whether the Conservancy had adequate funds at its disposal to implement urgently needed research on a sufficient scale in this field is well founded. On this point the Minister for Science was not entirely reassuring. He agreed that we have to learn more about the factors affecting our water supplies if we are to make the best use of them and pointed out that the Committee on Hydrological Research brought together the various interests and bodies with special responsibilities here, including the Geological Survey, the Meteorological Office and the Hydraulics Research Station of the Department of Scientific and Industrial Research, as well as the Nature Conservancy. The latter's special contribution was in the study of the influence of different forms of land use and land management on the evaporation and run-off elements in the hydrological cycle. The Conservancy was conducting investigations and experiments in the extensive watershed areas and was providing staff for a new Catchment Research Unit to be established initially at the Hydraulics Research Station at Wallingford.

If this debate brought out the critical importance of taking a comprehensive view of demands made on national resources—Lord Hailsham himself admitted the need for a co-ordinating Minister in respect of hydrological research—the earlier debate on the Manchester Corporation Bill also emphasized the need for greater co-operation and co-ordination. The issue in that debate was not so much whether the part of the Bill relating to waterworks should be approved or not—these clauses were almost unanimously opposed—but whether they should be deleted forthwith or examined in detail at the committee stage. It was not in dispute that the Manchester Corporation must get more water from somewhere if the situation by 1970 was not to become critical: the issue was rather whether the promoters of this Private Bill should be allowed to present their case to a Committee of the Whole House where the issues could be fully and expertly and impartially examined, though in practice this would be mormally limited to the arguments presented by the promoters.

Opponents of this part of the Bill—the only part which was opposed—did not always recognize the extent to which the Manchester Corporation had sought to meet its critics and ensure that the scheme did as little damage as possible, but this is not really relevant to the major issue any more than is the alarm caused by the impression of haste and secrecy which was conveyed to some by the way in which the scheme was handled by Manchester authorities. The real issue is that a single waterworks authority, however important, should be able to advance an independent proposal threatening to spoil a particular national interest—that of amenity in a national park—and it is made the more intolerable by the admission that the proposal offers only a temporary solution and that ultimately resort must be had to distillation of sea-water or like means. As the late Lord Birkett emphasized, in what was possibly the decisive speech in the debate, we should be assured that really serious search had been made for alternative sources before proposals involving a national park were advanced, and full explanation and reason should be given for the rejection of alternatives. Such a proposal should only be possible on the basis of a national water policy and of an authoritative survey of water resources and of future demand. The decision should be a national decision and not one advanced by any individual authority or on Departmental judgment.

This view was cogently argued by Lord Silkin, who was prepared to let the clauses go forward for examination in committee, and by Lord Rea, and, as was pointed out by Lord MacAndrew, it is a principal conclusion of the Central Advisory Water Committee's sub-committee's final report on the growing demand for water that it should no longer be left to independent initiatives to develop Britain's water resources according to their separate needs. "A separate central authority, accountable to the Minister, should be set up to promote an active policy for the conservation and proper use of the country's water resources." The sub-committee saw no reason to doubt that the rainfall in England and Wales is sufficient to ensure an adequate supply of water to all parts of the country provided proper means of conservation and distribution are developed to keep pace with growing demand, and while Prof. Gordon Manley has expressed the view that ultimately it may be necessary to meter all supplies, he considers that in the meantime the proposed regional authorities will supply a much-needed organization.

The core of the situation is that water is now far more than a local problem, and, as the sub-committee concludes, it should no longer be left to independent initiatives to develop the country's water resources according to their separate needs. That is made equally plain in the debates on the Manchester Corporation's Bill and the Nature Conservancy. The future of Ullswater, alike from the point of view of amenity and of water supply, is a national matter and should be treated as such. The Minister of Housing and Local Government has rightly asked for reasonable time to study the report on water supplies which was only published a week before the debate, and, while the sub-committee considered anything in the nature of a national water grid was impracticable, its recommendation for the creation of new river authorities to manage the water resources of river basins as a whole, and responsible for the preservation, control and development of water resources, both surface and underground, by storage and other means, would prevent the recurrence of such a situation as was created by the Manchester Corporation Bill. Much would, of course, depend on the resources, as well as the powers, available to the proposed authorities; some provision is clearly required for reconciling impartially other national interests, such as amenity, where they clash with the national need for water and ensuring that cost is considered on a national and not a local or even regional basis.

Again, if such alternative sources of supply as the distillation of sea-water or the re-use of water by industry are to receive fair consideration, adequate resources for investigation must be available, and this should reasonably be a national charge. Prof. E. D. Howe, director of the Salt Water Conversion Laboratory of the University of California, recently expressed the opinion that in spite of substantial improvement in costs and in the performance of desalting equipment effected in the past ten years, basic scientific and engineering investigations were badly needed into existing and hitherto unused processes for obtaining fresh water from sea-water. Two British companies already specialize in such plant and between them have installed most of the world's land-based sea-water converters, but cost is clearly recognized as a major difficulty, though it is believed that the cost could be reduced to 2*s.* per 1,000 gallons within five or ten years, particularly if the Government assisted with development contracts. There is no prospect of a revolutionary method of obtaining fresh water from sea-water at a negligible cost.

Meanwhile, the Minister of Housing and Local Government has agreed to call a conference of authorities which are directly affected by the proposals of Manchester regarding Ullswater and Bannisdale to consider how the need for additional water supplies for the Manchester region can be met, and over this Conference the Parliamentary Secretary to the Ministry, Lord Jellicoe, is to preside. At a conference in Manchester on March 5 of nine water authorites which take water in bulk from Manchester, a technical working party was appointed to study the water supply situation in the north-west region, and it is hoped that the preliminary views of this working party will be available for Lord Jellicoe's conference. Even more important, however, is an early Government decision on a national water policy and the precise means by which such a policy should be implemented.

In replying on the debate in the House of Lords on February 8, the Minister for Science seemed to lean to an investigation of the situation by a Select Committee, particularly in regard to alternative sources of water supply, though as regards the distillation of sea-water he was scarcely more encouraging than Lord Jellicoe, who merely quoted from the report of the sub-committee on the growing demand for water that while the distillation of sea-water might find some application in coastal areas in general it was likely to be more economical to transfer water from other catchments to the extent to which it was available. The use of the term economical, however, avoids the real issue, and in neither debate did the Minister for Science sufficiently emphasize the importance of a national policy in regard to national resources whether of water supply, of land, of fauna or of flora, and the futility of piecemeal decisions. Both debates in the House of Lords and the report of the Nature Conservancy itself point decisively to the urgent necessity of a national policy in such matters and as corollary to the need for an organization adequately staffed and equipped to provide the facts on which wise decisions can be made in time and not after events have taken charge of the situation.

A NEW HISTORY OF CHEMISTRY

A History of Chemistry

Vol. 2. By Prof. J. R. Partington. Pp. xxiv+795, with 38 illustrations. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1961.) 105*s*. net.

THE appearance of a comprehensive and detailed history of chemistry by Prof. Partington must be hailed as a momentous event in this important field of humanistic science. The author's reputation as an outstanding authority has been long established by his *Short History of Chemistry* and a legion of specialized papers ranging over many aspects of a vast subject. The *Short History* of 1937, reaching a fifth issue in 1960, is universally acknowledged as a masterpiece of accuracy and selective presentation; and this work has now become the harbinger of a massive and fully-documented treatment. Since the classical books of Hoefer and Kopp, dating back to the 1840's, there have been other histories of chemistry, but in general these carry no detailed references to original sources. Moreover, both the science of chemistry and research in historical chemistry have made enormous advances in recent times.

The work is to be issued in four volumes, of which the first to appear (Volume 2) traverses the period 1500–1700, marking the slow extinction of alchemy and the gradual opening of the road leading on to modern chemistry, through the era of phlogiston. Volume 3, 1700–1800, already in the press, deals with the progress of chemistry in France, Scandinavia, Germany, Great Britain and Ireland, passing through the later years of the phlogiston theory to Higgins and Dalton. Volumes 1 and 4, both in preparation, will be concerned with the earliest (to 1500) and latest (from 1800) periods.

To his formidable task the author brings the fruits of extensive reading and research. A work of this scope, marked by sound scholarship, meticulous documentation, accuracy of detail, and copious quotations from original sources, would normally call for the united efforts of a team of specialists; it is therefore amazing to find a single author producing a massive work of this character.

Space allows of the mention of only a few specific points, taken in illustration from the twenty chapters. Essentially, the treatment is based on a biographical plan, lending significance to the humanistic aspect of the subject. Chapter 1 ("Empirical Prelude") leads off with the versatile Leonardo da Vinci, followed by Eck of Sulzbach, Cardan and Porta, with sections on books of poisons and books of secrets. Of Cardan, it is related that he lost a fortune at chess, sold his wife's jewels, and acquired a great reputation by travelling in 1552 from Milan to St. Andrews and curing Archbishop John Hamilton, to the great envy of the Scottish practitioners. Chapter 2 ("Technological Treatises") tells us that Biringuccio, whose *Pirotechnia* appeared in 1540, had an extensive knowledge of metallurgy and technology, including "such politically useful branches as gun casting, the manufacture of gunpowder, and making false coin". Here, as in general throughout the volume, there is a summary of the bibliography of the author's important writings; a note on his life and career; a full documentation of leading statements; and also quotations in the original language and in English.

A detailed bibliography relating to Paracelsus (Chapter 3) illustrates the great attention that has been devoted to this vivid and blustering man, not least in modern times. Among his iatrochemical successors (Chapter 4) comes Thurneisser, a character little less picturesque than Paracelsus himself. Son of a Basel goldsmith, "in consequence of having sold a gilded brick to some Jews, he left for England in 1548," at the age of seventeen; travelling extensively, he developed into a charlatan and business man, resembling Paracelsus in "Geist und Betriebsamkeit". In this chapter it is pleasing to find mention of Mylius and Stolcius, two of many intriguing alchemical figures whom historians of chemistry have generally ignored.

"Basil Valentine" (Chapter 5) is described as, after Geber, "the literary forgery which has misled and perplexed chemists for the longest period". Dealing later with Francis Bacon and others, the author considers the development of philosophical thought in the supersession of the views of Aristotle, with their medieval accretions, by the new ideas of Bacon, Descartes and Gassendi, stressing thereby the importance of including a concise account of philosophical systems which have influenced chemistry.

Judging from the quality of the initial volume, there can be no doubt that this erudite and authoritative work will stand out as a landmark in the literature of historical chemistry. JOHN READ

SURVIVAL AND SEXUAL SELECTION

Theory of Sexual Selection

By L. S. Davitasvili. In Russian. Pp. 538. (Moskva: Akademia Nauk S.S.S.R., 1961.) n.p.

SINCE "very many biologists who work on the evolutionary problem reject the theory of sexual selection, or merely ignore it", the author has set himself the task of collecting carefully any facts which might serve to support the theory as originally formulated by Charles Darwin. Darwin's term "secondary sexual characters", as well as that of J. Huxley, epigamic characters, are considered too broad, and he proposes a category of perigamic characters defined as "such traits of the structure and behaviour of animals which arise and develop through sexual selection". A chapter is devoted to criticisms of other writers on the subject—largely Russian—though the views of Pycraft and J. Huxley are also discussed, not very favourably. In formulating the basis of his own approach to the problem, the author admits that "of course, nobody saw how species are transformed under the influence of sexual selection", but he considers it legitimate to regard as highly probable such influence in the cases when certain unusual structures cannot be explained by the requirements for obtaining food, movement or defence from enemies.

Fourteen chapters are devoted to a review of the supposed perigamic characters in a number of animal groups, with particular emphasis on the extinct vertebrates—the author being a palæontologist. Recent cephalopod molluscs, with their highly developed nervous system, colour changes of males and their fights during mating period are regarded as undoubtedly offering examples of sexual selection; more daringly, perigamic importance is postulated for the external protuberances and, especially, the ligulæ of Ammonoidea. The importance of sexual selection in the living Arthropoda (Crustacea and insects) is regarded as beyond doubt and, therefore, only a short chapter is devoted to them; recent fishes, of course, offer much support to the author's theories, but he also ascribes perigamic function to such features of fossil fishes as the spiral 'nose' of *Helicoprion*. Again, recent reptiles, with their extraordinary crests, folding collars, bright coloration, etc., serve to encourage his search for unusual structure among the fossil members of the group, and the extraordinary crests of some dinosaurs (especially the *Pelicosaurus*) are discussed at length; the views of Romer, Colbert, Yonge and others regarding possible functions of these structures are dismissed and there is no choice left but to postulate their importance for sexual selection. Armoured dinosaurs, stegosaurs, ceratopsids, etc., are treated in the same way and even the enormously thick skulls of Pachycephosauridae are regarded as perigamic characters, which would be useful during fights between males, if they had this habit.

Coming to birds, the author rejects suggestions of J. Huxley and Boback that bridge plumage of males distracts attention of enemies from sombrely coloured females and is, therefore, favoured by natural selection. Mammals, again more particularly fossil ones, offer a wealth of cases of extraordinary development of horns, tusks, etc., which are all interpreted to favour the author's views, and he criticizes severely those of J. Huxley, Simpson, Schmalhausen and Hersh who regarded such structures as a result of allometric growth and hypermorphosis.

The author's final conclusion is that "the sexual selection is merely a special form of natural selection". The book is well produced, with abundant, though not original, illustrations, and the bibliography of 20 pages includes references to some foreign and Russian papers as recent as 1960.

PATHOLOGY OF THE BANANA

Banana Diseases, including Plantains and Abaca

By Prof. C. W. Wardlaw. Pp. vii + 648. (London: Longmans Green and Co., Ltd., 1961.) 130*s*. net.

WARDLAW'S *Diseases of the Banana*, published in 1935, has until now remained unchallenged as the best available text-book on the subject; but inevitably much has happened in a quarter of a century to make it out of date. The banana industry, the plant pathologists who serve it, and all who are scientifically interested in tropical agriculture, must therefore be glad that Prof. Wardlaw has been able and willing to write a new book to replace his earlier one. He has unique qualifications for the job and has done it beautifully, combining his first-hand knowledge of developments in the field with an impressive diligence in the handling of the literature —up from 559 references in 1935 to more than twice as many to-day. Like its predecessor, *Banana Diseases* deals with the pathology of the plant in the field and of the fruit in transit from field to consumer. The post-harvest diseases and the wastage they cause in shipment occupy about one-fifth of the text.

Three chapters set the background for a detailed discussion of the various infectious diseases of the crop in the field by presenting an outline of the botany and cultivation of the banana, followed by an account of the numerous non-infectious disorders caused by unsuitable climatic and cultural conditions such as soil deterioration, poor drainage, drought and mineral deficiencies.

Of the individual diseases in the field, banana wilt, or Panama disease, still takes the most prominent place. Though controlled in practice in an increasing number of producing countries by change of variety from Gros Michel to various clones of the Cavendish Group, Panama disease continues to challenge the pathologist with baffling scientific problems of considerable economic importance. Consequently, a great deal of research has been done on it since 1935 and investigations continue. The two chapters devoted to Panama disease provide an excellent critical summary of the present state of knowledge on this subject.

Second place is now held by leaf spot, or Sigatoka disease, and the two chapters on this contain some of the most important new material in the book. They include the astonishing story of the critical years in which leaf spot suddenly threatened the survival of the trade in the Western hemisphere, and the heroic measures by which it was brought under control—probably, as the author says, one of the greatest achievements in the history of phytopathology. They also present a thorough account of subsequent improvements in control measures, including the introduction of oil spraying. It is especially in this section that the reader is made aware how far the industry has moved in recent years towards more intensive methods.

Chapters on the virus diseases, and on the lesser. but not unimportant, bacterial and fungal diseases of the plantations, are all equally up to date.

Detailed accounts of the many fruit diseases that become important after harvesting are prefaced by an outline of the processes of harvesting, packing, transport and ripening, and a chapter on the causes and extent of wastage in harvested fruit. The changes in the techniques on the handling side of the industry have been less conspicuous than those on the growing side; but there have been steady improvements, which are well summarized.

There are short but useful appendixes on nematode diseases, the weevil borer, and deterioration of Manila hemp. The book is elegantly produced, as its scientific quality deserves, and well illustrated.

E. E. CHEESMAN

HORTICULTURE: 1958 VINTAGE

Advances in Horticultural Science and their Applications

Proceedings of the 15th International Horticultural Congress, Nice, 1958. Vol. 1. Edited by Jean Claude Garnard. Pp. lxxxi + 546. (London and New York: Pergamon Press, 1961.) £20 net per set of three volumes.

INCREASINGLY, reports of international conferences are coming to be accepted as works of review and reference. In this respect a report on a horticultural congress is at a disadvantage; the subject-matter is varied in character and must cater for the interests of growers and of research workers with widely differing backgrounds. Nevertheless, if the format and price of the three volumes reporting the Horticultural Congress at Nice are to be justified, it is as works of reference that they must be assessed.

The four "general lectures" which are printed in this first volume were presumably intended to give members of the Congress opportunities to hear recognized authorities give short accounts of their own fields of work. In printed form such lectures are of more limited value and in parts read too much like potted versions of the subjects under discussion. Some interesting hypotheses and concepts concerning morphogenesis in plants are introduced by R. Bouillenne in "Hormones Végétales, Organogénèse, Sexualization et Floraison" and by S. J. Wellensiek in "Theoretical Backgrounds of Flowering". It is unfortunate that while presented in the form of short reviews these papers include no lists of references.

After the general lectures comes a series of symposia dealing with growth substances, fruit ripening, soilless culture, diagnosis and control of virus diseases, mineral nutrition, uses of plastics in horticulture and heterosis and breeding of horticultural plants. Matching this range of subjects is the range of approaches adopted by the contributors. There are critical reviews incorporating the contributor's own research findings like the papers on fruit senescence by A. C. Hulme, and on chemical control of growth and flowering by J. P. Nitsch; there are papers which are primarily experimental like those on strawberry nutrition by C. Bould and on foliar diagnosis of mineral deficiencies by P. Prevot and M. Ollagnier, and there are papers which are largely descriptive like those on "Commercial Experiences in Soilless Culture" by S. R. Robins, and on "Plastics for Horticultural Use" by E M. Emmert.

The remainder of this first volume is taken up with an impressive number of papers on vegetable crops, their culture, breeding, physiology and diseases. Here, most of the contributors present results of their own experiments, and the papers together form a valuable collection of information which, except for this publication, must have been scattered through many journals. The strong international character of the Congress is evident both in this section and in the symposia.

Although misprints can be found, the standard of production of the book is generally high, the figures and tables are very clear, and the abstracts in English, French and German which precede each contribution are a helpful feature. The table of contents would have been better placed immediately after the preface instead of after about sixty pages of information concerning the Congress; some of this might have been better in an appendix.

The delay in the publication of the proceedings of the fifteenth Congress, held in 1958, is regrettable, but regret can be tempered with relief that they have appeared before the sixteenth International Horticultural Congress takes place in Brussels later this year. G. P. HARRIS

ANIMAL COLOUR AND ANIMAL COLORATION

The Nature of Animal Colours

By H. Munro Fox and Gwynne Vevers. (Textbooks of Animal Biology.) Pp. vii + 246 + 8 plates. (London: Sidgwick and Jackson, Ltd., 1960.) 42*s*. net.

IN view of recent discoveries and important advances in our knowledge of animal colours, especially due to the application of new and improved techniques, a comprehensive survey of this field is both timely and welcome.

It is more usual to consider animal coloration in relation to the environment and its value to behaviour and survival, but with its reminders of the existence of internal colours and functions of pigments other than visible coloration, this book, concerned with the nature, origin and fate of the colours, provides a valuable corrective and so opens up wider perspectives.

Of the two categories of animal colour—structural and pigmentary—the former, dependent on the physical nature of the surface and the effects of interference, diffraction and light scattering, has a brief but adequate description, while the latter, dependent on chemical constitution in relation to absorption spectra, is dealt with in detail.

The various chemical groupings of the pigments are reviewed in turn, and this supplies information on their occurrence, structure, varieties and variations, physical and chemical characters and functions. These accounts are up to date, admirable in scope and detail, valuable for the many items of historical and general interest brought together and particularly useful not only for the information provided but also for indications of its limitations and even its complete absence.

Melanin is not the only dark pigment, as the accounts of protein tanning and the ommachromes show, and the latter are unique since genetical research was responsible for their recent discovery. The well-known tyrian purple related to the melanins is not concerned with coloration in animals as it appears in a secretion after it leaves the body. Carotenoids, widespread in animals and combined with proteins or dissolved in fats to give a variety of colours, are obtained in their food, since they cannot be synthesized by animals. They are related to vitamin A and visual pigments. Chlorophyll, the main plant pigment, is absent from animal tissues except for a few symbiotic algae, but derivatives from food form green pigments in a few animals.

Hæmoglobin, as abundant as melanin, is a blood pigment with its primary function respiratory, and only relatively few animals owe their surface coloration to its presence. The same applies to other blood pigments of more limited distribution and the differences in chemical structure are concerned more with their efficiency as oxygen carriers than colour. Colourless respiratory pigments do not appear to exist. Hæmoglobin is broken down to bile pigments. These bilins can colour the integument or internal organs of some invertebrates or calcareous structures such as birds' egg shells, gallstones or molluscan shells.

Apart from activity in the tanning of proteins and synthesis of melanins, quinones appear as pigments in echinoids and scale insects (cochineal), but little is known of their origin or function.

These few notes give some indication of the range and treatment of the subject and the successful blend of biochemistry and natural history. There is a vast amount of information and more than six hundred references are listed. The presentation is good with a style clear and precise which maintains and stimulates interest to the end. There are some fine illustrations on eight colour plates.

This book offers both teacher and student much in concise form, and with the final chapter on the study of pigments in the laboratory and the appendix giving a synopsis of animal colours should appeal, as the authors hope, to the sixth form at school and a wider circle from the naturalist to the research worker. E. A. SPAUL

THE SYSTEMATICS OF MAN*

By BERNARD CAMPBELL

"SYSTEMS do not exist in Nature, but in men's minds." This observation of the physiologist Claude Bernard is scarcely acceptable to-day, but it does emphasize the strong inclination which the brain displays to systematize subjectively. Systematics is the investigation of the order and pattern which exists in Nature, and it is not a simple matter to develop such a science on a strictly inductive basis without being influenced by preconceived hypotheses.

But this is not the only fundamental problem facing systematists. It is necessary to record precisely the system as it exists, and this is profoundly difficult to do. It is clear, for example, that a numerical symbolism is more suited than a verbal one, to continuous as distinct from discontinuous phenomena. The more continuous the data become (that is to say, the more data at our disposal) the more inadequate will appear the taxonomic verbal symbolism of binary nomenclature.

Although a number of taxonomists (for example, Mitchener[1]) and most botanists can only aim to provide a purely static or phenetic, rather than a phylogenetic or phyletic classification, as a result of the limited data available, it is clear that to be most useful a classification must carry the maximum information content, and so must, where possible, be phyletic. In the study of man we have a fossil record, and there is no reason to aim at anything less than a full phyletic classification.

Before consideration of the systematics of man, the term 'man' requires definition. Oakley[2] has defined him as the "tool-maker", and this is the most valuable definition that students of man possess. The presence of stone tools accompanying fossil discoveries is easily recognizable, and while it cannot always be certain that the men associated with the tools made them (rather than their neighbours) it is normally a safe assumption. It is, however, an ethological character, and a more appropriate definition for the purposes of this article is by a recognized taxon—the family of man—the Hominidae. This family is generally recognized to contain two genera of undoubted tool-makers—*Homo* and *Pithecanthropus*, and one further genus *Australopithecus*, the present members of which were probably tool-makers and almost certainly tool-users. Verworn[3] called tools "petrified ideas", and it can be assumed that these remarkable ideas were introduced by members of the genus *Australopithecus*. All three genera have been recognized in all three continents of the Old World with the exception of *Australopithecus*, which is not at present known from Europe.

The Hominidae are characterized by a combination of the following main evolutionary trends:

(1) Bipedalism, together with associated skeletal changes.

(2) Changes in the position of the head on the vertebral column resulting from the erect posture, and associated modifications of the face, neck and jaws.

(3) Reduction and modification of the teeth. Hands and tools replaced the use of teeth for many tasks.

(4) Fœtalization: that is, the retardation of both ontogenetic development and the onset of sexual maturity, which have resulted in a prolonged juvenile learning phase and greater plasticity of behaviour.

(5) Lack of specialization, making possible the exploitation of a variety of environments. In particular the dentition and digestion are omnivorous, and the hand versatile, and prehensile.

(6) The development of social life, tool-making and speech.

(7) The increase in the absolute and relative size of the brain, and in particular the frontal region.

In a recent publication, Huxley[4] has described the Hominidae as Psychozoans, as distinct from Metazoans. The unique quality of the human animal implied by this 'grade' name leads to consideration of the peculiar problems of Hominid, or Psychozoan, taxonomy.

Special Problems of Hominid Taxonomy

Man cannot readily investigate himself objectively. This would appear to be for two reasons: first, man projects on man a reflexion of his own psyche, and secondly, man can communicate with man, and cannot treat his fellows as non-sentient objects. The discussion which is at present current as to whether a social science is a logical possibility can equally be applied to all anthropological sciences. In a recent review, Horton[5] has written: "The differences of attitude which underlie the category barrier human–non-human are far greater than those which underlie the category barrier living–non-living". It is scarcely possible for man to investigate man or his remains with the dispassionate and objective manner that he accords non-living matter, and attempts to accord living matter. In this sense, human taxonomy is a more difficult science than most, and the difficulties have been enormously accentuated by the fact that some of those who have published their opinions on the subject in the past have received little scientific training, let alone taxonomic training.

A subsidiary problem is that the experimental method, the cornerstone of scientific investigation, cannot be applied directly to help solve the problems involved in arriving at a taxonomy of living man. Transplant experiments in botany, and the use of controlled environments in zoological studies has given a new tool to taxonomists of living forms. Studies of *Homo sapiens* are limited to observations in an environment that cannot be controlled. The science is descriptive rather than experimental.

The first stage in taxonomic research involves a judgment of overall affinity: morphological, physiological, biochemical, genetic and ethological characters are involved. The second stage involves a qualification of such judgment to assess phyletic affinity.

It is clear that in many instances judgments of morphological affinity can be made by a taxonomist by inspection without much possibility of gross error, and this is especially true when a collection of specimens represents groups related, but not too closely related. Controversy arises when the issue is less clear. Hominid taxonomy has now reached the stage when many new taxonomic judgments are controversial, and it is therefore essential that the subject should be put on a more objective basis.

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*Substance of a lecture given before the Systematics Association on February 8 at the British Museum (Natural History).

Living Man an Animal

Man has not always recognized himself to be a kind of animal, and although Linnæus classified man with the apes in his *Systema Naturae* as Anthropomorpha, his concept of their relationship was based on phenetic similarity as had been Aristotle's when he classified man with other mammals 2,000 years earlier. It was La Mettrie in 1748[6] who not only presented a remarkable account of the similarities of man and animals, but also recognized the organic unity of Nature.

Those who accepted the idea of living man as an animal were then faced with the problem of whether he consisted of one or more zoological species. This difficulty was accentuated by lack of knowledge of the dark-skinned races of man, and by the overwhelming Biblical authority for the existence at some past moment of a single pair of human ancestors. On one hand, there was evidence of strange 'wild men', differing very much from ourselves, and on the other hand good reason for supposing that on Biblical authority we all shared a common and recent ancestry. Before the nineteenth century it took courage to suggest any deviation from the Biblical account of creation.

In his early editions of the *Systema Naturae* (1735–56), Linnæus clearly divided *Homo sapiens* into four varieties (Americans, Europeans, Asians and Africans) though in the tenth edition (1758) he adds wild men and monsters as two further sub-divisions. (At the same time he classified the orang-outan as a species of *Homo* in the tenth edition (as *Homo troglodytes*), but this species was transferred to *Simia* by the twelfth edition (1766).)

Buffon[7], writing at the time of Linnæus, was clear, too, in his mind that man originated from one species; but he believed that climate and other external factors had caused varieties to become specifically distinct in the passage of time (1749). Immanuel Kant[8], in 1775, argued that all human beings belong to the same natural species "since in mating they always beget fertile offspring however dissimilar the parents may be in appearance. For this unity of species, he wrote, "there can be but one natural cause—that all men belong to a single stock". Blumenbach[9], the father of anthropology, supported this view the same year, in his M.D. thesis.

But by the nineteenth century, the subject had become increasingly controversial and many authors, breaking free from the teaching of the Church, were arguing for a polyphyletic origin for mankind, which involved the recognition of many species derived from different pre-*sapiens* forms. Quenstedt, the famous palæontologist, wrote that "if Negroes and Caucasians were snails, zoologists would universally agree that they represented two very different species, which could never have originated from one pair by gradual divergence". But with belief in special creation still common, the zoologists of the past century faced a difficult dilemma: Bory de St. Vincent[10], Haeckel and many others were prepared to recognize twelve or more species of *Homo*. On the other hand, zoologists, such as Lamarck and Cuvier, still supported the idea of a single human species.

Darwin[11] brought an end to many of these difficulties however, and in 1871, though he formally left the choice of taxonomic characters open, he was in no doubt that all races of men were quite recently descended from a single primitive stock. Like T. H. Huxley, he always considered man to be a single species, and frequently commented on the physical and temperamental similarities between the races. But he considered the definition of the term species to be a matter of opinion.

To-day, a species is usually defined as "a group of actually or potentially interbreeding natural populations reproductively isolated from other such groups"[12], though some workers in this field still consider the problem of the definition of the species to be academic[13] or inappropriate to taxonomic method[14]. Wherever, among *Homo sapiens*, racial contact has been made, fertile hybrids have resulted. While some groups have no contact with others (for example, Australians have not met Eskimos) this would not invalidate the concept of a 'Rassenkreis'—a polytypic species of world-wide distribution. The extent of man's ecological adaptation and geographical distribution is, apart from his parasites, unique.

Choice of Taxonomic Characters for the Sub-division of *Homo sapiens*

Since Darwin's book was published, anthropologists have recognized the desirability of producing a phyletic classification of modern man, based on studies of comparative anatomy as well as a purely descriptive one. Many obvious racial characters, such as hair form, were first thought to be non-adaptive, and therefore stable. It is now considered that not only does *Homo sapiens* as a whole confirm Bergmann's, Allen's and Gloger's rules, but also that many other characters, not obviously adaptive, are likely to be so. As they became increasingly critical in their approach, anthropologists were concerned to assess the influence of nature and nurture without the possibility of controlled experiment. Evidence of the plasticity of the phenotype had to be derived from studies of monozygotic twins reared apart, and migrant populations. In spite of the amount of detailed work of this kind carried out[15], Harrison has written recently[16] that the prospect of anthropometric techniques based on the phenotype being confidently used for the solution of historical problems remains bleak.

The discovery of the ABO and other blood groups and the pioneering work of the Hirszfields in 1919 led anthropologists to suppose that they had here a tool with which they could produce a true phyletic classification of mankind. This supposition was based on the belief that this was an instance of neutral polymorphism, that blood groups were non-adaptive characters and therefore environmentally and historically stable. They are furthermore related in a simple and direct way to the genotype, and direct taxonomic access to genes avoided the problems of the plasticity of the phenotype. But since Fisher[17] showed that neutral polymorphism was an improbable condition, the hypothesis of the existence of such polymorphism has had to be slowly relinquished. Designation of a character as non-adaptive is often merely a cover for our ignorance. There now exists evidence of statistical correlation between the human blood groups on one hand, and fertility, fœtal disorders, certain kinds of ulcers and other illnesses, on the other[18]. Blood groups are adaptive, and they may well be historically less stable than polygenic characters such as skin colour, hair form, etc., on which the early anthropologists depended. The constant survival of the disadvantageous rhesus-negative gene can only be accounted for by the hypothesis of balanced polymorphism. We must conclude that phenotypic changes may quite rapidly arise both from the direct effect of a change in the environment, and as a result of interaction of the environment with a polymorphic gene[19], apart from the long-term and normal effects of selection on the pool of genetic variation in a population.

The state of our understanding of human variability is at present very fluid, and the data are rapidly accumulating. Workers are at present primarily interested in the geographical distribution of variation and the reason for it. Since it is not known which characters are of more or less value in human taxonomy, we are obliged to aim at a phenetic (or Adansonian) classification, and consider them all: a conclusion which will not intimidate palæontologists handling three or four geometrical variables of mollusc shells, but to a human palæontologist let alone a neotaxonomist, such a conclusion is not attractive. Nor is it known how to evaluate continuous anatomical against discontinuous biochemical characters. It is necessary, therefore, to follow the subjective opinions of experts, and to treat race as a purely contemporary phenomenon, with no regard to its phylogeny. History and archæology must be relied on to supply most of the required information about the origin of the present distribution of mankind.

Table 1 lists some of the most important characters which are at present being recorded in anthropological investigation divided into two groups: (*a*) Polygenic and continuously variable; (*b*) monogenic and discontinuously variable.

Table 1. CHARACTERS OF LIVING MAN INVESTIGATED IN ANTHROPOLOGICAL RESEARCH

Polygenic and continuous	Monogenic and discontinuous
Morphology of soft parts	Blood groups (11 systems)
Body proportions	Hæmoglobins
Stature	Haptoglobins and transferrins
Head and facial measurements	PTC tasting
Fat deposition	Colour blindness
Hair form, distribution and balding	Gross and lethal abnormalities
Tooth size, shape, eruption	Hereditary diseases and disorders
Skin colour, tanning capacity	
Eye colour	
Growth-rate	

Behaviour and Culture

The existence of culture complicates further the investigation of racial differences. In other groups behavioural characters are being given taxonomic consideration[20], and since the definition of a species is behavioural, it may be asked whether behaviour would not be of special importance to studies of *Homo*. The answer at present appears to be that human behaviour, since it is not instinctive, and is closely bound up with human culture, cannot be treated as a taxonomic character of living man. Whether culture, which is an extension of man's body among other things, should receive consideration in this context seems improbable since a strictly scientific investigation of such a character would be impossible, as it would be carried out by culture itself. Tool-making and fire-making may appear at first sight to be exceptions to this, since their existence can be objectively determined, but Oakley[21] has pointed out that early Hominids are likely to have been good imitators, with the result that cultural advances would spread very rapidly among them. In view of this it does not appear that these characters could ever be used to give precise taxonomic distinctions.

Living Sub-species of Man

The deme or local population is the basic unit of study in taxonomy, and, as the data from populations involves statistical treatment for its analysis, only statistical analysis can deliver fully objective results in taxonomy.

Some statistical work has been done at the level of local populations[22]; but in general no progress in this field can be made until agreement on the selection of taxonomic characters is arrived at from the mass of anatomical and biochemical data at our disposal. At the same time, these data of human variability are still too incomplete to achieve an analysis of man as a species, which will offer us objective sub-specific categories. The methods of statistical analysis which might prove suitable for this purpose are discussed in the second part of this article.

The existing classifications of living man, therefore, are based on a very limited number of characters, subjectively selected and assessed. Bernier[23] was the first man to produce a description of the different "species or races" of men. Linnæus listed the four main geographical groups in 1740 (*Systema Naturae*, second edition) and Blumenbach[9] added a fifth in 1775, the Malayans. In the nineteenth century, taxonomic schemes proliferated, from the idea of three races derived from the three sons of Noah, to Bory de St. Vincent's fifteen species, based on hair form[10]. There is, however, to-day a general consensus of opinion both among serologists and morphologists that there are five or six living sub-species of *Homo sapiens*. The terms 'race', 'stock' or 'variety' have been used indiscriminately, however, for the geographical sub-species of man. Anthropologists are often quite unaware of the current significance of these taxonomic terms.

The most generally accepted sub-species of *Homo sapiens* and their valid Latin trinomials are as follows: Caucasians *H. sapiens sapiens* L., 1758; Africans *H. sapiens afer* L., 1758; Asiatics *H. sapiens asiaticus* L., 1758; Amerindians *H. sapiens americanus* L., 1758; Australians *H. sapiens australasicus* Bory de St. Vincent, 1825; Polynesians *H. sapiens neptunianus* Bory de St. Vincent, 1825. (There are more than 130 binomials and trinomials referring to living man which arise through centuries of taxonomic confusion.) Trevor[24] recognizes five sub-species which exclude Amerindians and Polynesians, and include instead the Khoisan people (mainly the Bushmen and Hottentots of South Africa). A good case can be made for the exclusion of the former two rather recently formed sub-species, but it is not considered here that the separation of the Khoisan people from the African sub-species is justified.

These six groups appear to be typical geographical sub-species, each adapted to and living in (originally) a different geographical and climatic zone. Only in the past 400 years has the distribution been upset to the present extent; but it should perhaps be added that although hybrid populations were few and far between until the modern age, the gene flow was probably not fully interrupted at any point, except for short periods. Man is continuously variable, and so-called pure races are a figment of the imagination.

The two main discontinuities at the Bering Straits and the Torres Straits are comparatively recent. The American Indians arrived in America by land about 20,000 years ago and were cut off about 10,000 years ago: the Australians arrived by sea about 10,000 years ago, and so have never been entirely separated from other men. The islanders of South East Asia and the Pacific are for the most part inveterate navigators.

The hybrid populations which have grown up all over the world in modern times serve to emphasize the genetic compatibility of mankind.

Categories Below the Sub-species

Political capital has been made out of the idea of race, and in particular the idea of pure and superior races. Let it be said that no races are 'pure', nor are any known to be superior: it has not proved possible to devise intelligence tests equally applicable to people of different cultures.

Unless the term 'race' is discarded altogether, it must be used with reference to the next taxonomic category below the sub-species. Dobzhansky[15] defines races as "populations differing in the incidence of certain genes but actually exchanging or potentially able to exchange genes across whatever boundaries separate them". Such boundaries in the case of man are usually spatial and cultural. Races are in no sense static, and new races are to-day in process of formation as a result of hybridization (for example, in Hawaii, North and South America). Many classifications of human races have been drawn up, and estimates of the number of 'races' of man in the literature vary from four (when the authors used the term in place of the term 'sub-species') to more than 200. Coon *et al.*[16] recognized 30 races in a recent book; but this classification was somewhat over-simplified. Trevor[14] presents, perhaps, a more realistic account of this complex matter.

At present, therefore, although data of human variability are rapidly accumulating, and understanding of the interaction between man and his environment is increasing, there is no method of assessing the taxonomic value of the data. There does not exist a classification of living man which is objective, and likely to satisfy everyone. A certain amount of agreement, however, has been reached on the main geographical sub-species of living man, and some of the most distinct racial groups. The systematics of this variable and most investigated of animals presents problems which are at present unique to taxonomy.

Fossil Man

The first difficulty to be encountered in the taxonomy of palæontological material is, of course, the inadequacy of the methodology of taxonomy. While the reality of the biological species at any moment in time is clear, palæontological taxa are subjective categories created by the experienced taxonomist. In palæoanthropology the taxonomically 'convenient' gaps are slowly being filled, and while fossil data are still sparse, many of the missing links in the chain of human evolution have been found (Fig. 1). The record is fairly rich in parts but poor in others, and all the finds are incomplete: for example, we have from central and southern Africa five upper Pleistocene crania without mandibles, and from about the same period in north Africa, four mandibles without crania. Whether these fragments represent the same sub-species cannot be determined.

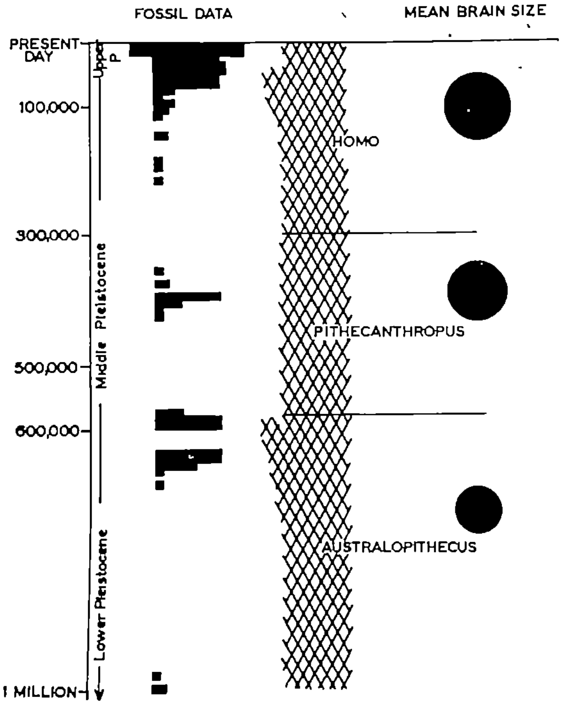

Fig. 1. The past million years of Hominid evolution, showing on the left the extent of fossil data of the three genera. (One small square represents one fragmentary skull, or approximate equivalent.) The central column shows the two points at which fossil evidence indicates that speciation may have occurred. The right-hand column represents the estimated mean brain size of the three genera. The Lower Pleistocene is now recognized to stretch back to between two and three million years; one further fossil has been obtained from this earlier period. The fossil data from recent times is greater than indicated

The difficulty of being objective in this branch of zoology has already been stressed. Each discovery is attended with so much publicity for the discoverer that he frequently become emotionally involved with the precious fossils and is unable to evaluate them in a rational manner. It is for this reason among others that the aim of palæo-anthropologists to-day is to develop an objective methodology in taxonomic work. Therefore, some account of methods of quantitative taxonomy follows, but first there are two more problems to be considered.

The first is the problem of samples. When we consider statistical populations we need to know the mean and variance of the population, and this can be evaluated from a sample of appropriate size. In many branches of palæontology samples of an appropriate size are available but not among the Hominidae. Two assumptions are therefore made: first, that the sample (which may be a single specimen) which we have is representative of the population being studied, and there are reasons for supposing that this may not strictly be the case; secondly, that the variance of the population being studied is similar to that of a modern population of the same kind. These are two rather gross approximations; but they are necessary if we are to extract any statistical information at all from our data, and, of course, they are subconsciously made by any taxonomist making a subjective evaluation from such data. Clearly, the amount of error introduced will be more serious in fine taxonomic distinctions, and much less so in assessing groups of higher taxonomic category.

Whether a number of skulls from a locality belong to one or more populations must be determined in the first place by the archæologist (cf. Brothwell[27]).

Lastly, character selection is of course another sampling problem which applies equally to the experienced taxonomist making subjective assessment of affinity and to the quantitative taxonomist. The difficulty of handling simultaneously the discontinuous and continuous characters of living man has been mentioned, and this problem fortunately does not at present arise in a consideration of fossils, though some blood-group data are now being obtained from the more recent skeletal remains.

It is necessary, however, to face squarely the problem of correlation between characters, since we are dealing with one organ, the skeleton. At the same time the choice of characters is limited, both by the nature of the material and its extent, so that their selection and treatment is of critical importance. It is my opinion that characters should be negatively weighted for both mathematical and functional correlation, and the method to be employed to achieve this is discussed later. If, on the other hand, correlated characters are treated as independent or omitted altogether, distorted results will be obtained from a comparison. Like the experienced taxonomist, we must consider them all, but in relation to each other.

Methods of Quantitative Analysis

The quantitative techniques which have been used in the evaluation of affinity in Hominid taxonomy will now be discussed. Not only is this approach essential to the future development of taxonomy, but anthropologists have been among the leaders in this field, and it is here perhaps that they can best make a contribution to the methodology of taxonomy.

I would first like to mention very briefly the technique of pattern analysis[18]. This is a not uncommon method of handling and comparing taxonomic data, and has been used in many branches of taxonomy. The technique is a quantitative aid to the assessment of affinity, and as it does help us to arrive at a more objective evaluation of the data, its use should be extended and developed.

The more precise techniques fall into two main groups: (1) discriminant functions; (2) measurements of morphological 'distance'.

A discriminant function is designed to determine on the basis of many characters which of two or more taxa a specimen or group belongs to. Proposed by Fisher[29], this multivariate function was used by Bronowski and Long[30] to determine the status of the South African man-apes (the genus *Australopithecus*) over which controversy had raged for many years, and still does. The question was whether the genus belonged to the Hominidae or the Pongidae. Using four measurements on the milk canines of the three groups, Bronowski and Long showed that the man-apes were much closer to the Hominidae than to the Pongidae, and it was therefore suggested that the genus *Australopithecus* should be placed in the family Hominidae. But this use of the discriminant function is open to criticism. In the first place the function was based and evaluated on four measurements of one tooth only, and critics were quick to point out that other characters would give a different answer. This was demonstrated by Ashton, Healy and Lipton[31], who calculated the position of all the *Australopithecus* fossils *vis-à-vis* the Hominidae and Pongidae for all available teeth using 4–8 measurements on each tooth, and they plotted the position of the fossils on two-dimensional scatter diagrams, one for each tooth. No final conclusions could be drawn from their analysis because the results were equivocal, and were not combined in any way. At the same time, the data only concerned the teeth.

While this paper showed very well the variable taxonomic status of the different teeth, it did not inform us of the taxonomic status of the group under analysis. There is, furthermore, a second criticism which must be levelled at the use of the discriminant function for assessing taxonomic status. The use of this function, in the above instance by Bronowski and Long, and in other similar instances, involves the assumption that the two families Pongidae and Hominidae are all-inclusive, and that all new discoveries must belong to one or the other. It did not allow the possibility that *Australopithecus* belonged to a new family, the Australopithecidae, a view which has been held by some anatomists. These two criticisms alone make the discriminant function a very difficult tool to apply. It is potentially most useful when the classification of the specimen is equivocal, and it is precisely in instances of this sort that the new specimen might represent a new taxon. Fisher recommended the use of the function for determining the sex of individuals, and it is indeed ideally suited to this problem, since the two alternatives are inclusive, and the means and variances of the two populations are available—and they are usually not available for problems involving fossils.

The use of two-dimensional scatter diagrams (based on two orthogonal canonical variates) which Ashton *et al.*[31] initiated does seem to be a very useful tool to the taxonomist, and has been utilized more recently by Blackith[32] on insects, and by Jolicoeur[33] to demonstrate the regional variation in the wolf using twelve skull dimensions. With the increasing use of electronic computers complex functions of this kind may be utilized for particular taxonomic problems where the necessary data and facilities are available. Something different is needed, however, from a function of discrimination, in order to facilitate the exact evaluation of taxonomic status. Such functions exist and can be divided into three groups: (*a*) coefficients of similarity; (*b*) simple 'distance' measurements; (*c*) generalized 'distance' measurements.

(*a*) Sneath[34], in his studies of bacteria, developed a simple coefficiency of similarity between taxa that was based on 105 characters (in this example) which either occur in two states (that is, present/absent, plus/minus) or can be treated as such. Sneath has estimated the affinity between his different strains of bacteria by calculating the extent to which different characters in each group occur together. Clearly, if two groups have a lot of characters in common they are likely to be more closely related than two groups with few characters in common. Treatment of continuously variable characters by this method is, however, mathematically unsound and subjective and the method as it stands cannot be satisfactorily applied to Metazoans. Another example of the calculation of the correlation of characters between species has been published by Mitchener and Sokal[35], who recorded 122 characters of 92 species of Megachilid bees, and its taxonomic value appears to be considerable, in spite of a number of criticisms which have been made. (See also Rogers and Tanimoto[36].)

In a modified form this technique might be applicable to living man when enough data are available which can be used for this type of analysis, but will be of little value in evaluating fossil relationships as most of the characters that we have at our disposal (and the exceptions are very few) concern one organ, the skeleton, and are either mathematically or functionally correlated, or most frequently both. The technique does not take into account the varied origin of this correlation and it depends on the data from a very large number of characters to give anything approaching a true picture of affinity. The central problem of taxonomy, moreover, has not been overcome, for the results are still subjective, both as a result of character selection, character coding (that is, continuous characters treated as discontinuous) and character correlation[36].

(*b*) Simple 'distance' measurements or functions of morphological difference have been in use for many years, and were developed by Heinke[37], Pearson[38], Zarapkin[39], Morant[40], and more recently by Penrose[41], Cain and Harrison[42] and Sokal[43]. Like the techniques already mentioned, none of these procedures, with the exception of Penrose's, allows for mathematical or functional correlation between characters, and so will give distorted results when used to analyse the characteristics of a single organ (the skeleton) even though it may reflect a great many other organs. Only Penrose, among them, has introduced a correction factor based on the mean correlation coefficient between every pair of characters (Table 2). The remainder can be of value in comparing the divergence between living taxa, but none of them takes the problem of taxonomic affinity right out of the sphere of subjective assessment, and almost all versions contain fundamental mathematical objections which seriously limit their value. For example, the most recent method proposed by Sokal[43] does not take into account intra-group variation, and the significance of inter-group difference cannot be fully assessed without relating it to the variance within each group. Cain and Harrison[42], on the other hand, handle intra-group variation by considering the "maximum and minimum likely values" of each character, and are thus able to produce a maximum and minimum measurement for the distance between groups. This method has been used recently by Sneath[44]. The formulæ of these various functions, which are very similar, are listed in Table 2.

Table 2. FORMULÆ OF SOME SIMPLE 'DISTANCE' COEFFICIENTS

Pearson (ref. 38): $C.R.L. = \Sigma_1^m d^2/m =$ Heinke's coefficient (1898).

Penrose (ref. 41): $C^2 = \dfrac{\Sigma_1^m d^2 - R[\Sigma_1^m (d)]^2/(1 - R + Rm)}{1 - R}$

Cain and Harrison (ref. 42): $M.C.D. = \Sigma_1^m \delta/m$

Sokal (ref. 43): $C^2 = \Sigma_1^m \delta^2/m$

m, No. of characters; d, difference between the mean values of a character in two populations expressed in units of the standard deviation of one of those populations; R, mean correlation coefficient between characters; δ, difference between the mean values in any two populations expressed as a function of the variability of the character among the different populations.

(*c*) The generalized distance statistic (D^2) was introduced by Mahalanobis[45]. This multivariate function, closely related to the discriminant function already mentioned, will analyse the relationship between continuous characters, discounting both the mathematical and functional correlation between them, and it will produce a true function of morphological distance on the basis of characters which can be quantified between the groups under consideration. Like all these measures, it does assume a common dispersion matrix in the different populations—an assumption which in the circumstances is likely to be justified (that is, normal distribution, similar variance, etc.). A very similar function, A^2, has been introduced by Defrise-Gussenhoven[46].

A further great advantage of a technique based on multivariate analysis is that after a certain point the addition of further data does not affect the accuracy of the result and, therefore, so long as sufficient characters are used, the subjective element involved in their choice is lost. Under proper conditions, therefore, this technique is an answer to many of our problems: its great disadvantage is that it involves a tremendous amount of computation, but with electronic computers, this is no longer of overriding importance. Owing to the complexity of D^2 it has not been used as much as it should be, nor has it yet been adapted for the analysis of discontinuous variation, though this should not present insuperable problems. Examples of the use of this function include Trevor[47], Jones and Mulhall[48], Mahalanobis *et al.*[22], Mukherjee *et al.*[49], Blackith[32,50,51] and Giles[52].

While there is certainly room for improvement and development of techniques of this kind, it would appear that an approach along these lines is the most valuable that exists to elucidate the taxonomic relationships of evolving organisms. Blackith[53] has recently reviewed the broad field to which multivariate techniques can be applied, and Jahn[54] has gone so far as to suggest that the future of taxonomy lies in the use of electronic computers.

From Affinity to Phylogeny

Having reached an assessment of phenetic affinity between fossils, the geographical, geological, and palæontological context in which they were found will give much of the information needed to assess their phyletic relationships. New dating methods appear to give us remarkably accurate data about the relative and absolute age of different fossils[55]. Where a fossil record exists direct evidence can give information about phyletic relationships which can only with difficulty be obtained from living forms[56]. It is recognized that estimates of phenetic affinity between contemporary groups will mask the existence of convergent evolution, but while this may be an important consideration in the systematics of macro-evolution, it does not appear to be a problem involved in Hominid taxonomy, since the evidence at present strongly suggests a monophyletic descent without much speciation. Convergence on a micro-scale within a species lineage is not a factor which can seriously affect our taxonomic conclusions.

In considering the process of Hominid evolution, Mayr[57] and Dobzhansky[25] have both expressed the opinion that speciation has not occurred since the Hominidae branched from the Anthropoid stock: that man has evolved as a single species throughout the period with which we are concerned. On the principle of Occam's razor, it is right not to postulate a plurality of vertical taxonomic divisions without evidence of them. However, at least one occasion when speciation has occurred can now be recognized.

Australopithecus robustus and *Pithecanthropus erectus* both existed, according to the latest estimates based on potassium argon dating methods, at about 0·6 million years B.P. (at Swartkrans and Djetis). Being allopatric, their biological status is uncertain, but on morphological grounds they are certainly specifically distinct. Furthermore, evidence suggests that the two species of *Australopithecus* (*A. africanus*

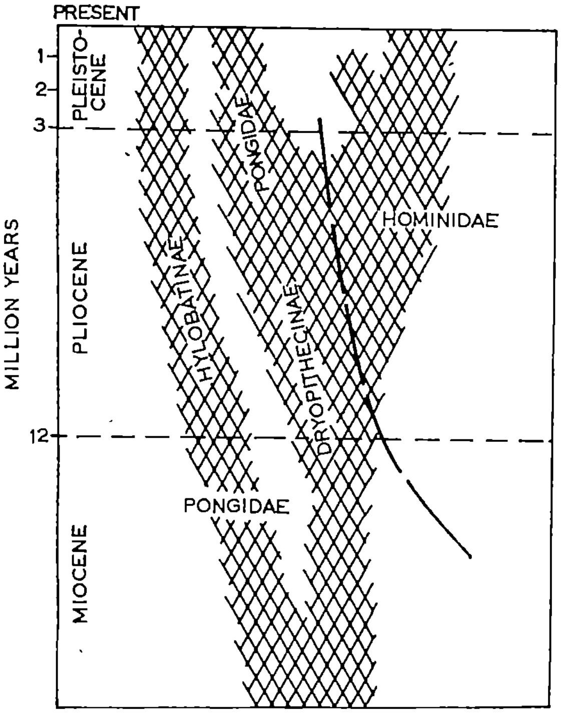

Fig. 2. An imaginative diagrammatic representation of the evolution of the Hominoidea, showing the two families, the Pongidae and Hominidae

and *A. robustus*) are not so much successive forms, but two contemporary and discontinuous groups, since they both appear to have existed over a long period in eastern and southern Africa (1·3–0·6 million years)[21]. *A. africanus* would therefore be likely to be near the lineage leading to *Pithecanthropus*, while *A. robustus*, a coarser and more ape-like form, is probably a side branch which became extinct after the *Pithecanthropus* stage had been reached in Java.

There is one other point at which speciation may have occurred where there appear to exist biological criteria to distinguish Neandertal man in western Europe from the contemporary sub-species of *Homo sapiens*. The latter replaced the former in France very suddenly about 40,000 years B.P., without any known morphological continuity except in past time. If intermediate fossil forms are not discovered in the future which retain genetic continuity with the extreme Neandertal forms, we shall have to recognize western European Neandertal man as a distinct biospecies from *Homo sapiens*. It seems preferable for the present, however, to classify this rather aberrant group as a sub-species of *Homo sapiens*. The same remarks apply to the sub-species *Homo sapiens rhodesiensis* and *Homo sapiens soloensis*, which were terminal sub-species in Africa and Asia of the Neandertaloid type of man. In these instances, however, the evidence of genetic discontinuity is less clear and it seems possible that these forms were absorbed by the advancing groups of modern *Homo sapiens*.

The classification which is presented here (Table 3) does not imply that all known fossils can be confidently assigned to one or other of the taxa. Many must remain in a suspense account, and the temptation to systematize with insufficient data must be resisted. In particular, three genera which have been created on the basis of three important fossils are not mentioned. *Telanthropus* from South Africa is perhaps a South African form of *Pithecanthropus*, but the fragments are too few to reach any certain conclusion. *Meganthropus palaeojavanicus* is perhaps a south-east Asian form of *Australopithecus*, but again the evidence is insufficient. The skull named *Zinjanthropus* is probably a member of the genus *Australopithecus*, but full details have not yet been published about this important find. The so-called *Chellean* man from Oldowai is probably a member of the African sub-species of *Pithecanthropus*, *P. erectus mauritanicus*.

Table 3

Family:	Hominidae Gray, 1825
Genus:	† *Australopithecus* Dart, 1925
Species	† *A. africanus* Dart, 1925
Subspecies:	† *A. africanus africanus* Dart, 1925
	† *A. africanus transvaalensis* Broom, 1926
	† *A. robustus* Broom, 1938
	† *A. robustus robustus* Broom, 1938
	† *A. robustus crassidens* Broom, 1949
Genus	† *Pithecanthropus* Dubois, 1894
	† *P. erectus* Dubois, 1894
	† *P. erectus erectus* Dubois, 1894
	† *P. erectus pekinensis* Black and Zdansky, 1927
	† *P. erectus mauritanicus* Arambourg, 1954
Genus	*Homo* L. 1758
	H. sapiens L., 1758
	H. sapiens sapiens L., 1758
	H. sapiens afer L., 1758
	H. sapiens asiaticus L., 1758
	H. sapiens americanus L., 1758
	H. sapiens australasicus Bory de St. Vincent, 1825
	H. sapiens neptunianus Bory de St. Vincent, 1825
	† *H. sapiens neanderthalensis* King, 1864
	† *H. sapiens rhodesiensis* Woodward, 1921
	† *H. sapiens soloensis* Oppenoorth, 1932
	† *H. sapiens krapinensis* Gorganovic-Kramberger, 1902

When we come to consider the horizontal taxonomic boundaries which sub-divide the Hominidae in time, the position of them and the rank to be applied to them is of course arbitrary. Mayr[57] would place the whole family in the genus *Homo*, while other workers have recognized ten or more genera. Compared with other mammalian groups it may be considered that the length of time of the existence of a taxon is small, and that too much splitting has occurred. It can be argued, however, that the rate of Hominid evolution was very high, and the different genera show the degree of difference appropriate to that category. The genera here used follow the scheme of Simpson[58]. There is, however, much to be said for sinking the genus *Pithecanthropus* to form the species *Homo erectus*, and many workers are now taking this view. This is the kind of problem which only mutual discussion can resolve.

Future discovery will no doubt make it necessary to create many new taxa, and great difficulty will doubtless arise over the classification of fossils from the Pliocene. If Fig. 2 represents in any sense the kind of situation that may be expected, it is difficult to see how the methodology of taxonomy as it stands at present can effectively handle a situation such as this may prove to be. So long as taxonomy is a matter of opinion it will remain a matter of controversy.

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A CHILDREN'S CANCER DEPENDENT ON CLIMATIC FACTORS

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THE incidence of many cancers varies enormously in different races. Notable examples are bowel carcinoma, so common in European races and so rare in the Bantu, and primary liver carcinoma with the reverse relationship. These and other tumours probably owe their differing racial incidence more to environmental than to genetic factors, as negroes in America show a tumour pattern approximating much closer to the white population there than to the pattern observed in Africa[1].

Until recently no cancer has, to my knowledge, been shown to occur only in certain clearly defined geographical locations, and within these boundaries to affect different tribes and races without discrimination.

Five years ago a number of hitherto unconnected tumours affecting children were recognized as being but different manifestations of a single tumour syndrome[2]. At about the same time it became evident that this tumour was very prevalent in certain parts of Africa while it was quite unknown in other parts[2].

The tumour has been histologically identified as a malignant lymphoma[3,4], but the condition is recognized rather as a clinical syndrome than as a specific tumour. Malignant lymphomata, presenting usually with generalized lymph-node enlargement, occur throughout the world. The unusual anatomical distribution of this tumour syndrome[2,4–7] is apparently unique, and with one exception, which will be referred to later, is confined to certain areas of tropical and sub-tropical Africa.

The age incidence differs from that observed in any other tumour. Nearly all cases recorded have occurred between the ages of 2 and 14 years, with a peak incidence at 5 years. The incidence appears to be approximately the same in both sexes[2,5].

This tumour syndrome accounts for more than 50 per cent of all children's cancers seen in Uganda[2,7]. More than 200 cases have been recorded during the last eight years.

This tumour appears to traverse all barriers of race and tribe. Although unknown in India, it has been observed in Indian children domiciled in Africa in the proportion expected from the relative population figures[5].

The clinical characteristics have already been reported[2,5–7]. The distribution of tumour deposits throughout the body is totally unlike that observed in any other tumour. Moreover, the virtually simultaneous development of multiple tumours in sites where tumours were hitherto least expected to occur presents a strikingly characteristic clinical picture. Although there is a remarkably constant pattern of distribution, the presenting features vary, and are dependent on the particular lesion most in evidence at the time.

The most characteristic feature of this syndrome is the remarkable tendency for tumours to grow in

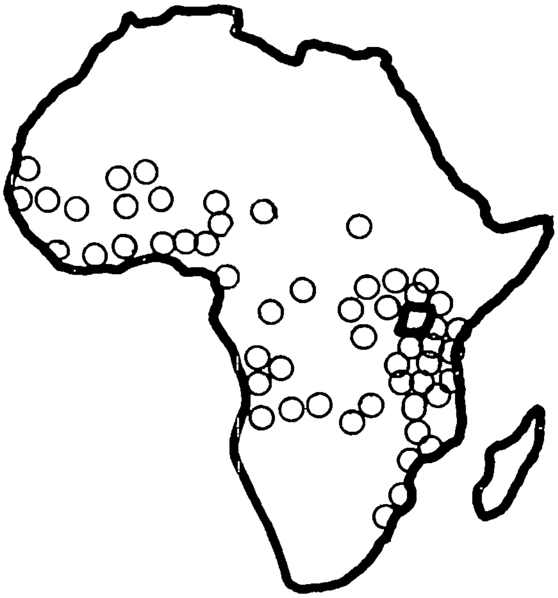

Fig. 1. Map of Africa showing the known distribution of the tumour syndrome. Each circle represents an area where this tumour has been recognized

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the jaws. In more than 140 of the cases recorded in Uganda, tumours were present in the jaws, and in a high proportion of these more than one quadrant, and in 16 cases all four quadrants were involved. The tumours grow rapidly and cause gross disfigurement. The maxillary tumours commonly invade the orbit, presenting as an exophthalmos and eventually destroying the eye. These lesions have frequently been mistaken for retinoblastomata.

Other striking features are the bilateral ovarian tumours, present in more than 70 per cent of female patients who have been submitted to laparotomy or come to autopsy, the bilateral thyroid tumours and the very high incidence of multiple renal tumours, which are also usually bilateral. The adrenals, heart and stomach are frequently involved, and lesions in the spinal canal make this tumour the commonest cause of paraplegia in children in the areas where the syndrome occurs.

Although tumours have been observed in many of the long bones, these lesions are relatively rare compared with the jaw tumours. Despite the fact that a more or less generalized enlargement of the lymphatic glands and spleen is the most prominent feature of malignant lymphoma as generally understood, clinical enlargement of these structures is exceptional in this syndrome. This unusual pattern and the rarity of pulmonary involvement constitute some of the most characteristic and unusual features. Untreated, the tumours grow exceedingly rapidly and terminate fatally, usually within six months.

Epidemiological aspects have also been discussed[2,5–8]. In view of the fact that the characteristic jaw tumours cannot easily be mistaken for any other condition these have been used to determine the geographical distribution of the tumour syndrome.

After wide distribution of illustrated leaflets accompanied by a questionnaire it became obvious that the tumour syndrome occurred right across tropical Africa from the coast of East Africa to Dakar in the extreme west (Fig. 1). In West and Central Africa the northern limit of the tumour belt is about 15° latitude; but this line falls below 10° in the east. Populations fall off, in many instances to virtual desert, along this line so tumour incidence naturally tends to disappear. The condition is common in West Africa as far south as the sea coast and has been recognized as far south as the middle of Angola.

A much more detailed study, including a 10,000-mile roads safari, has been undertaken in East and Central Africa. Geographical plotting of all cases observed in Uganda showed that the only factor, other than population, affecting tumour distribution was altitude. Near the equator the tumour does not appear to exist above an altitude of about 5,000 ft.

East and Central Africa with its varying contours produced mountain ranges and the two limbs of the Great Rift Valley were examined in some detail to determine the areas of tumour distribution. The findings of this survey can be summarized as follows: (1) Throughout Uganda, Kenya and Tanganyika the tumour can occur anywhere except at altitudes of more than about 5,000 ft. with the possible exception of the southern part of Tanganyika. (2) The off-shore islands of Zanzibar and Pemba are a notable and significant exception. No case of this syndrome has been observed in these islands with a population of more than a quarter of a million. (3) Throughout the Federation of Nyasaland and Southern and Northern Rhodesia, the syndrome is found only in or near the great river valleys, and on the shores of Lake Nyasa. (4) It is common throughout the coastal plain of Mozambique. (5) It is virtually unknown in South Africa. (6) This pattern of distribution does not coincide with population densities.

It is thus evident that the critical altitude above which the tumour is not observed falls progressively as the distance from the equator increases. Altitude is therefore only a limiting factor in so far as it reflects temperature. The actual limiting factor would appear to be a minimum temperature of about 60° F. in the coldest season of the year (July) (Fig. 2).

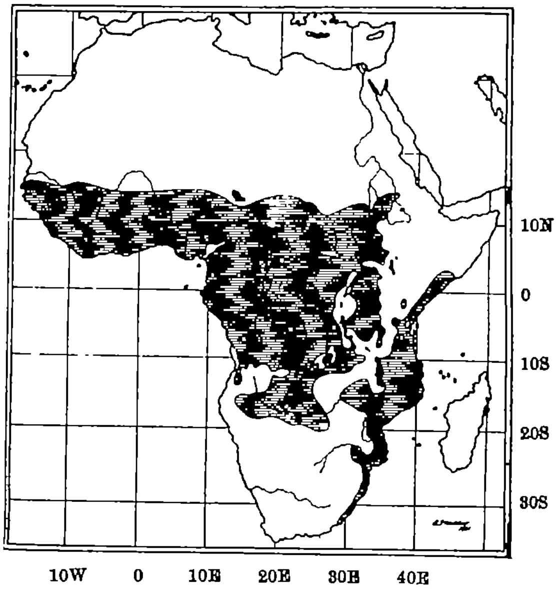

Fig. 2. Map of Africa from which have been eliminated (1) all areas where the temperature falls below 60° F., and (2) areas where the rainfall is below 20 in. a year

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There is no evidence that this tumour occurs in any country outside Africa with the exception of a few cases reported from New Guinea (Ten Saldam, personal communication, 1960).

The fact that the tumour distribution is dependent on climatic factors strongly suggests that some vector, perhaps a mosquito, is responsible for its transmission. This would naturally suggest that some virus may be the responsible agent.

No human tumour has yet been shown to be virus-induced but a number of virologists are now investigating the virological aspects of this tumour syndrome.

It was Prof. J. N. P. Davies who first suggested to me the possibility that this tumour might be virus induced. Dr. A. J. Haddow, director of the Virus Research Institute at Entebbe, has greatly assisted me in correlating tumour distribution to climatic factors.

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THE CROSS-CHANNEL ELECTRIC POWER LINK

THE linking by an undersea cable of the electric power networks of France and England, inaugurated officially on December 8, 1961, by the Minister of Power, the Right Hon. Richard Wood in England and the Minister for Industry, Jean Marcel Jeanneney in France, is an event of both technical and economic significance*.

The cable connexion lies across the channel from Le Portel, a suburb of Boulogne, to Lydd in Kent. By means of this link either country will be able to receive from the other up to 160,000 kW. of electrical power. The interchange of power between the two countries is rendered advantageous by the difference, between France and England, in the time of occurrence of the peak demand for electric power. It is estimated that the scheme will result in a saving of capital expenditure of about £8,000,000 in generating equipment and an annual saving in operating costs of £300,000. The total cost of the project has been some £5,000,000.

The power networks of Britain and of western Europe are alternating current systems operating at a frequency of 50 c./sec. The link joining the two systems uses direct current. Notwithstanding the fact that the use of direct current involves elaborate rectifier-inverter equipment at each cable terminal, this system has over-riding advantages. In the first place the transmission of electric power by alternating current in underground or submarine cables becomes difficult if long distances are involved, by reason of the large charging current drawn by the electrostatic capacitance of the cable. This difficulty is, of course, absent with direct current and in addition, for a given voltage, the cable for direct current is more economical of insulation. Secondly, two linked alternating-current systems must run in synchronism. Between alternating-current generators running in parallel there is an automatic synchronizing action, but this is associated with a flow of reactive current and, in consequence, the phenomena of load sharing are not simple. The problems of maintaining synchronism between the British and Continental systems would have been very great, none the less the initial plans for a cable link were drawn up on the basis of alternating current. Linkage by direct current leaves the two systems entirely free so far as small variations of frequency are concerned and enables the exchange of power to be made practically independently of internal variations in load or the occurrence of faults within either system.

An experimental high-voltage direct-current transmission system was undergoing development in Germany during the Second World War. After the War similar experiments in very-long-distance transmission were initiated in the U.S.S.R. It was, however, in Sweden that pioneer work was done which resulted in the first commercial installation. In 1943, Dr. Uno Lamm of the A.S.E.A. Electrical Co. in Sweden, assisted by a small team of research and development engineers, started work on the difficult problem of designing the heavy-current high-voltage mercury-arc rectifier valves required for such systems. Full-scale experiments and prolonged tests were involved. As a result of this a system was designed and built to supply 20 MW. to the island of Gotland in the Baltic by means of a single submarine cable carrying current at 100 kV. d.c. and utilizing a sea and earth return. The successful operation of this scheme engendered confidence in the utilization of direct current for the cross-Channel link.

Transmission is effected by two cables about 34 miles long operated with a potential difference of 200,000 V. The system is symmetrical with respect to earth so that the two conductors are 100,000 V. negative and positive to earth respectively.

On the English side power is drawn from the 275 kV. grid and on the French from the 225 kV. system. Each sub-station comprises transformers, a.c. switching equipment, control equipment and rectifiers. Rectification or inversion is effected at each end by two groups, connected in series, of six grid-controlled mercury arc rectifiers together with by-pass valves. The control of power flow is effected by impulses applied to the control grids of the valves. Normally, reversal of power flow is effected by the automatic operation of polarity reversing switches. Elaborate precautions have been taken to avoid the transmission of harmonic voltages into the a.c. system and to suppress the effects of high-frequency ripple in the d.c. system. Control engineers communicate directly with one another and with sub-station attendants over rented Post Office lines. Once the load and direction of power flow have been set, operation is entirely automatic and does not require the maintenance of a communication link between the two ends.

Apart from the economic advantages which the scheme offers, it will provide a most valuable opportunity for the acquisition of operating experience with a system which will find application in other parts of the world.

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* *Cross-Channel Power Link*. By J. H. M. Sykes. (Central Electricity Generating Board, Winsley Street, London, W.1, December 1961.)

Equipment is already being manufactured for a scheme linking the north and south islands of New Zealand across the Cook Strait. This cable will transmit 600 MW. at 500 kV.

Operation of the cross-Channel system was temporarily suspended as a result of damage to one of the cables about four miles from the English coast by a ship's anchor on January 12. It has been reinstated for the time being at half-power, utilizing the damaged cable as an earthed return conductor. J. Greig

OBITUARIES

Dr. Ezer Griffiths, O.B.E., F.R.S.

Dr Ezer Griffiths died at Teddington on February 14. His intellectual qualities combined with his open-hearted friendliness to win him the respect and affection of any community to which he belonged. His own interests and affections were deeply bound up with the National Physical Laboratory, which he served for nearly forty years.

Griffiths went to work at the National Physical Laboratory in 1915 being then twenty-seven years old and having already gained considerable experience as a research worker on thermal properties. A native of Aberdare, he had graduated at University College, Cardiff, with first-class honours in physics, gained the Isaac Roberts research scholarship and later elected a fellow of the University of Wales. With Principal E. H. Griffiths, he determined the heat capacities of several metals down to liquid air temperatures and also published papers on related subjects.

Early in 1916, full responsibility for the work of the Heat Section at the National Physical Laboratory fell on Griffiths and remained with him until his retirement in November 1953. Work on thermal insulation had already been started and was to assume major proportions, leading to the two outstanding investigations made by Griffiths in the 1920's into the transport to Great Britain of apples from Australia and frozen lamb from New Zealand. A series of special reports on this work was published in the years 1924–33 by the Food Investigation Board and can still be read with considerable profit. This was the work of which Griffiths himself was most proud and which he loved to recall in later years.

During the 1920's and 1930's Griffiths was also actively engaged on investigations for the Building Research Board and the Electrical Research Association. As a result of these researches, he published papers on heat transmission through walls, concretes and plasters. and on the thermal resistivity of solid dielectrics. Knowledge of the thermal resistivity of these component materials had been required in connexion with the calculation of the loss of heat from electrical machinery. For the Alloy Steels Research Committee he determined thermal expansion, total heat and thermal and electrical conductivity for twenty-one commercial steels over a wide temperature-range. This information was required in connexion with the heating and cooling of steel ingots.

Other subjects on which Dr. Griffiths worked were the use of the spectral line reversal method for the measurement of flame temperatures (1928 and 1929), the dependence of the mobility of ions in air or relative humidity (1929), the heats of combustion of carbon monoxide in oxygen and of nitrous oxide in carbon monoxide at constant pressure (1933), and the specific heat of pure iron to about 950° C. (1940).

During the Second World War, Dr. Griffiths and his Section were engaged on many special problems. These included tests on war emergency cold stores, in which the usual cork insulation had been replaced by slag wool filling in a cavity-wall construction; storage and transport of meat in the tropics, heat treatment of stored grain to guard against pest infestation; tests on tanks and on special military equipment under simulated tropical conditions; temperature measurements of jet propulsion turbine blades—these were undertaken during the initial development work on the jet engine—problems of flax drying and processing, measurement of humidity and temperature at high altitudes, measurement of gun-barrel temperatures in connexion with the problem of gun erosion and measurement of the distribution of temperature at various distances in the flame of large flame projectors.

Griffiths was elected a Fellow of the Royal Society in 1926. He received the Moulton Medal of the Institution of Chemical Engineers in 1935 for a joint paper (with R. W. Powell) on the fundamentals of evaporation. In 1950, he was awarded the O.B.E.

The refrigeration industry recognized their great debt to him and constantly sought his advice. He was elected president of the Institute of Refrigeration from 1936 until 1938, later serving as a member of the Council, and was chairman of the Research Committee at the time of his death. He was well known internationally as an authority on refrigeration, being president of the Institut International du Froid during 1951–59, and after that president d'honneur. He was elected president of the Institute of Engineers in Charge in 1961. Many other organizations benefited from his knowledge and willingness to serve the public good. He was a governor of the National College for Heating, Ventilating, Refrigeration and Fan Engineering, and at various times held office or served on committees of the Physical Society, the British Association, the International Union of Pure and Applied Physics and the Twickenham Education Committee.

G. B. B. M. Sutherland

Prof. Władysław Dziewulski

Władysław Dziewulski was born in Warsaw on September 2, 1878. He entered the University of Warsaw and graduated in 1901. This was followed by one year of study in the University of Gottingen (1902–3). In 1903 he went to the Astronomical Observatory of the University of Cracow as an assistant. In 1906 he was awarded the Ph.D. degree of that University for his work on the secular perturbations of Eros caused by Mars. In 1907 he went to Göttingen again for two years of study and worked, under Karl Schwarzschild's direction, on photographic photometry of stars. In 1909 he was appointed adjunct at the Cracow Observatory, and in 1916 became lecturer in astronomy in the University of Cracow. In 1919, Dziewulski was appointed professor of astronomy in the University of Wilno. There he organized the Astronomical Observatory and remained its director until December 1939, when the Polish University of Wilno was closed. He was elected rector of the University of Wilno in the year 1924–25.

The Astronomical Observatory of the University of Wilno, founded by Władysław Dziewulski, was destined mainly for astrophysical research. Dziewulski observed the Cepheid Variables visually and photographically. Beside the observations of variable stars, Władysław Dziewulski's astronomical researches were devoted to problems of statistical investigations of stellar space motions in the local system of the Galaxy. His contributions concerned the peculiar motions of stars of different spectral and luminosity classes, the moving clusters and the local rotation of the stars of *A* type. During 1922–26 his interests were directed to the problems of the determination of the solar apex by the Bravais method and of the determination of the vertex from the data of the *Boss Preliminary General Catalogue*. In 1945 Dziewulski went to Toruń, where the new University was founded. He took an active part in its organization and together with Prof. W. Iwanowska founded the Astronomical Observatory of the University of Toruń at Piwnice near Toruń. In that Observatory he continued his research on secular perturbations of minor planets, on the space motions of stars and on Cepheid Variables. He took a deep interest in the history of Polish astronomy of the nineteenth century, especially in the history of the Wilno Astronomical Observatory in the eighteenth and nineteenth centuries.

Władysław Dziewulski was a member of the Polish Academy of Sciences and Fellow of the Royal Astronomical Society. He was an excellent teacher and attracted many young pupils to astronomy. He was elected professor emeritus in 1960 but continued his research work until his death.

He died in Toruń on February 6, 1962.

EUGENIUSZ RYBKA

NEWS and VIEWS

The Anti-Locust Research Centre:

Dr. T. H. C. Taylor

DR. T. H. C. TAYLOR retired from the directorship of the Anti-Locust Research Centre, London, on March 31. Dr. Taylor has had a long and varied career in entomology; he graduated with first-class honours at the University of London in 1924 from what was then University College, Reading, and went to Fiji to undertake work on the biological control of the coconut moth which was then causing heavy damage in the plantations. In the course of his work for Fiji he travelled widely in Melanesia, Indonesia and the West Indies looking for suitable parasites. He and his collaborators, J. D. Tothill and R. W. Paine, working with the Government entomologist, H. W. Simmonds, succeeded in completely controlling the moth, and they described this classical example of biological control in a book, *The Coconut Moth in Fiji: a History of its Control by Means of Parasites*. Taylor and Paine went on to establish successful biological control of the coconut leaf-mining beetle and the coconut scale. In 1935 Taylor was transferred to Uganda, where he worked mainly on pests of cotton and coffee and their natural enemies, and during this period he published several papers and was awarded the D.Sc. degree. In 1944 he returned to England to join the Commonwealth Institute of Entomology, where he became assistant director in 1946. In 1953 he transferred to the Anti-Locust Research Centre as deputy director, and in 1959 he succeeded Dr. (now Sir Boris) Uvarov as director.

Dr. Taylor's years of office as director have been marked by enlargement of the scope and functions of the Centre, which has long been regarded as the main international body for locust research; important developments during this time have been the establishment of the Centre as one of the units of the new Department of Technical Co-operation and the increasing part it has played, notably in collaboration with the Food and Agriculture Organization, in promoting international co-operation in locust research and control. The recent constitutional changes in Africa have reacted on overseas locust organizations, and the Centre in general and Dr. Taylor in particular have played a large part in guiding the policy of newly emergent countries in the locust sphere.

Dr. P. T. Haskel

DR. TAYLOR has been succeeded as director by Dr. P. T. Haskell, who has been deputy director of the Centre since 1959. Dr. Haskell graduated at the Imperial College of Science and Technology in 1951 with first-class honours in entomology and was appointed assistant lecturer and then lecturer in entomology in the Department of Zoology. He gained the degree of Ph.D. in 1955 for research in insect acoustics, and in 1956 was awarded the Huxley Gold Medal for Biology for this work. He is most widely known for his work on insect acoustics and behaviour, and his recent book, *Insect Sounds*, is the only general text-book on the subject. Dr. Haskell joined the Anti-Locust Research Centre in 1955 to take charge of physiological research and devoted much time to building up such work in the Centre and also to stimulating physiological work on locusts in university departments at home and abroad. Since his appointment as deputy director he has travelled widely in Africa to familiarize himself with the problems relating to the most important locust species and to discuss and advise on problems of locust research, in which he intends to remain active. By both lecturing and writing he has contributed greatly to the dissemination of knowledge concerning locusts and their control.

Sir Henry Head, F.R.S.

THE centenary of the birth of Sir Henry Head, who achieved considerable distinction in the field of medicine, has been marked by a series of articles in a recent issue of *Brain* (84, Part 4; 1961), which he edited from 1910 until 1925. Sir Henry was born on August 4, 1861, and was educated at Charterhouse and Trinity College, Cambridge. He studied physiology under Hering at Prague and took a medical degree at Cambridge and University College Hospital. He was responsible between 1893 and 1896 for showing the areas of reflex cutaneous hyperæsthesia and hyperalgesia, due to visceral disease, and also the segmental distribution of the sensory nerve roots involved. The man and his ideas are described by

Lord Brain, while Dr. K. W. Cross explains how Head's collaboration with Hering at Prague later led to the discovery of his paradoxical reflex. Head's considerable contribution to our knowledge of sensation is examined by Dr. R. A. Henson, while his theories of aphasia are analysed by Dr. Macdonald Critchley. The symposium also includes a detailed bibliography of Head's original works.

U.K. Atomic Energy Authority

The United Kingdom Atomic Energy Authority has announced the following senior appointments: in the Production Group, Mr. S. F. Hines, formerly general manager, Capenhurst, is appointed production director, with his headquarters at Risley, and Mr. W. R. A. Taylor, formerly deputy general manager, is appointed general manager, Capenhurst, to succeed Mr. Hines; in the Reactor Group, Mr. P. W. Mummery is appointed deputy director, Atomic Energy Establishment, Winfrith. These appointments took effect from April 1.

S. F. Hines, O.B.E.

Mr. Hines was educated at St. Albans School and the University of London. In 1930 he joined the staff of the Royal Gunpowder Factory, Waltham Abbey, and in 1936 transferred to the Royal Ordnance Factory, Irvine. In 1939 he was appointed superintendent of the Factory at Pembrey, and in 1944 became superintendent of the Factory at Drigg, Sellafield and Bootle. In 1946 Mr. Hines was appointed general manager of the Atomic Energy Factory at Springfields and was responsible for starting up this plant for uranium purification and fuel element manufacture. In 1950 Mr. Hines became general manager of the new gaseous diffusion plant at Capenhurst.

W. R. A. Taylor, O.B.E.

Mr. Taylor was educated at Scunthorpe Grammar School and the University of Cambridge. During the War he was a member of the Directorate of Filling Factories, becoming chief technical assistant in 1945. In 1946 he joined the Department of Atomic Energy at Risley and in 1954 was appointed works manager, Springfields Works. In 1956 Mr. Taylor was appointed deputy general manager, Capenhurst Works (gaseous diffusion plant). In July 1956 he was a member of the Duke of Edinburgh's study conference.

P. W. Mummery, O.B.E.

Mr. Mummery was educated at Rotherham Grammar School during 1936–44 and Queen's College, Cambridge, 1944–47. He joined the Authority in August 1947 as a scientific officer at Harwell. On January 1, 1952, he was promoted to senior scientific officer, Atomic Energy Research Establishment, Harwell, in 1955 to principal scientific officer, and in 1957 to senior principal scientist. Mr. Mummery later became head of the Power Reactor Branch, Reactor Division, Harwell, and is at present head of Industrial Power Reactor Division, Atomic Energy Establishment, Winfrith, Dorset.

Reorganization of Radio Standards Laboratory at the U.S. National Bureau of Standards

The Radio Standards Laboratory at the Boulder Laboratories of the National Bureau of Standards, U.S. Department of Commerce, has been divided into a Radio Standards Division and a Circuit Standards Division to provide unified direction for a growing programme. Dr. J. M. Richardson will continue as head of the Radio Standards Laboratory, which is responsible for providing the central basis for the complete, consistent, uniform and accurate measurement of physical quantities pertaining to radio science, and assurance of international co-ordination of such measurements. Dr. Y. Beers, chief of the Radio Physics Division, will direct research concerning the interaction of electromagnetic fields with matter having possible application to radio standards, frequency standards, time scales, atomic and aggregate properties of matter, and constants of physics. The Division will also develop certain national standards and direct the dissemination and international co-ordination of the results. The Circuit Standards Division, headed by Dr. G. E. Schafer, will conduct research and develop national standards of physical quantities pertaining to radio circuits and measurement techniques, disseminate the standards, especially by calibration services, and direct their international co-ordination.

The Iron and Steel Institute

The following changes have been announced by the Council of the Iron and Steel Institute: *Honorary Vice-President*, Mr. D. A. Oliver (director of research, B.S.A. Group of Companies); *Vice-President*, Mr. E. W. Senior (director, British Iron and Steel Federation); *Members of Council*, Dr. N. P. Allen (superintendent, Metallurgy Division, National Physical Laboratory), and Sir Douglas Bruce-Gardner (deputy-chairman, G.K.N. Steel Company, Ltd., and joint managing director, Lysaghts Scunthorpe Works); *Additional Honorary Members of Council*, Mr. A. Jackson (technical adviser on steelmaking, United Steel Companies, Ltd.), and Prof. F. D. Richardson (professor of extraction metallurgy, Imperial College of Science and Technology).

Awards

The Council of the Iron and Steel Institute has made the following awards: *Bessemer Gold Medal for* 1962, Sir Charles Goodeve, director of the British Iron and Steel Research Association, president during 1961–62 of the Institute; *Sir Robert Hadfield Medal for* 1962, Mr. G. T. Harris, assistant managing director, Jessop-Saville, Ltd.; *Williams Prizes*, Mr. H. I. A. Archibald (Stewarts and Lloyds, Ltd.) for the paper "The Application of a Digital Computer to the Pre-operational Problems of Programming the Extraction and Supply of Iron Ore", and Mr. M. C. Harrison and Mr. P. Truscott (Steel Company of Wales, Ltd.) for the paper "Operation of the Oxygen/Steam-blown Converters at the *VLN* Steel Plant of the Steel Company of Wales, Ltd."; *Andrew Carnegie Silver Medal for* 1961, Mr. J. S. White (University of Liverpool) for the papers "Kinetic Features of Upper and Lower Bainite Formation in a Plain Carbon and a 3 per cent Ni-Cr Steel" and "The Effect of Up-quenching on the Kinetics of Bainite Formation".

International Co-operation in Outer Space

The U.S.S.R. has nominated Prof. V. A. Bugaev and the United States has nominated Dr. M. Alaka to be consultants to the Secretary-General of the World Meteorological Organization in studies related to the preparation of a report on global benefits to be derived from meteorological satellite research. These two scientists began work in Geneva on March 13, 1962. This co-operation between the world's two leading countries on research in outer space results from a resolution of the General Assembly of the

United Nations, as well as from the mutual desire of both countries. The resolution of the United Nations recommends the early and comprehensive study in the light of developments in outer space of measures (*a*) to advance the state of atmospheric science and technology so as to provide greater knowledge of basic physical forces affecting climate and the possibility of large-scale weather modification, and (*b*) to develop existing weather forecasting capabilities and to help member States make effective use of such capabilities through regional meteorological centres. It also requests the World Meteorological Organization to submit a report on arrangements to achieve those ends. This report will be studied by member Governments and by the Economic and Social Council prior to further consideration by the General Assembly of the United Nations. It is expected that Dr. H. Wexler, a leading American meteorologist, will shortly join Prof. Bugaev and Dr. Alaka.

Teaching of Science and Mathematics: Nuffield Foundation Programme

THE Nuffield Foundation has set aside £250,000 towards the cost of a comprehensive long-term programme to improve the teaching of science and mathematics in schools. Co-ordination of the whole programme will be undertaken by the director of the Foundation, Dr. Leslie Farrer-Brown, in consultation with the Curriculum Study Group in the Ministry of Education and all branches of the education service, with the professional institutions concerned, and with industry. The five sections chosen as starting points for the programme are: physics, chemistry and biology for 11–15-year-olds in grammar schools and streams; secondary school mathematics; and primary and secondary school science in general. The aim in the first three sections will be to provide a complete range of aids designed by teachers for teachers. The work will be based on the extensive new thinking now being undertaken by bodies such as the Science Masters Association, H.M. Inspectorate of Schools, the Scottish Education Department, the professional scientific institutions and some of the larger industrial concerns. A full-time organizer will be appointed as a Nuffield Fellow to lead the work of each section. Each organizer will be backed by a small consultative committee, and detailed work on the programme will be undertaken by teams of practising teachers. The physics project will be started during the next few weeks under the leadership of Mr. D. McGill, who has been seconded for the purpose by the Scottish Education Department. The chairman of the Physics Consultative Committee will be Sir Nevill Mott. The intention is to complete the physics project within three years. In the first phase, the teams will concentrate on producing preliminary drafts of the required teachers' guides, class texts, and laboratory notes, and on commissioning demonstration and laboratory apparatus and visual aids for the course as a whole. The Associated Electrical Industries Film Unit and Design Unit have made their services available to the Committee for policies concerning visual aids. The second phase will comprise trying out the texts under classroom conditions and submitting them to further detailed criticism. In the final phase, arrangements will be made for the amended texts and approved versions of the apparatus and aids to be produced on a scale sufficient to meet the expected demand. Active steps are now being taken to begin work on the other four sections of the programme.

International Exchange of Skilled Persons

IN a written answer to a question in the House of Lords on March 19, Lord Hastings stated that full information about the United Nations Operational and Executive Personnel Scheme (OPEX) would be found in the report of the Secretary-General on programmes financed by the regular budget. The scheme was launched in January 1959, with an annual budget of 200,000 dollars, which was increased to 300,000 dollars for 1960, and to 850,000 dollars for 1961 and 1962. Up to September 1961, eighty posts had been established, of which seven were in the newer independent Commonwealth countries, three officers had completed their assignments and twenty-seven were in post. At their meeting in September 1960, the Commonwealth Economic Consultative Council decided that the best way to foster exchanges between Commonwealth countries of persons with specialized skills and experience would be for the Commonwealth Governments to improve their bilateral arrangements, and that appropriate arrangements should be devised, where they did not already exist, for filling executive as well as advisory, technical and specialized posts in Commonwealth countries which had recently achieved independence. Shortly after this decision was announced, Britain extended her offer of technical assistance to those Commonwealth countries which had recently become independent to including filling senior and important posts on the permanent establishment of the Governments of those countries, for a limited period, pending the training of a local successor. This offer was made to Ghana, Malaya, Nigeria and Sierra Leone, and so far about twenty appointments had been made, covering a wide variety of fields, including finance, engineering and telecommunications.

British Student Population

IN a written answer in the House of Commons on March 27, Sir Edward Boyle stated that of 15,256 students in arts, science and technology in 1952, 16·7 per cent left, for various reasons, without success, 1,688 leaving for academic reasons; for 1955, corresponding figures were 18,239, 13·9 per cent and 1,804; and for 1957, 21,793, 14·3 per cent and 2,584. Sir Edward also stated that a target of 150,000 students for the next five years had been selected to provide the greatest practicable opportunities for the large age-groups reaching university age in 1966–67; it would provide university places for as high a proportion as was available at present.

British Colleges of Technology

IN answer to a question in the House of Commons on March 28, the Secretary of State for Scotland, Mr. J. Maclay, said that of the Government's programme of technical college building to be started by the end of March 1964, projects had been started to date to the value of £13·5 million, including projects already completed to the value of £1 million and others to the value of £1·5 million expected to be completed by the end of 1962. In a written answer on March 26 the Minister of Education, Sir David Eccles, stated that twenty-one colleges were at present recognized as regional colleges of further education and he was reviewing the list in consultation with the regional advisory councils for further education. Seventeen of these were included in the first list compiled in 1956 and four were added between 1956 and 1959; one, the Brunel College of Technology, was being designated a college of advanced technology on April 1. In October 1960, 10,280 students were enrolled in full-time and 2,943 in sandwich courses, and of these, 4,270 and 2,767 were in advanced courses. There were also 33,077 part-time day and 43,527 evening students, and of these, 7,415 and 7,029, respectively, were in advanced courses.

Approach to a National Library in England

R. Irwin, director, School of Librarianship and Archives, University College, London, contributes to the *Library Association Record* (March 1962) an article, "The Approach to a National Library in England", which is of some general interest. Mr. Irwin describes briefly, but lucidly, the various steps by which something of a national library system was built up and the early background to such efforts, notably in the late sixteenth and early seventeenth centuries. Due attention is given to the relation of such developments to the advance of science itself. The scientist should find Mr. Irwin's article of special interest, though he takes the record no further than the founding of the Science Museum in 1857.

Library Services in Manchester

The one hundred and ninth annual report of the Libraries Committee of the City of Manchester, covering the year ended March 31, 1961, records a stock of 1,162,952 volumes at the end of the year, 162,387 volumes being added and 164,169 withdrawn (Pp. 38 + 4 plates. Manchester: The Public Libraries, 1962). Issues decreased by 316,502 to 6,985,430, but reference library issues again increased, by 95,467, to 1,829,225. Concern is again expressed over the dilution and turn-over of staff; of 276 full-time staff, 54 resigned during the year, bringing the total of resignations in the past five years to 256. In his report, the City Librarian notes the concentration of photographic services in a single unit and the steadily increasing demand for photocopies. Consolidation continued in the Technical Library, in which items issued for reference increased by 51,312 to 873,772, although issues from stock decreased by 30 per cent. In its first full year of operation the Patents and Microtexts Library served 15,010 readers, consulting 119,436 patent specifications, 3,179 microfilms and 717 microcards, while the Technical Information Service issued 1,940 items and handled 14,004 telephone and 598 telex inquiries. Accounts of the numerous other aspects of the work of the Manchester Public Libraries are included in the report.

The Smithsonian Institution

The annual report of the Smithsonian Institution, the United States National Museum, for 1961 adheres in general format to previous years and records extensive building operations (Pp. viii + 188. Washington, D.C.: Government Printing Office, 1961). An extension to the east wing and air conditioning of the existing Natural History Department is in progress and the corner-stone of the Museum of History and Technology was laid in May 1961. It is anticipated that this Museum will be completed in July 1962, and that it will commemorate the national heritage and illustrate the historical development of the basic elements in the life of the country. Original objects associated with famous Americans and historical events include the original Star-Spangled Banner and the desk at which Jefferson drafted the Declaration of Independence. The history of science and technology will be traced in exhibits of the original apparatus of the scientists, relating their great discoveries to the machines in which the inventors and engineers applied new scientific knowledge for the benefit of the community. It is satisfactory to record that ample provision is being made for the conservation of the objects in the Museum.

Protection of Nature: South Downs

The National Parks Commission has issued designation orders to make the South Downs in Sussex and Hampshire, from Beachy Head to Winchester, an area of outstanding natural beauty (*Intelligence Unit. Council for Nature Press Bull.* 25). The orders are subject to confirmation by the Minister of Housing and Local Government. The Forestry Commission has leased to the Yorkshire Naturalists Trust twelve acres of the famous Grass Wood near Grassington in Wharfedale. This wood, once famous as a site for the rare lady's slipper orchid, is an ashwood on limestone, and has been visited by generations of Yorkshire naturalists.

Hayley Wood

The Cambridgeshire and Isle of Ely Naturalists' Trust has taken an option to buy the 122-acre Hayley Wood, the largest unspoilt boulder-clay wood remaining in the country, ten miles west of Cambridge. This type of woodland is famous for its displays of spring flowers, including wood anemones, dog violets, early purple orchids, bluebells, and instead of the more usual primroses, oxlips, for which this part of the eastern counties is famous. The Trust is appealing for £7,500 to buy the wood and establish a fund to enable the traditional system of management to be maintained.

Downe

Charles Darwin's Orchid Bank at Downe in Kent has recently been acquired as a nature reserve by the Kent Naturalists' Trust. It is a site of great scientific interest which shows a wide range of vegetation types including ancient chalk grassland with a very rich flora; and, as it is only sixteen miles from the centre of London, the need for conservation is urgent and considerable. It is also of unique historical importance, for Darwin is known to have carried out field studies there from his nearby home, Down House, where he lived for forty years. The reserve is eleven acres in extent, and in addition to chalk grassland, it includes chalk scrub with juniper (now rare in Kent) and beechwood on chalk; and where clay-with-flints overlies the chalk is a strip of woodland with oak, beech and hornbeam. Eleven of the thirteen species of orchid mentioned by Darwin as occurring within a mile of his house can still be found in the reserve; and in addition *Aceras anthropophorum*, not included in his list, has recently been found there. The bryophyte flora is also of interest. The Trust has set up a management committee to organize necessary conservation measures; these include fencing (a form of chestnut paling traditional in Kent is being used) and the cutting back of scrub which is invading the grassland. This committee is also preparing a long-term scientific programme of ecological observation.

Steel Foundry Year

The British Steel Founders' Association has announced that open days will be held to mark Steel Foundry Year by the following members of the Association: Catton and Co., Ltd., Leeds (May 23); Head Wrightson Steel Foundries, Ltd., Thornaby-on Tees (May 24); F. H. Lloyd and Co., Ltd. (May

30–June 1); Edgar Allen and Co., Ltd., and Hadfields, Ltd., Sheffield (June 21); Brown, Lenox and Co., Ltd., Pontypridd (July 4); English Steel Castings Corporation, Ltd., Sheffield (July 5); Stewarts and Lloyds, Ltd., Glasgow (September 20); Osborn Foundry and Engineering Co., Ltd., Sheffield (September 21). A conference will also be held in London on May 10. Further information can be obtained from Mr. K. V. Robinson, British Steel Founders' Association, 13 Broomgrove Road, Sheffield 10.

Selby Fellowship

The Selby fellowship has been established by the Australian Academy of Science to bring graduates of high promise and proved capacity for research to work in the laboratories of universities or research institutions in Australia. The fellowship may be awarded in any branch of physical or biological science and is tenable at any university or research institution in Australia, normally for one full year of work. Candidates must have taken their first university degree, and have had preliminary research experience, at some university or universities in a country other than Australia. In general, they should hold a Ph.D. degree or have equivalent status and experience, but the fellowship will not normally be given to those who have already held a senior research award. Applicants should be less than thirty years of age on July 1 in the year in which their applications are received. The fellowship will carry a stipend of £2,000 (Australian) per annum. The costs of the Fellow's travel to Australia, and back to his country of origin at the conclusion of the fellowship, will be provided, up to a limit of £A500, including dependants, if any. Applications should be sent to the Assistant Secretary, Australian Academy of Science, Gordon Street, Canberra City, A.C.T., before July 30.

The Night Sky in May

New moon occurs on May 4d. 04h. 25m. U.T. and full moon on May 19d. 14h. 32m. The following conjunctions with the Moon take place: May 1d. 19h., Mars 3° N.; May 6d. 00h., Aldebaran 1° S.; May 12d. 06h., Regulus 1° S.; May 25d. 03h., Saturn 0·6° S.; May 27d. 04h., Jupiter 2° N.; May 30d. 15h., Mars 4° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Aldebaran on May 6d. 18h., Venus being 6° N., and Mercury with Aldebaran on May 10d. 10h., Mercury being 8° N. Mercury is well placed for observation during the first three weeks of May, when it is visible low in the west after sunset, setting about 2 hr. after the Sun. Venus is an evening star, visible in the west after sunset. It sets at 21h. 35m., 22h. 15m. and 22h. 40m. on May 1, 15 and 31, respectively; its stellar magnitude is $-3·3$. Its distance decreases during the month from 142 to 127 million miles and the visible portion of the apparent disk decreases from 0·918 to 0·847. Mars is too close to the Sun for observation. Jupiter rises at 2h. 50m., 1h. 50m. and 0h. 55m. at the beginning, middle and end of the month, respectively. It is in Aquarius, its stellar magnitude is $-1·9$ and its distance from the Earth in the middle of the month is 480 million miles. Saturn is in Capricornus, rising at 1h. 50m , 0h. 55m. and 23h. 55m. on May 1, 15 and 31, respectively. Its stellar magnitude is $+0·8$ and its distance from the Earth on May 15 is 900 million miles. There are no occultations of stars brighter than magnitude 6 visible at Greenwich. Comet Seki-Lines (1962c) will have faded to magnitude 8 by early May, and to magnitude 11 by the end of May.

Announcements

Sir Harold Jeffreys, formerly Plumian professor of astronomy in the University of Cambridge, and Fellow of St. John's College, Cambridge, has been granted the Grove Karl Gilbert Award in Seismic Geology by the Carnegie Institution of Washington to carry out a programme of correcting his long standard "Travel-time tables".

Mr. F. P. Doyle has been elected to the Board of Directors of the Beecham Research Laboratories, Ltd., as research director. Mr. Doyle succeeds Dr. J. Farquharson, who relinquishes his post on medical advice. Dr. Farquharson will later take up an appointment as special consultant directly responsible to the research director, Beecham Group, Ltd.

The annual general meeting of the American Philosophical Society will be held in Philadelphia during April 26–28. Further information can be obtained from the Secretary, American Philosophical Society, 104 South Fifth Street, Philadelphia.

An international conference on "Instrumentation for High Energy Physics", sponsored by the International Union of Pure and Applied Physics, will be held at CERN, Geneva, during July 16–18. Further information can be obtained from Mr. E. W. D. Steel, Scientific Conference Secretariat, CERN, Geneva 23.

A special meeting, arranged by the Iron and Steel Institute, will be held in Germany during June 27–July 11. The main meeting will be held in Dusseldorf during July 1–8, and there will be special excursions to Saarland and Trier, to Heidelberg and Frankfurt/Main, and to Salzgitter and Bremen. Further information can be obtained from the Secretary, the Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

The Industrial Safety Division of the Royal Society for the Prevention of Accidents is organizing a conference with the theme "Safety by Research", which will be held in Blackpool during May 10–13. The programme will include: publicizing the results of safety research; safety at work: the trade union point of view; a new look at accident prevention and its implication for practice; the development and influence of British standards on safety. There will also be an exhibition of industrial safety equipment. Further information can be obtained from the Royal Society for the Prevention of Accidents, Industrial Safety Division, Terminal House, 52 Grosvenor Gardens, London, S.W.1.

A symposium on "Electromagnetic Theory and Antennas", sponsored by the International Scientific Radio Union, the Technical University of Denmark, the Danish Academy of Technical Sciences and the Danish National Committee of the International Scientific Radio Union, will be held in the Technical University of Denmark, Copenhagen, during June 25–30. The programme will include the following topics: propagation of guided and unguided electromagnetic waves in anisotropic media; scattering in random media; propagation along and radiation from modulated structures; properties of partially coherent electromagnetic fields; recent developments in diffraction theory; antennas. Further information can be obtained from the secretary of the symposium, H. Lottrup Knudsen, Øster Voldgade 10 G, Copenhagen K.

FOOD POLICY AND PLANNING IN HUNGRY COUNTRIES

ESTIMATES of the proportion of the world's population that is hungry for all, or the greater part, of the year vary from about 20 to about 70 per cent. The main reasons for this very large margin of uncertainty are two. In the first place, reliable food consumption surveys, far from easy in highly developed countries, are, in most of the underdeveloped countries, extraordinarily difficult to carry out, and the results may be particularly misleading where a major part of a population is engaged in agriculture. In the second place, existing, well-defined standards for adequate nutrition applicable to Western populations have to be interpreted with great care in relation to populations, the individuals of which differ in physical size from Western individuals, or where there is a marked different age distribution, or where many of the individuals carry out quite different types of physical work under different environmental conditions from those in the technologically advanced countries. Nevertheless, to any but the most fleeting Western visitor, it is abundantly obvious that undernutrition is widespread in Far Eastern countries, and malnutrition, especially among young children, is particularly marked.

Pursuing his inquiries further, the observant Western visitor may find that in some at least of these countries the responsible authorities, while cognisant in a distant way that hunger, or even seasonal near-starvation, may afflict a substantial percentage of the people under their charge, scarcely know where to begin in an endeavour to raise nutritional standards.

The fourth Food and Agriculture Organization Regional Conference for Asia and the Far East (1958), recognizing "that the technical issues involved in food policies and planning need to be clarified early from the nutritional angle on a regional basis for the guidance of the governments", recommended to the Director-General of the Organization that a meeting should be convened as soon as possible to consider "the various aspects of the problem of linking the nutritional needs of the people with national policies and plans relating to food and agriculture". The meeting was duly held in Bangkok, Thailand, in June 1960, and its recently published report gives an account of its proceedings and recommendations*. The countries represented at the meeting were India, Thailand, Philippines, Ceylon, Indonesia and Taiwan. Present also were representatives from the World Health Organization, the United Nations International Children's Emergency Fund, the Economic Commission for Asia and the Far East, and a strong group from the Food and Agricultural Organization.

For any country intending to introduce a national policy for nutritional improvement, the essential requirement is reliable information on the actual situation, quantitative and qualitative, of both food production and food consumption in the various districts and among the different socio-economic groups in that country. The first chapter of the report endeavours to make the beginnings of an appraisal of food consumption per head per day, both for the countries represented at the meeting, and also for some of the unrepresented countries, Japan, Korea, Burma and Malaya. The conclusion is reached that "the average levels of food consumption do not appear to be satisfactory", a statement which seems to me, who has seen conditions in part of the Far East, to provide a classical example of meiosis. In fact, if the figures quoted in the report for quantitative food consumption are reliable, the average calorie intake of all except one of these countries is highly unsatisfactory; further, the paragraphs in the same chapter reporting the widespread incidence of deficiency diseases—among which beriberi is reported to be still common in the Philippines, Burma, Viet-Nam and North Thailand—indicate that qualitatively also, many of the Far Eastern diets are seriously inadequate. Another comfortless statement in this chapter is that except in Japan (where per caput fish supplies have more than doubled since the War, and where milk supplies are rapidly increasing) the intake of animal protein has declined in these countries in recent years. The report points out that "clinically-evident cases of malnutrition represent only a fraction of their prevalence. Cases without frank manifestation but with impaired vital functions . . . are usually disregarded, with the result that the magnitude of the problem is hardly realized".

The establishment of food consumption targets, both long-term and medium-term, is dealt with in the second chapter. Among the general principles outlined, one is of especial significance—that in planning for increased food supplies, improved indigenous production should be the basis, a fact which appears still to be only partially recognized in several of the Far Eastern countries. As regards medium-term objectives, a compromise between what is nutritionally desirable and what is economically feasible is recognized as inevitable, though what is economically feasible, in a sovereign country at least, is a matter which is to a large extent under 'indigenous' control.

The third chapter is mainly concerned with improvement in nutrition through food policies and plans. The complex of interrelated factors influencing food consumption and nutrition varies so much from one country to another, or even from one region to another within the same country, that generalizations made in this chapter tend to be so broad as to become truisms. Thus, few can doubt the scarcely epoch-making statement made in the report that the most important economic factor which determines what people eat is their purchasing power. The equally important factors, in several Eastern countries, of religious prohibitions and traditional taboos, which still add gratuitous handicaps to the nutritional burdens at present borne by the humbler citizens in these countries, or bound up as these handicaps are with the difficulties, particularly among the illiterate, of "motivation towards change", receive little emphasis.

The usual, unexceptional recommendations are made—better education in nutrition, home economics, and dietetics, increase in the number and influence of food and nutrition organizations, the need to train individuals who can correlate nutritional needs with agricultural food production from land-

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* Report of the Technical Meeting on Nutrition in Food Policy and Planning in Asia and the Far East. FAO, Rome, 1961, pp. 40. H.M.S.O., 2*s*. 6*d*.

or sea. Few of these individuals exist in the hungry countries at present.

In the fourth and final chapter the need for improved staffing, equipment, status and influence of nutrition research centres in the Far Eastern countries is stressed, and the establishment in each country of a strong committee representing health, agriculture, education, economics and social welfare, capable both of advising the Government and stimulating action along effective lines, is recommended.

Clearly visible through all the cautious phraseology of this report is the general agreement that a significant improvement in most of the Far Eastern countries depends on influencing governments to give indigenous efforts toward the achievement of such improvement both high priority and adequate funds. These efforts would include the far closer correlation of agricultural, economic and nutritional policy, and the training forthwith of adequate numbers of indigenous technologists in these fields.

Truly, the improvement of national diets in the hungry countries is a problem which must depend for its solution on indigenous effort, with, especially in the early stages, specialist assistance, 'know-how' and finance from United Nations agencies and other outside sources. The technical knowledge required is already available in the world; if it is adequately applied, very few indeed of the hungry countries need remain so.

H. D. KAY

TEACHING BIOLOGY IN AMERICAN HIGH SCHOOLS

EARLY in 1959 the American Institute of Biological Sciences, an association of forty-seven societies with an aggregate membership of 84,000, set up the Biological Sciences Curriculum Study under the chairmanship of Prof. Bentley Glass of the Johns Hopkins University. Arnold B. Grobham, of the University of Florida, agreed to serve as full-time director of the project, and space for a permanent headquarters was obtained on the campus of the University of Colorado. With financial support assured by the National Science Foundation, the aim of the Biological Sciences Curriculum Study was to examine the defects of present biology teaching at all levels and the provision of specific programmes and materials to bring biology curricula up to date and to focus them more sharply on what a consensus would hold to be the major objectives of biology teaching.

At the outset, it was decided to begin by tackling the problem of biology at the high-school level. Although the defects of biology teaching in the elementary school or in the colleges may be fully as great, it is in the secondary schools that greatest choice of occupation is made; it is here that one finds the last opportunity to teach the sciences, and specifically biology, to nearly every future citizen. Clearly, much of the difficulty in interesting students in the study of biology at more advanced levels derives from the failure of high-school biology teaching to awaken the interest commensurate with its importance.

A steering committee was formed, comprising some thirty persons, who included not only college and university biologists recognized for their research as well as their interest in education but also high-school teachers of biology, writers of text-books and teachers' hand-books, and high-school administrators. During the course of the first year, the steering committee formulated a general plan of procedure and discussed the problems posed by the existence of an antiquated biology curriculum in the secondary schools. There was general agreement on several key points. Some of the findings of the committee are outlined in a recent issue of the *American Scientist* (49, No. 4; December 1961).

The biological sciences are at present advancing at so rapid a rate as to double the amount of significant knowledge every 10–15 years. While this fact makes it imperative to revise courses and methods of teaching at more and more frequent intervals, it also makes it increasingly impossible to 'cover' in any course all that is significant and that a general citizen might profitably know. Most high-school text-books are twenty years behind the advancing front of knowledge, and in some very significant matters, because of social or religious opposition, fully a century in arrears. Appropriate scientific treatment must be accorded such 'controversial' subjects as organic evolution, the nature of the individual and racial differences, sex, reproduction in the human species, and the problems of population growth and control.

Even worse than the failure to keep biology for the class-room up to date is the prevalent fault of teaching the life sciences (and indeed all others) as constituting essentially a body of established 'true' information, knit by concepts and laws of nature assumed to be unalterable and prescriptive. That observations are always limited, that laws of Nature are mere summations of experience, and that science consequently is for ever adding new observations, modifying its concepts, and refining or replacing its laws of Nature, are principles honoured in the breach. The conception of science as a body of methods of inquiry—involving accurate and confirmable observations, quantitative treatment of data, and controlled experimentation—gets lip service, but rarely, if ever, enters into the experience of the student at the high-school level. In particular, so-called laboratory work has become a travesty of genuine scientific activity.

It is the essence of the life sciences that they are diverse in point of view and methodology. In consequence, there is no single best way to organize a biology course. There is a vast difference between the analytical, physico-chemical approach of the biochemist or biophysicist and the organismal approach of the student of behaviour and the supra-organismal view of the ecologist who must deal with communities and ecosystems. It is both valid and necessary to explore a variety of ways of approaching and organizing the subject-matter of biology.

Whatever the approach and whatever the organization, the essential character of scientific activity and the great biological themes must permeate the treatment. On one hand, the nature of science as an increasingly important element of human history must be stressed. On the other, the warp and woof of the treatment of the subject-matter must consist of the great biological themes such as the interdependence of structure and function, regulation and homeostasis, the genetic continuity of life, its evolution, diversity of type bound up with unity of pattern, and the relation of organism to environment. These must be treated at all levels of organization, from the molecular level to that of the ecosystem, and at all levels of process, from the chemical reaction through the growth and development of the individual to the evolutionary changes with time.

General education must provide each citizen with an understanding of the nature of the scientific process that has become the source of the most radical social changes and the principal strength and resource of every modern nation. This is of the gravest importance in a democracy, where every ultimate decision rests with the people. This conclusion calls for a thorough and radical change in the nature of most up-to-date science teaching.

A sound understanding of biology is the educational right of everyone who in later years will have to cope with individual problems of health and nutrition, with family problems of sex and reproduction and parenthood, and with the citizen's problems of the wisest management of natural resources, the biological hazards of nuclear radiations in peace and war, and the extent of Governmental support of science as the primary source of national strength and wealth. It was agreed by the steering committee that biology in the high school curriculum might best remain at the tenth grade level, where it is now conventionally taught and where the great majority of students take it, rather than be shifted to a later year where it would have the advantage of following instruction in physics and chemistry but would be taken by a small minority of high school students.

The steering committee adopted the objective of preparing materials for a biology course that would be suitable for all high-school students, irrespective of ability or future plans. This choice did not exclude the possibility of preparing, in addition, biology courses designed either for selected groups of younger students or for those more advanced.

During the summer of 1960 the first drafts of three distinct biology courses, including text-books, laboratory manuals and teachers' guides, were prepared by three teams of writers and supporting staffs to produce the 'yellow', 'blue' and 'green' versions, short names for courses on the respective genetic and developmental, biochemical and physiological, and ecological and evolutionary approaches. In the summer of 1961, a revision of the texts and laboratory work was made.

These books are supported by a teachers' handbook, a set of volumes which includes, among numerous other aids to the teacher, a discussion of the background, emphases, and the aims of the Biological Sciences Curriculum Study, and the distinctions between the three versions and conventional high school courses.

The separate volume of *Biological Investigations for Secondary School Students* presents 100 biological problems at present unsolved, selected and edited for the use of the truly gifted student, from hundreds of suggestions submitted by research biologists in the United States. Each problem is outlined with sufficient detail for the student to begin a long-term project. Copies of this volume, the text-books, laboratory manuals, teachers' guides and the teachers' handbook are obtainable from the American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D.C.

YOUTH SERVICES IN BRITAIN

In opening a debate on the youth service in the House of Lords on February 21, Lord Stonham, while critical of the scale of the Government's contribution of less than £1 million to be distributed among nearly 5 million young people during the year, congratulated the Minister of Education on the action taken to implement the recommendations of the Albemarle report. The National College for Training Youth Leaders was opened at Leicester in January 1961; 84 students completed the course last year, 140 more had started, and there was already a waiting list for next year's course. The Youth Service Development Council had met 14 times since it was founded, usually with the Minister of Education in the chair; the requirements for the status of qualified youth leaders were announced last July and a salary scale ranging from £680 to £1,000 a year was agreed. For the period 1960–63 the Minister had authorized starts on building projects to a value of £7 million, including furnishing and equipment, four-fifths for local authorities and one-fifth for voluntary bodies, and £535,000 had been paid to voluntary organizations in each of the past two years.

For the Government, the Lord Chancellor, Lord Kilmuir, stated that direct financing of the Youth Service by the Exchequer had increased from £229,000 in 1959–60 to £775,000 in the current year. Under the building programme some 750 new and expanded youth clubs and centres were being provided and there was good reason to believe that the Albemarle target of 1,300 full-time qualified youth leaders would be achieved by 1966. Besides the National College, there were full-time courses of training at University College, Swansea, and West Hill Training College, Birmingham, and 10 teacher-training colleges had introduced training for youth leadership as an optional part of their three-year course. Proposals to a value of about £14 million had been submitted in respect of building programmes and because of this the Minister had had to introduce a system of local priorities, irrespective of whether the jobs were to be undertaken by local educational authorities or by voluntary bodies. There had been two conferences of senior Government experts on youth questions: one in Paris, in March 1960, and the other in Brussels, in March 1961; and there would be a third in Strasbourg in March 1962; as well as one at Leicester in April on training full-time leaders. A continuous exchange of information and technical assistance was developing on all matters connected with the youth service and there was the prospect of establishing at Strasbourg in the next two years a European Youth Centre concerned with training senior youth leaders, organizers and administrators, through courses of six months, as well as providing shorter courses and research activities.

In replying on the debate, Lord Newton added that the Youth Service Development's Council publication *Youth Service* had now a circulation of 23,000 and that the grants to national voluntary organizations for 1961–62 totalled £236,000 for 35 organizations. Grants for special developments in experimental work amounted so far to £57,000 and included grants to the Youth Theatre, to the Council for Nature, for appointment of an officer to attract more young people to the work of the Council's conservation corps, and to the National Association of Youth Clubs and the Young Women's Christian Association for research into techniques of approaching and attracting potential delinquent 'unclubables'. Voluntary Service Overseas had been financially assisted through the Department of Technical Co-operation. At the time of the Albemarle report local education authorities were employing directly, or contributing to the salaries of, some 700 full-time youth leaders. By the middle of 1961 this total had increased by at least 100, and authorities and voluntary bodies had plans to employ another 450 over the next three years, while the number of youth officers and organizers employed by local education authorities had increased from 350 to 425 and would probably increase by another 50 in the next three years.

THE CARNEGIE TRUST FOR THE UNIVERSITIES OF SCOTLAND

THE sixtieth annual report of the Executive Committee to the Trustees of the Carnegie Trust for the Universities of Scotland for the year 1960–61 * records payments of more than £135,000 to universities and other institutes under the quinquennial arrangements, in addition to expenditure on schemes directly administered by the Trust. The major projects have been, in the University of St. Andrews, £9,881 for a building for the Biology Department at Queen's College, Dundee, and £5,055 for the Bell-Pettigrew Museum at St. Salvator's College; at the University of Glasgow, £36,400 for the Stevenson Physical Education Centre and £5,700 for the new Arts Faculty building; £26,325 for the Physics Department building at the University of Aberdeen; and £25,000 for the Holland House Hall of Residence at the University of Edinburgh.

Early in 1961 assurances were given to the universities that the University Grants Committee would not expect them to contribute from their own resources to approved building programmes for 1962 and 1963 and to the provisional programmes for 1964 and 1965. Accordingly, the Trust has informed the universities that during the quinquennium 1962–67 it would not make grants to remedy any deficiency in the Treasury Grants for teaching buildings as it had done in the 1957–62 quinquennium. The provision of new teaching buildings is regarded as a Government responsibility, and in the new quinquennium the Trustees consider that in student residences, playing fields, unions, staff common rooms, etc., there is a large field in which they can substantially help the Scottish universities to supply the proper amenities of university life, provision of which would otherwise be seriously delayed if made at all. In the quinquennium 1962–67, the Executive Committee has decided to release from resources £250,000 for capital expenditure on approved schemes, while retaining normal recurring grants at an annual level of £60,000. Of the allocation from reserves, £72,000 each will be available to the Universities of Glasgow and Edinburgh, £48,000 each to St. Andrews and Aberdeen, and £10,000 to the Royal College of Science and Technology, Glasgow.

Expenditure on the research scheme exceeded £60,000, subventions in aid of publication of works of scholarship increasing to £3,167 (excluding two special provisions made in the previous year), and from October 1961, Glasgow and Edinburgh are each to receive £3,000 annually, and St. Andrews and Aberdeen £2,000 annually, to facilitate travel in connexion with research by members of the staff of the universities. The usual notes on the work of Fellows, Senior Scholars and Scholars during the session 1960–61 are given in an appendix, together with lists of research grants and of publications.

The system of assistance to students with fees continued without significant change from its modified form when the Scottish Education Department altered its bursary regulations in 1957. The number of beneficiaries fell from 538 in 1959–60 to 455, and payments from £18,185 to £15,575, and the Trust's scheme will continue to be administered on the same principles as before until the results of the new Government scheme, following the acceptance of many of the recommendations of the Advisory Committee on Awards to Students, can be properly assessed. Repayments by beneficiaries fell to £1,482 by thirty beneficiaries, compared with £2,069 by thirty-nine in 1959–60. In the summer of 1961, a system of vacation awards to students who had shown exceptional merit at their university, which had been tried in one faculty in 1960, was extended to all faculties at each of the Scottish universities. In all, sixty-one awards were made, totalling £2,640, to senior students generally either about to enter their final year or about to go on to research immediately after first graduation. In arts most awards in 1961 were used to enable their recipients to study a special topic, or to pursue language or other studies at a foreign university. This scheme has been a great success and it may be desirable to increase expenditure on it.

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* The Carnegie Trust for the Universities of Scotland. Sixtieth Annual Report for the year 1960–61. Pp. vi+68. (Edinburgh: The Carnegie Trust for the Universities of Scotland.)

HAZARDS OF DUST

THE stage is set for action when, in a time of rapid social change, a recently nationalized industry which employs nearly three-quarters of a million men is found to be also manufacturing hundreds of cases of an industrial disease. If, in addition, the Nation depends fundamentally on the product of this industry which possesses, therefore, a trade union of great power, then in the colloquial phrase "something will happen".

After an initial few years of feeling its way through situations, some of which were almost laughable—such as when the embryo medical research programme was directed by a former professor of physics—the National Coal Board has now come to be equipped with strong medical and scientific services managed by hard-headed men who know what they are about.

A report, entitled, *The Study of the Composition of Respirable Dust in the Pneumoconiosis Field Research*, comes from the scientific service alone*. It summarizes the considerations which have led up to the present views on the study of the composition of airborne dust in coalmines. It discusses how the collieries should be selected for dust sampling and the statistical design of the sampling method. The use of a thimble versus a thermal precipitator technique for sampling is balanced up and the writers come down on the side of the thermal precipitator. They then summarize what is known about the coal and mineral content of airborne coalmine dust and the techniques necessary for its analysis. For this, they suggest that a phase-contrast method be used with an optical microscope. The man-hours necessary for dust surveys of different types are calculated, and the relation of the physics and chemistry of coalmine dust to the human disease of pneumoconiosis is considered.

The authors realize that what they are talking about are shadow pictures of men's chests rather than functional disease, and they feel that the X-ray absorptions of dusts of differently ranked coals should be studied. They also point to a fundamental gap in our knowledge of the pathological effects of dust on the animal cell by suggesting that this should be investigated experimentally. These are merely two of the more interesting ideas from a number of projects for future work.

But it must be admitted that it is not easy to get the sense out of this report and the abstract says nothing about what is really between the covers.

Another report, entitled, *The Relation between Pneumoconiosis and Environmental Conditions*, describes the most impressive survey of its kind which has ever been carried out anywhere in the world*. The chest X-ray appearances of more than 30,000 coalworkers have been set against all the different types of work which they had carried out up to the time the pictures were taken. The survey took in 26 different collieries chosen to cover the range of relevant geographical and environmental conditions. The sample of men was nearly 95 per cent complete.

This extensive national study shows that there are wide variations in the prevalence of pneumoconiosis and in its relation to the age of the mineworker, but with regional similarities; that the higher the rank of coal the greater the prevalence of chest X-ray abnormality; and that underground coal-getters are the occupational group most at risk. There is now, therefore, for the first time a pneumoconiosis prevalence map for the coalfields. Apart from this, the work has confirmed what was known or strongly suspected already, and the authors reasonably look forward to concentrating the survey in the future, in an attempt to answer the 'why' rather than the 'what' of the problem.

This is a clearly presented document with 31 tables of data in the body of the report, as well as many more in the appendixes, in which there are also 23 well-drawn coloured charts.

As a source-book of data on dust disease in British coalmines it is a *sine qua non*. R. C. BROWNE

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* *The Study of the Composition of Respirable Dust in the Pneumoconiosis Field Research.* By J. W. J. Fay and D. Hicks. Pp. ii+25+6 tables. Appendix 1–3. (London National Coal Board, 1962.)

* *The Relation between Pneumoconiosis and Environmental Conditions: an Analysis of the Results of the First Series of X-ray Surveys in the National Coal Board's Pneumoconiosis Field Research.* By J. R. Ashford and S. Rae. Pp. viii+40+31 tables. Appendix 14+23 figures. (London. National Coal Board, 1962.)

SCIENCE IN THE PACIFIC

SPONSORED by the National Science Foundation (Washington, D.C.), a new series of *International Science Information Studies* examines the main scientific publications, research facilities and information services of selected countries of the world. The first three volumes have been written by Mr. John O. Sutter and are devoted to four Pacific nations, namely, the Republic of Indonesia, the Federation of Malaya, the State of Singapore, and the Republic of Viet-Nam*.

The object of the series is to help in overcoming some of the obstacles to communication between scientists brought about by distance, language and different cultural patterns Each volume starts with a brief account of the geographic, political and economic background of the area and then describes the facilities available for education in science, concluding with an account of scientific research and information activities in the country. In appendixes, factual information is given about research institutes, libraries, translation services and scientific publications.

Mr. Sutter's investigations show that in Malaya and Singapore there are forty-four scientific or semi-scientific serial or periodical publications, ranging in content from descriptions of original research to material of a popular science type. In the Republic of Viet-Nam, there are relatively few periodicals of a scientific nature, though French-established institutes publish articles on oceanography, medicine (Pasteur) and rubber research. In Indonesia, there is an ever-increasing flow of publications, some of which contain papers on original research. Before the Second World War these were in Dutch, but now they are in Indonesian with some articles in English or with English summaries.

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* National Science Foundation, Washington. Pacific Scientific Information. No. 1: *Scientific Facilities and Information Services of the Republic of Indonesia.* Pp vi+136. 1 dollar No. 2: *Scientific Facilities and Information Services of the Federation of Malaya and State of Singapore.* Pp. vi+43. 50 cents. No. 3: *Scientific Facilities and Information Services of the Republic of Viet-Nam.* Pp. vi+36. 50 cents. By John O. Sutter. (Honolulu: Pacific Scientific Information Center, B. P. Bishop Museum. Published for the National Science Foundation.)

POSSIBLE MECHANISM OF OLFACTION

By DR. ANDREW DRAVNIEKS

Armour Research Foundation of Illinois Institute of Technology, Chicago

OLFACTORY receptors detect the presence of 10^5–10^7 molecules of certain odorivectors per cm.^{3} of air, discriminate between small differences in the molecular structures of these odorivectors, and convey the observations to the nerves in the form of electrical potential pulses several tens of mV. strong. Such action may be possible by a selective utilization of dispersion (London–van der Waals) forces, together with some dipole interactions, in absorption on ferroelectric substrates. Circumstantial evidence may be enumerated.

Relative rapidity of appearance and disappearance of the olfactory sensation leads to the conclusion, from estimates by the absolute rate theory, that the energies of adsorption must be in the range of 5–15 kcal. and that the activation energy must be not more than a few kcal. From a typical isotherm[1], only low coverages ($\ll 0{\cdot}01$) can result on a receptor surface. Even if every adsorbed molecule would produce a dipole change of 2–4 Debye, much higher than thus far observed[2], the resultant surface potential change would be only a fraction of a mV.

However, an amplification of such change is possible on a ferroelectric substrate. Below the Curie point, and before a spontaneous polarization occurs, a mechanical strain exists in the ferroelectric lattice. If the odorivector molecules adsorb on a number of sites, the polarizing field of the permanent or induced dipoles of the osmophoric groups serves to nucleate the lattice shear. This triggers the ferroelectric transformation throughout the ferroelectric domain and results in a spontaneous polarization. The probability of triggering within a given time interval will be proportional to the number of the adsorbed molecules per domain. The resulting Barkhausen pulse[3] may serve as a source of a large signal to the olfactory nerve. The receptor may be restored to the non-ferroelectric lattice form periodically by a metabolic process, followed by a descent back into a strained state as the final preparation for a new pulse. The odorivector molecules may remain on the surface during all parts of the cycle, or else the ferroelectric shear transformation may destroy the specificity of the adsorption sites and cause a temporary decrease in coverage.

The indications that ferroelectric substances occur in organisms have been enumerated by Athestaedt[4]. Some of the more recently discovered ferroelectrics such as guanidine and glycine sulphates and thiourea approach organic structures. It appears likely that co-operative charge separation may occur in proteinic structures.

Inspection of the energy term in the adsorption isotherm[1] shows that a difference of 1–2 kcal. in the energy of adsorption may change the coverage and consequently the probability of triggering in the low-coverage range by a factor of 10–100. At a coverage below a certain threshold the pulses will be too infrequent or too indistinct. Above certain coverage the pulses may be too frequent to be transmitted by the nerves; this would occur also with substances that are adsorbed strongly and unspecifically, such as most molecules with a molecular weight above 300, which usually have no odour[5]. Thus, the selectivity may be based on a sensitivity of the proposed pulsing mechanism to a band of adsorption energies somewhere in the 5–15 kcal. range but by itself only 1–2 kcal. wide. With several receptors[6,7], each tuned to a different adsorption energy band, differentiation between odours becomes possible. Other sub-differentiation may be based on discrimination of the frequency of pulsing.

The energy of adsorption necessary to reach the required energy band may be collected from the dipole and the dispersion-type interactions. Here, many correlations pointed out by various olfactory theories fall in place at least qualitatively.

The bond dipole moments for the functional groups range between 1·4 and 3·5 Debye, and since they are located on the periphery of the molecule, they can interact strongly with the highly polarizable site of the ferroelectric substrate. Two point dipoles at a distance $r = 3$ Å. would interact with an energy of 4·5 kcal., and when the actual dimensions of the dipoles are commensurable with the separation distance, this value may be 50–100 per cent higher[8]. Thus, Müller's dipole theory[9] and various theories of the molecular shape[10-13] elaborated by Beets[14,15] in his profile-functional-group concept, all fall into proper perspective, since the stericity of the whole molecule determines the closest distance of approach between the molecule and receptor dipoles and also influences the effective moment of the functional group.

On the other hand, the critical additional increments in the adsorption energy, to shift its value into the pulse-stimulating band, can be supplied by the frequency-sensitive dispersion forces[16]. This provides a link to the phenomenological aspects of the vibrational theories. Wright's[17,18] objections that Dyson's[19-21] osmic frequencies are energetically implausible would not apply in utilization of these vibrators—together with the interactions of dispersion electrons—for generation of additional attractive energy between the odorivector and the oscillators in the receptor surface. Even if the oscillators are at the lowest quantum-level, they possess a zero-energy $1/2h\nu$ and are considered responsible for the van der Waals interactions in solid lattices[22]. Since the dispersion interactions are additive, with the energy proportional to r^{-6}, many of the oscillators in the odorivector will contribute to the total adsorption energy, but those located in the peripheral functional groups would contribute most significantly. Hence some but not consistent[23] correlation between Raman spectra and the odour; in some cases the dispersion interaction caused by the osmic frequency vibrator may not be critical in shifting the adsorption energy into the specific band. London[24] has given mathematical framework for adsorption by dispersion forces for the case of a spherical molecule and an infinite surface with a quasi-continuous arrangement of oscillators. For explanation of the specificity in olfactory adsorption this simple picture must be elaborated by consideration of interactions between specific oscillators in molecules of a complex shape and distinguishable specific oscillators in the receptor surface; probably even the directions of oscillations must be considered.

It is possible that some receptors may utilize hydrogen bonding[25] as one of the sources of adsorption energy.

Even Zwaardemaker's findings[26] on generation of spray electricity by solution of odorivectors tie loosely into the picture of the dispersion-force adsorption, since the ionization energies of adsorbed molecules enter a form of London's equation[24]; but on the other hand, co-determine the ease of charge separation in rupturing the double layer at surface on which the molecules are adsorbed.

Thus we propose that ferroelectric receptor surfaces cycled through the spontaneous polarization threshold may serve as transducers for a selective sorting-out of adsorbable odorivectors. The energy of adsorption would determine the delay and consequently the frequency of the Barkhausen pulses if the act of depolarization by an outside energy source is fast enough. The odorivectors will be recognized principally by the pulse frequency. In olfaction, similar mechanism coupled to transmitting nerves responsive only to a limited range of frequencies may be functioning.

I am attempting to demonstrate experimentally the relation between the adsorption and the ferroelectric effects in inorganic systems.

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THERMAL EXPANSION MEASUREMENTS ON GRAPHITE–BROMINE COMPOUNDS

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LARGE increases in the thermal expansion coefficient of graphite have been observed on the addition of small quantities of bromine. When graphite is exposed to an atmosphere containing bromine vapour at room temperature, lamellar graphite–bromine compounds are formed. Bromine enters the graphite lattice by diffusion between the layer planes, and under given pressure–temperature conditions, the limiting composition is dependent on the degree of perfection of the graphite structure. Changes in both single crystal and bulk graphite dimensions on bromination are reported in the literature. X-ray analysis of the limiting composition of near-perfect crystals[1] shows a large increase in the *c* dimension of the graphite structure due to the presence of bromine layers between the graphite layer planes. The increase in bulk graphite dimensions has been determined with varying bromine content[2]. The contribution of the *c*-axis expansion to that of the bulk polycrystalline graphite in any given direction will depend on the orientation of the crystallites and the amount of microporosity reducing the effect of the single crystal expansion.

Desorption at room temperature by removal of the bromine atmosphere leaves a residual bromine content. These residual compounds have been observed by several authors, and are discussed by Ubbelohde and Lewis[3]. The bromine may be held by normal lamellar bonding with outward diffusion paths blocked, or at defects in the graphite structure. This article presents results on the effect of this residual bromine on the thermal expansion of a number of graphite specimens.

The effect was observed on two types of artificial extruded graphites, *A* and *B*, differing in their orientation characteristics and basic raw materials, but graphitized at very similar temperatures. Thermal expansion measurements were made in a silica dilatometer over the range room temperature to 700° C. on specimens cut parallel and perpendicular to the extrusion axis. The specimens were suspended from a calibrated quartz spring for brominating, and length and weight increases were followed with a cathetometer. Table 1 shows the maximum values of strain and bromine composition (atomic ratio Br/C × 100) for the initial bromination of these specimens, and the effect of heat treatments on the residual compound obtained after allowing the brominated

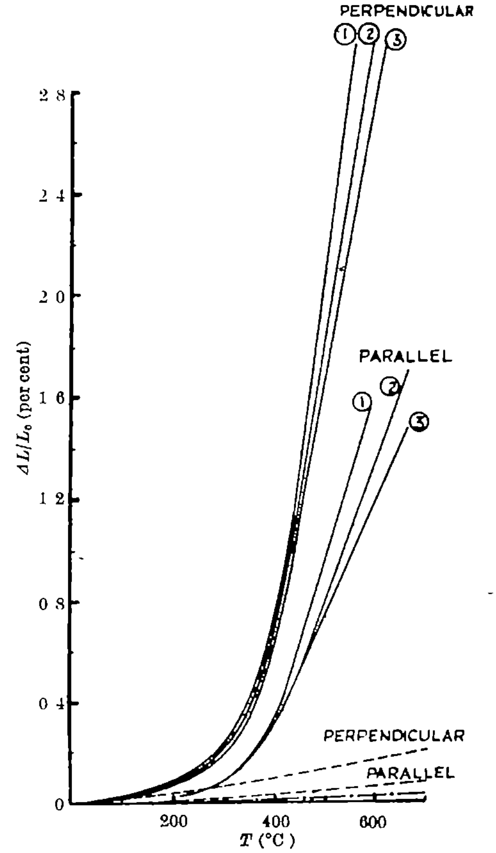

Fig. 1. Effect of residual bromine on the thermal expansion of Graphite *A* parallel and perpendicular to extrusion. (Curves relative to silica)

- - -, Before bromination; —, after bromination. Atomic ratio (Br/C per cent): perpendicular (1) 0·85–0·76; (2) 0·76–0·71; (3) 0·71–0·67, parallel (1) 0·88 – –; (2) – – 0·74, (3) 0·74–0·76. – –. Silica

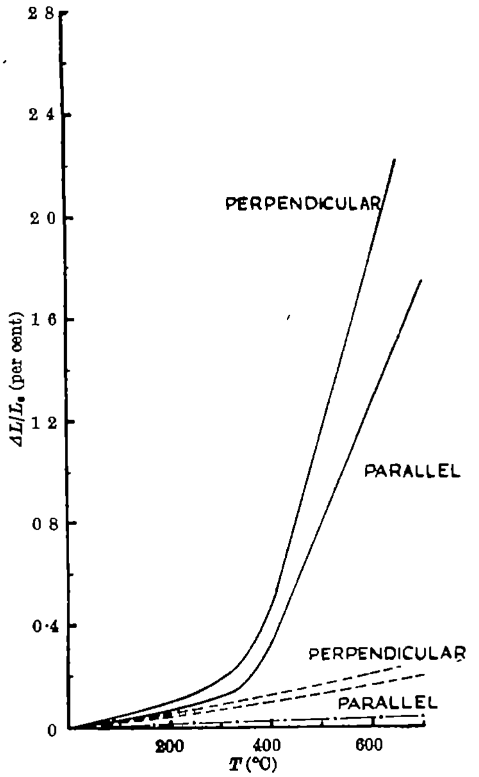

Fig. 2. Effect of residual bromine on the thermal expansion of graphite *B* parallel and perpendicular to extrusion. (Curves relative to silica)
– – –, Before bromination; ——, after bromination. Atomic ratio (Br/C per cent): perpendicular, 0·91–0·82; parallel, 0·94–0·82. –·–, Silica

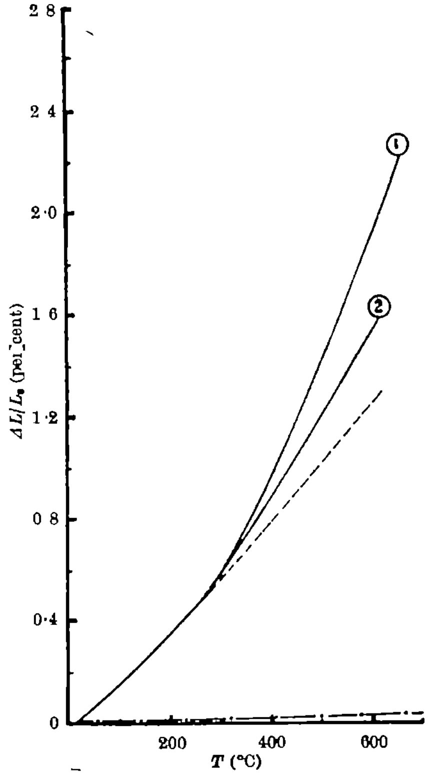

Fig. 3. Effect of residual bromine on the thermal expansion of a natural graphite compact parallel to pressing. (Curves relative to silica)
– – –, Before bromination; ——, after bromination. Atomic ratio (Br/C per cent): (1) 0·04–0·01; (2) 0·01 – –. –·–, Silica

specimens to stand in air at room temperature for several days. The residual bromine content was found to be only partially removed on heating to 700° C. under vacuum.

Thermal expansion measurements on the brominated specimens (Figs. 1 and 2) show a large increase over the virgin sample even below 300° C., but above this temperature, the thermal expansion-rate increases rapidly to a much higher value. The length returns to about the original (residual) value on cooling, and repeat runs show a lower expansion coefficient together with some loss of bromine.

Measurements were also made on a specimen of a pressed natural graphite compact cut parallel to the pressing direction and containing a small quantity of residual bromine. This material is of high density and well orientated, giving a high contribution of the *c*-axis in the parallel direction. It should therefore give a good indication of single crystal behaviour. There was no significant change in thermal expansion coefficient up to about 300° C. (Fig. 3), but a rapid increase was again observed above this temperature. No prior heat treatment was given to this specimen after bromination and a large fraction of the bromine content was removed during the first thermal expansion run. A second run showed the same characteristics as the first with a lower expansion rate above 300° C.

The results from the natural compact show that below 300° C., the thermal expansion coefficient of the residual bromine compound is the same as that of graphite when the bromine concentration is low. Above 300° C. the thermal expansion coefficient of the bromine compound is higher than that of natural graphite, and increases with bromine concentration.

The thermal expansion coefficients of the artificial graphite residual compounds are higher than those of the original graphites even below 300° C. Helium density measurements show that the artificial graphites contain closed porosity, whereas the natural graphite does not. It is this porosity which accommodates part of the *c*-axis expansion, thus making the volume thermal expansion coefficient of the artefacts much less than that of a single graphite crystal. However, in the bromine residue compounds formed from the artificial graphites, the *c*-axis has expanded and closed some of the microporosity. It is considered that the closure of this porosity makes the artificial

Table 1. SPECIMEN HISTORY—BROMINE CONCENTRATION (ATOMIC RATIO Br/C × 100) AND STRAIN

Graphite	Density (gm./c.c.)	Direction of cut with respect to extrusion or pressing	Maximum composition and strain during bromination		After bromination and standing in air		Residual composition and strain							
							After 4 hr. at 700° C. under vacuum		After thermal expansion 20–700° C.					
									Run 1		Run 2		Run 3	
			Br/C (per cent)	$\Delta L/L$	Br/C (per cent)	$\Delta L/L$	Br/C (per cent)	$\Delta L/L$	Br/C (per cent)	$\Delta L/L$	Br/C (per cent)	$\Delta L/L$	Br/C (per cent)	$\Delta L/L$
A	1·74	Parallel	5·48	2·9	1·18	0·4	0·88	0·3	—	—	0·74	0·2	0·70	0·3
		Perpendicular	6·02	7·5	1·17	1·0	0·85	0·8	0·76	0·7	0·71	0·8	0·67	0·7
B	1·70	Parallel	5·25	3·1	1·32	0·7	0·94	0·3	0·82	0·4	—	—	—	—
		Perpendicular	6·00	5·3	1·32	0·9	0·91	0·6	0·82	0·5	—	—	—	—
Natural compact	2·00	Parallel	0·26	0·90	0·04	—	—	—	0·01	—	—	—	—	—

Table 2. Comparison of Volume Thermal Expansion Coefficients of Natural Graphite Crystals and Artificial Graphite Residual Bromine Compounds

Graphite	Volume expansion coefficient ($\times 10^6$) (500°–600° C.)
A (residue compound)	240–330
B (residue compound)	180
Single crystal (ref. 4)	29

graphite–bromine compounds less able to accommodate further thermally induced *c*-axis expansion, and this is at least partially responsible for the higher thermal expansion coefficients observed in these compounds below 300° C. Above 300° C., as in the case of the natural compact, the thermal expansion coefficients of the artificial graphite residual compounds are much larger than that of a normal graphite crystal, and increase with bromine concentration.

This fact is well illustrated in Table 2, where mean volume thermal expansion coefficients of the artificial graphite–bromine compounds, over the temperature range 500°–600° C., are compared with that of a single graphite crystal using the data of Nelson and Riley[4].

One further point emerges from Table 2 and the data presented in Figs. 1 and 2. The volume thermal expansion coefficient of graphite *B* is higher than that of graphite *A*, yet the volume thermal expansion coefficients of the residual bromine compounds of graphite *A* are higher than that of compound *B* when the two types of graphite contain similar quantities of bromine. This indicates that the residual bromine compounds formed in the two artificial graphites are not identical.

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ROLE OF *OLPIDIUM* IN THE TRANSMISSION OF BIG VEIN DISEASE OF LETTUCE

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THE symptoms of big vein (yellow vein banding, sometimes with leaf puckering) are similar to those commonly associated with certain types of plant virus infection. Probably mainly because of the symptoms and since the disease was known to be soil-borne it was formerly thought to be caused by a soil-borne virus[1]. Numerous attempts have been made to isolate viruses from the roots of infected plants, but the only one frequently isolated was tobacco necrosis virus[2–4], which consistently failed to induce big vein symptoms when inoculated into healthy plants. Tests of mechanical transmission with leaf sap[5] and transmissions by aphids[6] and eelworms[5] were also negative.

In 1958, it was found[7,8] that the roots of big vein affected plants were invariably infected with a species of *Olpidium* and that zoospores of the fungus taken from such plants would induce the disease if they infected the roots of healthy plants. These investigations suggested that root infection by the fungus was the sole cause of the disease. Many workers have doubted this, however, because the fungus is confined to the surface cells of the roots and because the leaf symptoms are unlike those normally associated with *Olpidium* infection (stunting and wilting of the plants —usually slight[9], but occasionally severe[10]).

New evidence that the cause of big vein was of a virus-like nature was obtained when the disease was transmitted by grafting the tops of plants together in the absence of root infection by *Olpidium*[11,12]. It has also been reported[11] that Haeske[13] obtained transmission with *Cuscuta gronovii* Willd.

Further evidence is now presented that this virus-like causal agent is introduced into the plants by the fungus.

The investigation became possible when an *Olpidium*, indistinguishable in morphology and extent of root invasion from that present in the roots of big vein affected plants, was found in the roots of normal lettuce plants growing at this Station. In numerous serial transfers to healthy lettuce the fungus consistently failed to induce big vein. It was accordingly used in two experiments to determine the role of *Olpidium* in the transmission of the disease. This was done by culturing it for three weeks in the roots of plants affected with big vein, but originally having no *Olpidium* in their roots, and then transferring it to other lettuce plants on which the occurrence of big-vein symptoms could be assessed. Details of the experiments are as follows.

In one experiment, nodal cuttings were rooted from the stems of a wild *Lactuca* sp. (U.S. Department of Agriculture Plant Introduction Service No. 177418) and grown in pots of autoclaved soil. Twelve of them were taken from plants showing big vein symptoms and 8 were from healthy plants, and none had *Olpidium* in their roots. A 'healthy' isolate of *Olpidium* was inoculated into the roots of these plants by pouring on the soil a zoospore suspension obtained by immersing in water the roots of a healthy *Lactuca* plant infected with such an isolate (treatment 1). Similar cuttings, 12 with big vein and 8 healthy, were kept as controls and were not inoculated with *Olpidium*, and the roots of 8 further healthy cuttings were inoculated with zoospores of a 'big vein' *Olpidium* isolate (that is, one known to induce big vein symptoms). Precautions were taken against chance infection with *Olpidium*.

After 3 weeks each of the 48 cuttings in the experiment was removed from its pot and its roots were examined for the presence of *Olpidium*. The fungus was present only in the inoculated roots. Several 2-cm. portions from the root system of each plant were then detached, washed free from soil and placed in a vial of tap water for 10 min. Zoospores were released from the inoculated roots. All the roots were then removed and five 10-day-old *Lactuca* seedlings were stood in each vial for 24 hr. The seedlings were then grown in autoclaved soil for 10 weeks and examined for root infection by *Olpidium* and for the presence of big vein symptoms on their

Table 1. TRANSMISSION OF BIG VEIN BY *Olpidium* CULTURED IN THE ROOTS OF AFFECTED PLANTS

Treatment No.	Derivation of suspensions: Type of *Olpidium*	Derivation of suspensions: Status of culture plant	No. of test seedlings	Effect on seedlings: With *Olpidium* infection: No. with big vein	With *Olpidium* infection: No. without big vein	Without *Olpidium* infection: No. with big vein	Without *Olpidium* infection: No. without big vein
1	'Healthy'	Big vein	43	21*	4	0	18
2	'Healthy'	Healthy	28	0	24	0	4
3	Nil	Big vein	40	0	0	0	40
4	Nil	Healthy	30	0	0	0	30
5	'Big vein'	Healthy	27	24	0	0	3

* Infection from 8/12 separate suspensions

leaves. The results of these observations are shown in Table 1.

The result of treatment 1 shows that the zoospores liberated from the roots of the culture plants showing big vein transmitted the disease to 21/25 of the seedlings they infected. These zoospores, however, had arisen from a fungus which, when inoculated into the culture plants 3 weeks previously, would not transmit big vein and which still would not transmit the disease after being cultured in the roots of healthy cuttings (treatment 2). The result of treatment 3 shows that transmission from the roots of big vein affected plants did not occur in the absence of *Olpidium*.

The design of another experiment was similar to that of the first, but plants showing big vein were obtained by grafting scions from *Lactuca* plants affected with big vein on to healthy *Lactuca* stocks, which then developed leaf symptoms of the disease. Similar plants grafted with healthy scions were used as controls. Eight weeks elapsed between grafting and the inoculation of the plants with *Olpidium*. The results of this experiment are shown in Table 2.

The results of treatment 1 are much less conclusive than those of the corresponding treatment in the first experiment, but some transmission was obtained. It is thus apparent that the graft-transmissible causal agent of big vein was also transmitted by *Olpidium*. It is thought likely that the difference between the two results is related to the fact that when cuttings from big vein plants were used the roots developed from infected tissue, whereas most of the roots of the grafted plants developed while the plants were healthy. The proportion of roots from which the disease could be transmitted may therefore have been low.

Table 2. TRANSMISSION OF BIG VEIN BY *Olpidium* CULTURED IN THE ROOTS OF AFFECTED GRAFTED PLANTS

Treatment No.	Derivation of suspensions: Type of *Olpidium*	Derivation of suspensions: Status of grafted culture plant	No. of test seedlings	Effect on seedlings: With *Olpidium* infection: No. with big vein	With *Olpidium* infection: No. without big vein	Without *Olpidium* infection: No. with big vein	Without *Olpidium* infection: No. without big vein
1	'Healthy'	Big vein	18	3*	12	0	3
2	'Healthy'	Healthy	15	0	15	0	0
3	Nil	Big vein	24	0	0	0	24
4	Nil	Healthy	21	0	0	0	21
5	'Big vein'	Healthy	27	18	0	0	9

* Infection from 2/5 separate suspensions.

To test for the presence of tobacco necrosis virus the primary leaves of young cowpea plants were inoculated with macerates of the roots of samples of plants from every treatment (except No. 4) in both experiments (total 27 plants). All these inoculations were negative.

Several explanations of the observed results are possible, but it is considered likely that the graft-transmissible agent of big vein (possibly a virus) was carried by the zoospores of *Olpidium*, either externally or internally, from the infected roots of the culture plants to the healthy roots of the test plants. The fact that the roots of the culture plants and the *Olpidium* zoospores released by them were in the test suspensions at the same time makes it possible, however, that the roots exuded the infectious agent into the water and the subsequent *Olpidium* invasion of the roots of the test plants provided entry points for the infectious agent. Teakle[14] has reported that the entry of tobacco necrosis virus into lettuce roots may be facilitated by the concurrent invasion of the roots by *Olpidium*. In our opinion, however, the tobacco necrosis virus is not the causal agent of big vein. Further experiments are in progress to determine the exact mode of transmission of the disease.

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A CONSTANT PATTERN OF GROWTH IN YOUNG APPLE TREES

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HATTON[1] has described the rootstock effect in apples where, by using suitable rootstocks, trees of the same scion variety may be produced which differ up to ten-fold in their annual increments and four-fold in accumulated crop[2]. A similar, though smaller, scion effect may be produced by growing a range of scion varieties on a single rootstock variety[3]. Because of the wide range of possible experimental effects, such material is suitable for analysing the growth (in a broad sense) of woody species[4]. In such species the large and always increasing woody skeleton presents a complicating factor not encountered with annual or herbaceous crops, for its growth may account for more than 50 per cent of the annual increment[5]. The pattern of growth of the successive annual growth regions of this skeleton is obviously an important consideration in determining the most efficient form of tree for fruit production. and also in

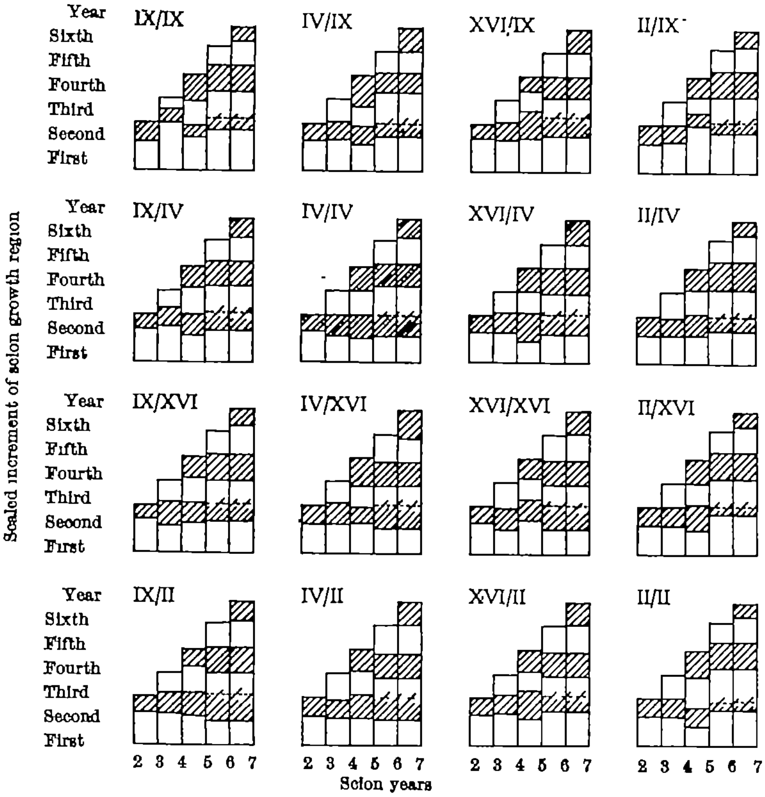

Fig. 1. The distribution of increment to the growth regions for each of the 16 rootstock/scion combinations. The combinations are indicated by Roman numerals, those in the rows having the same rootstock and in the columns the same scions.

crops where the skeleton itself forms the main economic product such as timber and various bark crops. Duff *et al.*[6], in their interesting analyses of 'pattern' and 'configuration' in *Pinus resinosa*, have suggested how external and intrinsic factors interact to determine the amount of cambial activity at a given site, but the fraction of the total increment involved in each region cannot be estimated from their data.

The term 'annual growth region' may be clarified by the following example: in a ten-year-old tree all the current extending shoots form the tenth-year annual growth region, and the total annual woody increment is made up of the extension and thickening of this tenth-year annual growth region plus the tenth-year's thickening on the ninth-, eighth- . . . first-year growth regions. It follows that there are as many of these growth regions as there are years of tree growth. The increments of each region can be determined by the difference between samples of trees analysed at the beginning and end of the growing season. Thus, although the determination of the pattern of growth increments to the woody regions of the tree is not difficult in theory, it does not appear to have been previously attempted, probably because of the length of time required and failure to realize the experimental potentialities of clonally propagated rootstocks. I report in this article a result from such an experiment.

The experiment has already been described in detail[7]; briefly, its main part consisted of adequately replicated plantings of the sixteen rootstock-scion combinations formed by grafting together in pairs the four apple rootstock varieties *M*.II, *M*.IV, *M*.IX and *M*.XVI, used both as rootstocks and as scions. Of these varieties, *M*.IX is slow growing and, as a rootstock, dwarfing, and *M*.XVI is fast growing and invigorating; the other two are intermediate, *M*.II approaching or even surpassing *M*.XVI but maturing to a smaller tree. The experiment lasted seven years, during which time fifteen sets of samples were dug up for the determination of the dry weights of the parts. Replication was six-fold at each sampling. Since the first year's scion growth was complicated by the removal of the rootstock's shoot system, only the five-year period from the end of the first-year's scion growth to the final harvest will be considered. To avoid excessive soil disturbance in the later samplings, root weights were then estimated from sub-samples of certain treatments. Pending a closer analysis of this estimation, root weights have been omitted, and the data refer to scions only.

The total increase in scion dry weight from the second to seventh year of scion growth is given in Table 1. It will be noticed that the heaviest treatment weighed eight times as much as the lightest, and that differences between rootstocks were more marked and consistent than between scions. The differences between treatments were initiated early in the experiment and augmented gradually by similar differences from year to year without any consistent interaction of rootstock and scion variety.

To compare the distribution of the increment of one combination with that of another, some form of scaling must be used, and for this the annual growth region is taken as the unit. For example, the woody increment during the second year of scion growth can occur on two growth regions—the second-year extension shoots and the thickening of the first-year's shoots. The woody increment is therefore scaled to two units, and the two components adjusted proportionally to their weights to add up to this; the distributions for other periods are calculated similarly. The scaled increments for the individual combinations for each period are given in Fig. 1. No samples were taken between the fourth and sixth years, and in order to compute the distribution over that period, the increment for the two-year period was scaled to eleven units (5 + 6), 2/11 being assigned to each of the first five growth regions and 1/11 to the sixth, since it was only present for one year. It was not possible to separate satisfactorily the second and third year growth regions at the final sample, and in the diagrams the separation between them for the final interval has been indicated as the average of the first three intervals.

Table 1. INCREASE IN SCION DRY WEIGHT (KGM.) 2ND–7TH YEAR OF SCION GROWTH

Rootstock	Scion *M*.IX	*M*.IV	*M*.XVI	*M*.II	Rootstock mean
M.IX	0·8	1·4	0·9	1·2	1·08
M.IV	2·0	5·2	3·4	4·5	3·78
M.XVI	2·3	3·5	3·8	4·8	3·60
M.II	3·6	4·5	6·1	6·7	5·23
Scion mean	2 18	3 65	3·55	4·30	3·42

The most remarkable features of the results are the similarity of the patterns for all treatments, despite the large differences in absolute increment, and the uniformity of the distribution from year to year. The larger fraction received by the first-year region and the smaller by the second can be explained as the response to pruning the rootstock shoots just prior to the outgrowth of the first-year scion shoot; the result of this was that this shoot made extra growth[8], resulting in a permanent increase in the cambial surface for that region. In the second year the extension growth was perforce proportionately less, but it is noteworthy that these proportions were generally maintained in subsequent years.

The constancy of the pattern from year to year, despite differences in season, indicates a close relation between extension growth and cambial activity. The relation is likely to be complex, depending itself on the relations between growth-rate and taper of the individual shoots, lengths and number of shoots in successive years, and the thickening of successively older regions. Data bearing on these points are now being examined. These results covered only the first seven years of growth, and it cannot be assumed that the constant distribution will apply throughout the life-cycle of the three.

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A SPERM FACTOR AFFECTING THE RECEPTIVITY OF *DROSOPHILA MELANOGASTER* FEMALES

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ONCE a virgin female *Drosophila* has mated she is usually unwilling to accept another male for some time. The length of this refractory period varies between species and probably between different strains of the same species. Smith[1] has shown that *D. subobscura* females rarely mate a second time, and has partly analysed the mechanism behind the change from receptive virgin to unreceptive fertilized female. He finds that the act of copulation itself prevents further mating for a period of 4–24 hr., but females become receptive again unless the copulation has provided them with sperm. If they have been adequately inseminated they remain unreceptive for the rest of their lives, even after their supply of sperm has become exhausted by egg-laying. Thus, although some factor from the sperm 'switches off' the receptivity mechanism, the subsequent absence of sperm does not lead to its being switched on again.

Drosophila melanogaster females are known to re-mate rather readily. If left with males in small vials they will often mate again on the day following their first mating. However rape is quite common in this species, and such rapid re-mating probably does not mean that normal receptivity returns after one or two days. Accordingly, I have repeated some of Smith's analysis using *D. melanogaster*.

A large batch of virgin females was fertilized when 3 days old, by which age their ovaries are fully grown and they are at their peak of receptivity[2]. They were kept in groups of 25, each in a well-yeasted vial and were shaken into a fresh vial every day. Under such conditions they lay eggs abundantly and continuously for many days. On each day subsequent to fertilization a batch of 50 was tested for receptivity. They were shaken into a half-pint milk bottle with 50 males (always 4 or 5 days old) for a period of 30 min. and pairs were removed as they began to copulate. Males court very vigorously in the test bottle, but unreceptive females have more chance to avoid them than if confined in a vial.

As a kind of base-line, 3-day-old virgin females in the same test show 30–35 pairs mated within 5 min. and 40–45 within the whole 30-min. period. Table 1 shows the comparable figures at different times after fertilization. Measured in this way, receptivity is virtually zero for 4 days, but after this the number of females accepting males rises and 9 days after fertilization the population is once more as receptive as when it consisted of 3-day-old virgins.

Table 1. NUMBER OF FEMALES ACCEPTING MALES DURING A 30-MIN. TEST, 50 FLIES OF EACH SEX, ON SUCCESSIVE DAYS AFTER FERTILIZATION

Days after fertilization	1	2	3	4	5	6	7	8	9	10	11
No. ♀♀ mated within 5 min.	0	0	2	2	5	7	10	22	32	31	28
No. ♀♀ mated within 30 min.	0	0	2	2	16	26	21	36	37	43	40

This recovery of receptivity is unlikely to be due to any ovarial changes. Throughout this period the ovaries remain in full production; gross examination reveals no change in size or composition and the females go on laying eggs without pause. Nor is it likely to be an effect of ageing so that, for example, flies go through cycles of receptiveness every 9 days. The receptivity of virgins gradually declines from their peak at 3 days and females do not inevitably become receptive 9 days after fertilization. I delayed the onset of egg-laying in females by keeping them on a diet of 1/5 *M* sucrose alone from soon after eclosion. Bodenstein[3] has shown that *melanogaster* females can mature a few eggs without access to protein as adults, but their ovaries remain very small and very few eggs are laid after fertilization. Such females when fertilized at 3 days old are just as receptive as normally fed flies, but they do not become receptive again after 9 days. They remain totally unreceptive for at least a fortnight, which was as long as observations were continued. Their receptivity returns after a week of normal diet with the accompanying rapid growth of the ovaries and onset of egg-laying.

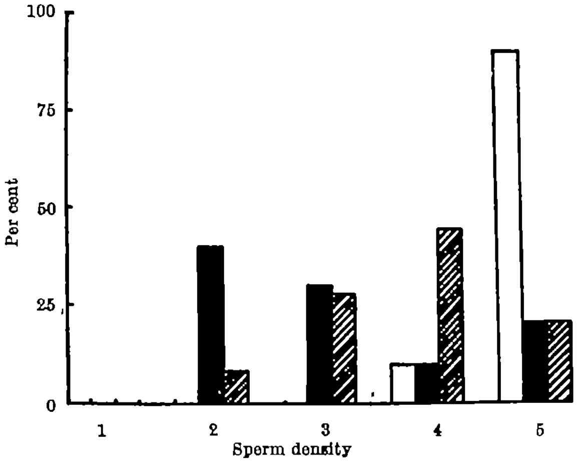

Fig. 1. Percentage of females, 6 days post fertilization, which have various densities of sperm remaining. Density ranges from 1, seminal receptacle full, to 5, empty. *A* (white), 10 ♀♀ which were receptive to ♂♂; *B* (black), 10 ♀♀ which were unreceptive; *C* (shaded), random sample of 25 ♀♀, also 6 days post fertilization

It is impossible to separate the effects of egg-laying from those of sperm-exhaustion, for they inevitably go together. Nevertheless, it is reasonable to suppose that, unlike *subobscura*, the receptivity of *melanogaster* females returns when that factor from the sperm which switched it off disappears when the sperm are all gone. As regards the switching-off mechanism, I have been able to confirm Smith's results and find that *melanogaster* females mated to males which cannot ejaculate sperm become receptive again within a day. Certainly, at the time when receptivity is returning to fertilized females, an increasing proportion of them prove, on dissection, to have used up all their sperm.

More direct support for this hypothesis was obtained by dissecting females after the daily receptivity tests and comparing the amounts of sperm remaining in those which mated quickly with that in females which remained unreceptive throughout. Even with the receptive females which do mate again, the original quantity of sperm which they held can be ascertained provided they are quickly separated from their second mates. No sperm is ejaculated during the first 10 min. of the 15–20-min. copulation period[4]. Only the seminal receptacle of females was examined, for the spermathecæ are often obscured by fat and sperm difficult to see. The sperm density was allocated to one of five grades, roughly estimated and ranging from 1, completely full, to 5, empty, with at most only a single sperm visible here and there.

Fig. 1 shows, as a typical example, the frequencies of the different grades of sperm density among females 6 days after fertilization. Three groups are distinguished : *A*, the first 10 females to accept males in the course of the 30-min. test, (they did so within about 6 min.); *B*, 10 females which remained unmated throughout ; *C*, a sample of 25 females the behaviour of which was not measured, also 6 days post-fertilization. It is clear that, compared with the random sample of females, a higher proportion of the receptive ones have exhausted their supply of sperm, while those that remain unmated tend to be those with most sperm left.

There are two ways by which insemination could lead to the receptivity mechanism being switched off, and sperm exhaustion lead to its being switched on again. The female's seminal receptacle is innervated[5], and the sperm may thus signal their presence because of the synchronized lashing movement which they maintain. Alternatively, some chemical may be produced, either by the sperm themselves or by their interaction with the female tissues, which operates via the hæmolymph on the nervous system. It is hoped to distinguish between these two possibilities by further experiment, but present evidence favours the chemical explanation. Fig. 1 shows that a few females which have exhausted their sperm are still not receptive, and the proportion of such cases rises with successive days, though the total number of unreceptive females gets less. This would suggest that a female does not become receptive immediately her supply of sperm is exhausted; it probably takes a day before the receptivity mechanism is switched on again. Changes due to neural factors alone usually happen quickly, but this delay is understandable if the concentration of some inhibitor chemical has to fall below a certain level. The measure used for the return of receptivity is rather crude, but it does not seem that the gradual depletion of her supply of sperm leads to a gradual increase in a female's receptiveness. She remains unreceptive, but then within about a day becomes fully receptive again. This suggests that if a chemical is produced, it is produced continuously and maintains an adequate concentration over quite a wide range of sperm density. It falls below the threshold concentration only when the supply of sperm is almost entirely exhausted.

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GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS IN A FIBROBLASTIC CELL LINE DERIVED FROM HAMSTER KIDNEYS

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THE use of cell lines for the growth and assay of the virus of foot-and-mouth disease has been limited. Schwobel and Siedentopf[1] described the cell line *ENS*.53, which was derived three years previously from pig kidney tissue. Satisfactory growth of virus was obtained, but cytopathic effects were absent unless high concentrations of virus were used. The authors stated that the cell line was, therefore, not suitable for the detection of small amounts of virus.

Thorne and Cartwright[2], working at this Institute, used the cell line developed by Stice (Cutter Laboratories, California) in experiments on the adsorption of virus. They found this line to be of low sensitivity for the virus strain which they studied. Other investigations at this Institute by Martin confirmed the low levels of sensitivity to other virus strains and Burrows, working with the two-hundredth passage, found that by this time the cell line was completely insensitive.

Attempts at this Institute to establish cell lines from pig kidney tissue have resulted so far in cells of fibroblastic appearance, which were insensitive to foot-and-mouth disease virus.

Recently, we have shown that a line originating from baby hamster kidney, designated *BHK* 21, is capable of supporting virus growth. These cells were isolated in the Institute of Virology, Glasgow, and have been described by Macpherson and Stoker[3].

For the experiments described, cells were grown as monolayer cultures in 4-oz. prescription bottles, Roux bottles and Petri dishes. The growth medium used was Eagle's basic medium, modified to contain twice the normal concentration of amino-acids and vitamins, plus 10 per cent autoclaved tryptose phosphate broth and 10 per cent unheated bovine serum. Following infection with foot-and-mouth disease virus, a cytopathic effect was observed similar to that seen with infected primary cultures of pig kidney cells. The cells became rounded and granular and detached from the glass surface. Infection with cattle-passaged virus produced a well-marked cytopathic effect in 24 hr., but destruction of the cell sheet was not complete in some experiments until approximately 72 hr. after infection. With virus adapted to pig kidney cells (204th passage) which was passaged 11 times through *BHK* 21 cells, cytopathic effect was observed 4 hr. after infection and the cell sheet was almost completely destroyed 4 hr. later.

The increase in virus concentration was followed in infected cultures from which unadsorbed virus had been removed and the cell sheet washed five times with phosphate-buffered saline. A sample of the medium was taken immediately (0 hr. sample) and further samples after convenient time-intervals. The virus was titrated in unweaned mice and the titre expressed as $\log_{10}$ mouse ID_{50}/ml. The results of a typical experiment are given in Table 1. The viruses used were: (*a*) a type *SAT* 1 attenuated vaccine strain (*RV*.11); modified by passage in mice, and (*b*) a type *C* strain adapted to pig kidney cells. Both viruses were produced in high titre, the cell sheets being almost completely destroyed after 72 hr. The results of a similar experiment comparing the growth of a type *SAT* 1 cattle strain of virus in primary cultures of pig kidney cells and *BHK* 21 cells are given in Table 2. Virus was produced more rapidly from pig kidney cells and the cell sheet was destroyed in less than 24 hr., whereas with *BHK* 21 cells, although the maximum titre was obtained 22 hr. later than with pig kidney cells, the yield of virus was apparently ten-fold greater. The *BHK* 21 cultures contained many more cells than the primary pig kidney cultures. It cannot be presumed that the higher yield of virus with these cells could be attributed to a greater number of cycles of virus multiplication until the results of a more detailed investigation of the quantitative aspects of the virus-cell system, now being undertaken, become available.

Table 1. GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS IN *BHK* 21 CELLS

Time of sampling (hr.)	Strain *RV*.11 (type *SAT* 1) *mo* 34 *m* 2 $\log_{10}$ mouse ID_{50}/ml	Strain *C*.997 (type *C*) *P.K.* 200 $\log_{10}$ mouse ID_{50}/ml.
0	*N.D.*	3·2
6½	6·0	6·0
24	6·5	6·5
30	6·8	6·8
48	7·3	6·5
54	7·5	6·5
72	7·2	7·0

N.D, not detected; *mo* 34 *m* 2, 34 passages in adult mice and 2 passages in unweaned mice; *P.K.* 200, 200 passages in cultures of pig kidney cells.

Table 2. COMPARISON OF THE GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS IN *BHK* 21 CELLS AND PRIMARY PIG KIDNEY CELLS

Virus: Strain *RV*.11 (type *SAT* 1) unmodified cattle virus

Time of sampling (hr.)	*BHK* 21 $\log_{10}$ mouse ID_{50}/ml.	Pig kidney $\log_{10}$ mouse ID_{50}/ml.
0	*N.D.*	*N.D.*
2	*N.D.*	1·5
4	3·0	4·5
6	5·5	6·2
8	5·0	6·0
24	6·0	5·0
	Many rounded cells still adhering to glass	Cell sheet destroyed
28	7·2	—
31	7·0	—
48	7·0	—

N.D., not detected.

The formation of plaques in an infected monolayer with an agar overlay has also been observed. Virus adapted to *BHK* 21 cells produced plaques of up to 7-mm diameter, 2 days after infection. Plaques have also been observed after infection of these cells with virus adapted to pig kidney cells, unmodified cattle virus and two strains adapted to mice. Virus titrations and neutralization tests of immune sera have also been made by means of cell metabolic inhibition tests with these cells. Owing to their rapid metabolism, results have been obtained after 3 days, which compares favourably with the 4–5-day period necessary with pig kidney cells.

These preliminary investigations with the *BHK* 21 cell line indicate that it will probably be of considerable value in foot-and-mouth disease research. This cell line has the desirable characteristics of rapid growth combined with the production of densely packed sheets of cells. Cultures grown in Roux bottles have yielded up to 1·5 × 10^8 cells, which is approximately five-fold more than might be expected from a similar culture of pig kidney cells. With strains of virus well adapted to these cells it should be possible to produce very high-titre virus preparations.

In addition to the use for virus assay purposes, it is likely that these cells will also be of value in the preparation of both inactivated and live attenuated virus vaccines. At present, live foot-and-mouth disease virus vaccines are prepared at this Institute from the tissues of unweaned mice infected with attenuated strains developed by serial passage in mice. If these mouse attenuated strains of virus could be grown in tissue culture, many of the problems associated with vaccine production would be overcome. So far, primary cultures of pig kidney cells have not proved satisfactory in that the virus titres produced were low. The results in Table 1 indicate that at least one virus strain modified by passage through mice can be grown in *BHK* 21 cells to titres acceptable for vaccine production ($10^{7.5}$ ID_{50}/ml.) without further adaptation to these cells.

Although it has been known since 1937 that hamsters are susceptible to foot-and-mouth disease there is no information on the cultivation of the virus in cells derived from hamster tissues.

Hayflick and Moorhead[4] have reported on the establishment of 25 strains of diploid fibroblasts derived from human fœtuses. These cell strains the development of adaptive behaviour in rats (descriptions in preparation).

The modified Aronson keyboard (Fig. 1) carries twenty typewriter keys individually connected to switches activating the Laupheimer converter. Devices to lock and to clear keys are provided.

The Laupheimer converter registers, accumulates and transmits pulses from the keyboard, or from switches activated by an animal, to a tape-punch. The pulses produce a single input-item, called a 'bit'. Thirty-six bits equal one 'data-word' (Fig. 2, top). The 36 bits of each word are accumulated in an appropriate binary register, transferred into a distribution (serialization) register, and transmitted to the punch at the rate of one, two or three words per second, thus producing a tape with serially punched words. 1–36 punches appear in any given word. A column of bits is called a 'character'. For compatibility with the IBM 1620 computer, the converter automatically inserts a 'parity' punch as needed so that each character contains an odd number of punches, and also automatically punches the thirty-sixth bit in each word to designate the end of that word.

A special switch (Fig. 1) permits the converter to record 'tagging' information (for example, the date, animal, group). From a Friden tape reader, the converter can receive pulses which it punches as tag words to record information about stimulus or time. Through a programmed tape in the reader, the converter can also stop the reader. A standard Friden, 8-channel, motorized punch, model No. 2, is used.

Atsl admits both a simple ordering of the data as to latencies, frequencies, durations, sequences and concomitances of behaviour items, and (by re-ordering measures of counts and intervals between items) the elucidation of complex relations among items. Statistical techniques can be programmed sequentially or simultaneously with the reduction of data. Data from the experiment for which *Atsl* was developed[5] have thus been prepared for publication.

Atsl permits investigation of many aspects of behaviour simultaneously, both by concurrently recording animal-generated and observer-generated data and by increasing the number of recorded items. A complete list of behavioural items of empirically proved significance can thus be obtained as a valuable basis for the specialized investigation of selected independent and dependent variables of such important behavioural phenomena as animal orientation, motivation and development.

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MECHANISMS OF NON-DISJUNCTION IN MAN

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SINCE my previous communication[1], further advances in human cytogenetics have provided information about mechanisms of non-disjunction in man.

Primary Meiotic Non-Disjunction

Types of Primary Meiotic Non-Disjunction. Meiotic non-disjunction in a subject with a normal karyotype may be regarded as primary, and three types of primary meiotic non-disjunction may be recognized. The term 'non-disjunction' is used to describe the abnormal behaviour of sex chromosomes during meiosis although the cause may be non-conjunction or failure of pairing rather than failure of separation of chromosomes or chromatids.

Simple non-disjunction. Simple non-disjunction during the first or second meiotic division of oogenesis may give exceptional gametes of nullo-X- or XX-bearing oocytes and hence abnormal zygotes of XO, YO, XXX or XXY sex chromosome constitution. Similarly, simple non-disjunction during spermatogenesis may give exceptional gametes of nullo-X- (sperm with no sex chromosome, either X or Y), XY-, XX- or YY-bearing sperm and hence abnormal zygotes of XO, XXY, XXX or XYY sex chromosome constitution.

Double non-disjunction. Double non-disjunction, consisting of simple non-disjunction both in the male and in the female, may therefore give abnormal zygotes of OO, YY, $XXXY$, $XXXX$ or $XXYY$ sex chromosome constitution.

Successive non-disjunction. Successive non-disjunction during consecutive meiotic divisions of oogenesis may give new types of exceptional gametes: XXX- and $XXXX$-bearing oocytes and hence $XXXY$, $XXXXY$, $XXXX$ and $XXXXX$ zygotes. Similarly successive non-disjunction during spermatogenesis may give XXY- XYY- or $XXYY$-bearing sperm with $XXXY$, $XXYY$, or $XXXYY$ zygotes. Several types of normal and abnormal zygotes are thus possible in consequence of non-disjunction at one or other or both meiotic divisions of oogenesis, of spermatogenesis, or of both together (Table 1).

Investigation of Primary Meiotic Non-Disjunction. Reported examples of heteroploidy in man include cases with sex chromosome constitution XXY[2–5], XO[6,8,9], $XXYY$[10], and $XXXXY$[11,12]. The XO and XXY karyotypes together with colour blindness

Table 1. POSSIBLE GAMETES AND ZYGOTES WHICH MAY RESULT FROM NORMAL AND ABNORMAL BEHAVIOUR OF THE SEX CHROMOSOMES AT ONE OR BOTH MEIOTIC DIVISIONS OF OOGENESIS OR SPERMATOGENESIS OR BOTH

Female gametes	Male gametes								
	O	X	Y	XY	XX	YY	XXY	XYY	$XXYY$
O	OO	OX	OY	OXY	OXX	OYY	$OXXY$	$OXYY$	$OXXYY$
X	XO	XX	XY	XXY	XXX	XYY	$XXXY$	$XXYY$	$XXXYY$
XX	XXO	XXX	XXY	$XXXY$	$XXXX$	$XXYY$	$XXXXY$	$XXXYY$	$XXXXYY$
XXX	$XXXO$	$XXXX$	$XXXY$	$XXXXY$	$XXXXX$	$XXXYY$	$XXXXXY$	$XXXXYY$	$XXXXXYY$
$XXXX$	$XXXXO$	$XXXXX$	$XXXXY$	$XXXXXY$	$XXXXXX$	$XXXXYY$	$XXXXXXY$	$XXXXXYY$	$XXXXXXYY$

THE *ATSL*: AN OBSERVER-TO-COMPUTER SYSTEM FOR A MULTIVARIATE APPROACH TO BEHAVIOURAL STUDY

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AND
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MOST techniques for the analytical study of behaviour are restrictive in the number and aspects of items studied. The animal's whole behaviour should be appraised, for under different conditions not only the behavioural items but also their significance, priority and interrelations change. To permit such an appraisal, the Aronson keyboard was devised in our laboratory[1].

This keyboard consists of a bank of microswitches, each microswitch representing one behavioural item and wired to a pen on an operations recorder. Thus, a trained observer can record as many as forty items while continuously viewing the subject[1]. Although this improves the scope and efficiency of data-collection and recording, analysis of records is time-consuming.

Accordingly, beginning in 1956, the possible modifications were investigated. One result was the development of the Laupheimer converter, which couples the keyboard to a standard tape-punch to produce a paper-tape usable in the IBM 1620 computer. The modified Aronson keyboard, the Laupheimer converter, a paper-tape punch and a computer comprise the data-collection and recording system which we term the *Atsl*. The data obtained through *Atsl* can be processed by a digital computer with appropriate programming, and *Atsl* can be used in any behavioural experiment, utilizing either observer-generated or animal-generated data or both. (At the Institute of Animal Behavior, Rutgers University (director, D. S. Lehrman) a modified version of *Atsl* is in use.) Fig. 1 shows the Aronson keyboard and the Laupheimer converter, two of the components of *Atsl*, as devised and constructed for a project investigating

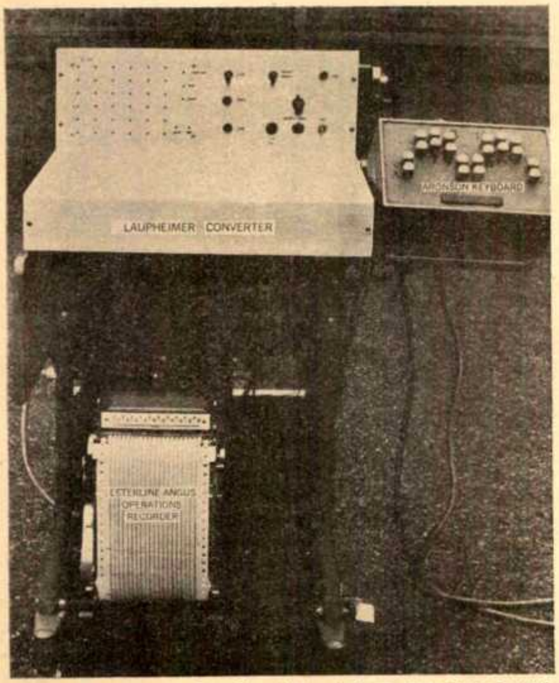


Fig. 1. Aronson keyboard and Laupheimer converter of *Atsl*. The operations-recorder is optional

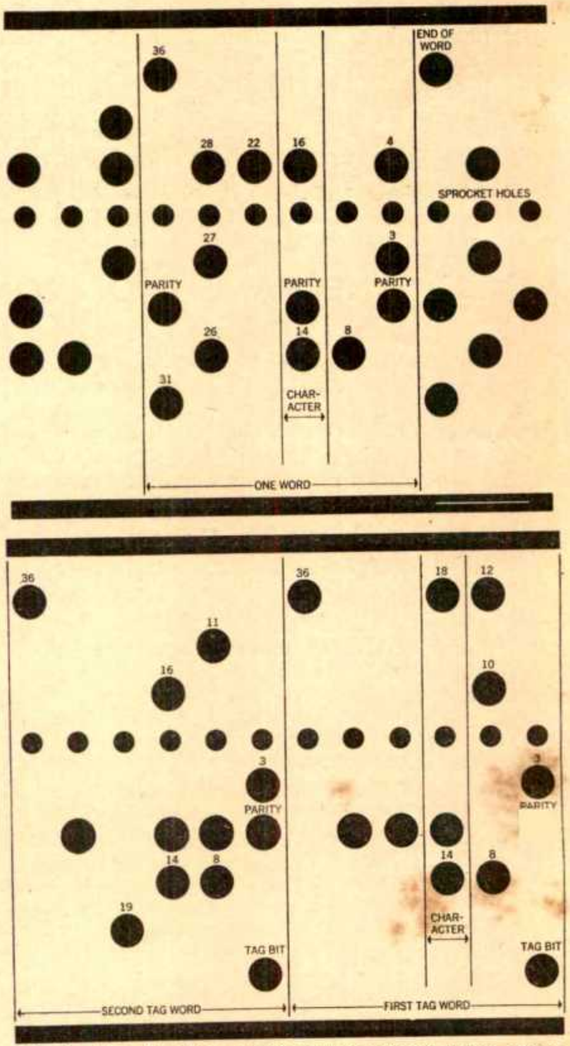
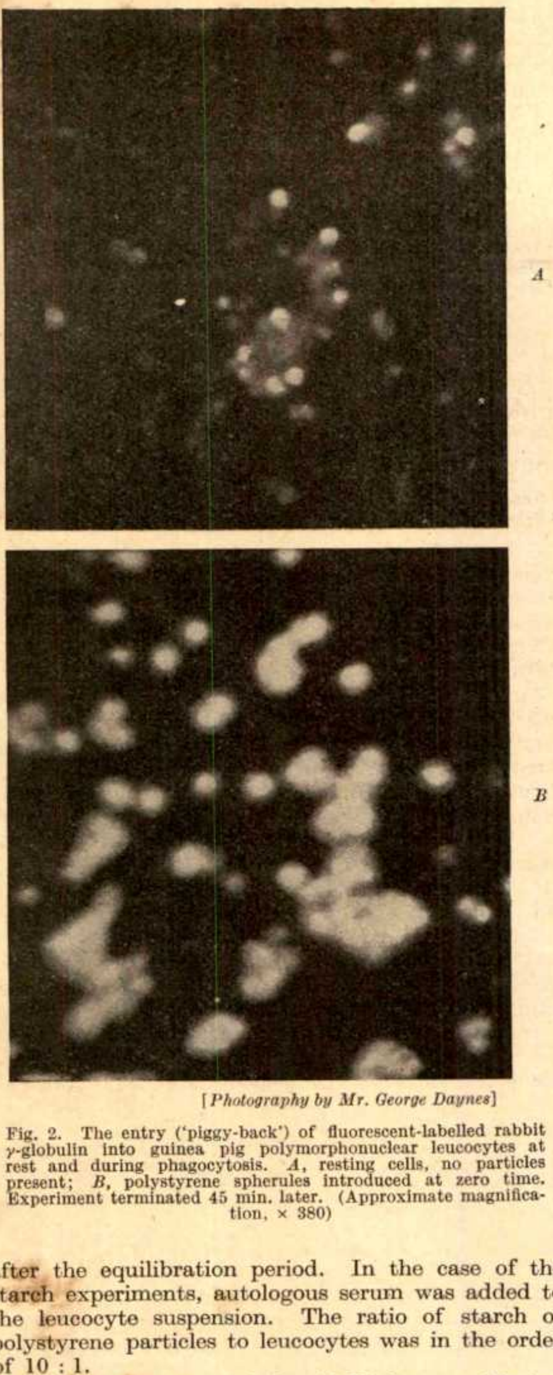


Fig. 2. Top, data-tape showing bits (numbered holes), parity bits and end-of-word bit. Characters are punched seriatim. The first 3½ characters represent observer-generated data for one animal: bit 3, stimulus; bits 4, 8, location; bit 14, 'nosing'; bit 16, 'rising'. The remaining 2½ characters represent animal-generated data from another animal in a respiration chamber; bit 22, consumption of 1 c.c. of oxygen; bits 26, 27, 28, 31, animal's location. Bottom, tag words. The first word gives the date, May (bits 10, 12), 17 (bits 14, 18), 1961 (bit 8). The second tag word gives the time of observation



[*Photography by Mr. George Daynes*]

Fig. 2. The entry ('piggy-back') of fluorescent-labelled rabbit γ-globulin into guinea pig polymorphonuclear leucocytes at rest and during phagocytosis. *A*, resting cells, no particles present; *B*, polystyrene spherules introduced at zero time. Experiment terminated 45 min. later. (Approximate magnification, × 380)

after the equilibration period. In the case of the starch experiments, autologous serum was added to the leucocyte suspension. The ratio of starch or polystyrene particles to leucocytes was in the order of 10 : 1.

It has been reported by McKinley *et al.*[6] that malonate will inhibit the respiratory activity of leucocytic homogenates while it will not affect the respiratory activity of intact leucocytes. The inability of malonate to suppress intact leucocytic respiration has been attributed to its failure to permeate the cell. Thus, the effect of three different concentrations of malonate on leukæmic leucocytes in the absence and presence of particles was studied and is shown in Fig. 1. Leucocytic respiration in the absence of particles is essentially unaffected. However, in the presence of particles the increased respiratory activity associated with phagocytosis is completely inhibited with 10^{-1} *M* malonate. At lower concentrations of malonate the inhibition is evident but to a lesser degree. Pre-incubation of leucocytes with malonate, before addition of particles, followed by washing of the cells to remove exogenous inhibitor, resulted in increased oxidation normally associated with phagocytosis. This further indicates that the malonate does not penetrate in the absence of particles.

Fluorescent-labelled rabbit γ-globulin was found to be present in cells only to a slight degree after incubation with guinea pig polymorphonuclear leucocytes and was thus used in order to obtain visual evidence for the 'piggy-back' phenomenon. The fluorescent material was added with the polystyrene spherules to the leucocyte suspension. Controls lacking particles were run simultaneously. After a 45-min. incubation period, the contents of the flask were removed, washed twice, and aliquots were placed on slides to air dry. They were then examined for fluorescence using ultra-violet microscopy.

The marked increase in fluorescence observed following the addition of fluorescent rabbit γ-globulin to phagocytizing cells is shown in Fig. 2*B*. The almost complete absence of fluorescence in the non-phagocytizing leucocytes can be seen in Fig. 2*A*. The fluorescent material did not enter the cell by coating the polystyrene spherule as we have been unable to show fluorescence surrounding or associated with the spherule after their incubation with only the fluorescent γ-globulin and subsequent washing and examination for fluorescence.

The possibility that malonate is not entering the cell in a 'piggy-back' fashion along with the particle, but instead acting on or near the cell surface, is of course realized. However, the indication that phagocytizing cells are able to concentrate fluorescent-labelled γ-globulin whereas non-phagocytizing cells are not, suggests that both markers are entering the cells along with the particle in the same membranous vesicle.

These observations, that malonate and fluorescent-labelled rabbit γ-globulin cannot enter a cell unless the cell is engulfing particulate material, are of interest. The evidence suggests that these substances do not enter a cell to any marked degree unless the cell is ingesting particles. Presumably the material is entering the cell along with the particle within the membranous vesicle surrounding the particles; the membrane is then thought to disintegrate releasing the particle and material. Thus, material, initially impermeable, can gain entrance to the cytoplasm with the particulate substance. This phenomenon may offer a new approach in permeability studies and may provide information concerning the nature of the cell membrane.

Further, this 'piggy-back' phagocytosis may offer an additional dimension in the field of chemotherapy. Agents unable to enter a cell can be made to do so and perhaps exert a chemotherapeutic effect that ordinarily would not be possible due to impermeability.

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maintained their diploid karyotype for approximately 50 passages and showed a wide spectrum of sensitivity to different viruses similar to that of primary cultures[3] of the tissues of origin. Macpherson and Stoker have shown the *BHK* 21 cells to be diploid and it is possible that a situation analogous to that of the human diploid cell strains exists if primary cultures of baby hamster kidney cells are also shown to be sensitive to foot-and-mouth disease virus.

We have also examined a clone of *BHK* 21 cells (clone No. 13) received from Macpherson and Stoker which appears to be as sensitive to foot-and-mouth disease virus as the original sample of uncloned cells. All future investigations will be made with the cloned cells. Investigations are now proceeding into methods of large-scale cell production and the growth of high-titre virus.

We thank Prof. M. G. P. Stoker and Dr. I. A. Macpherson for suggesting and supplying the *BHK* 21 cells and also for much valuable information.

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'PIGGY-BACK' PHAGOCYTOSIS

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ACTIVE transport, passive transport, phagocytosis or pinocytosis are terms used for describing different processes whereby substances are able to penetrate cells. In this communication we present evidence suggesting a new phenomenon for the entrance of impermeable compounds into cells. This mechanism of penetration appears to involve entrance with macromolecules and is referred to as the 'piggy-back' phenomenon[1,2]. Conceivably many agents, some potentially chemotherapeutic, are without effect because of their inability to enter a cell. Experiments have been designed to show that some substances which ordinarily would not enter a cell can be made to do so under phagocytic conditions.

Guinea pig polymorphonuclear leucocytes prepared according to the method of Sbarra and Karnovsky[3,4] and normal human and leukæmic leucocytes prepared by the method of Christlieb *et al.*[5] were used.

Guinea pigs weighing 300–400 gm., usually males, were injected with 15 ml. of a 12 per cent sterile suspension of sodium caseinate. 16–18 hr. later the peritoneal exudates were prepared by washing the peritoneal cavity with 40–50 ml. of saline. The cells were collected in 50-ml. celluloid tubes and centrifuged at 50*g* for 10 min. in the cold. They were then re-suspended in a desired volume of phosphate-free Krebs–Ringer solution. Aliquots were measured for total cellular phosphorus. Phosphorus determinations were found to be a more reliable measure of cellular protoplasm than the conventional counting methods (100γ of cellular phosphorus represents 5·53 × 10⁷ cells[3]). Phosphate buffer was now added to yield a suspension of cells in the usual Krebs–Ringer phosphate medium buffered at *p*H 7·4.

Human leucocytes were obtained from apparently healthy laboratory personnel and leukæmic cells from patients suffering from chronic myelogenous leukæmia. 50 ml. of venous blood was drawn directly into a 50-ml. 'luer-lok' syringe, containing 1·5 ml. heparin plus 2·5 ml. of 'Dextran' solution. The syringe was placed vertically in the cold with the 'luer-lok' side up; the major portion of the erythrocytes settled out after 45 min. The supernatant fluid containing the leucocytes was then expressed from the syringe into celluloid tubes. The cells were collected by centrifugation for 10 min. at 50*g*, and the sediment washed once with Krebs–Ringer phosphate medium and brought up to a desired volume. Autologous serum was obtained from the subject donating the leucocytes. Leucocyte counts were made by conventional counting methods.

The phagocytic experiments were carried out in the conventional Warburg manometric apparatus at 37° C. Krebs–Ringer phosphate medium was the suspending medium. Insoluble starch granules of *Amaranthus cruenthus* and polystyrene spherules, 1·171μ in diameter, were used as particles. Starch particles or polystyrene spherules suspended in Krebs–Ringer phosphate medium, 30 μmoles of glucose, and either malonate in different concentrations (10^{-1}–10^{-3} *M*) or fluorescent-labelled rabbit γ-globulin were added to the leucocytic suspension from the side-arms

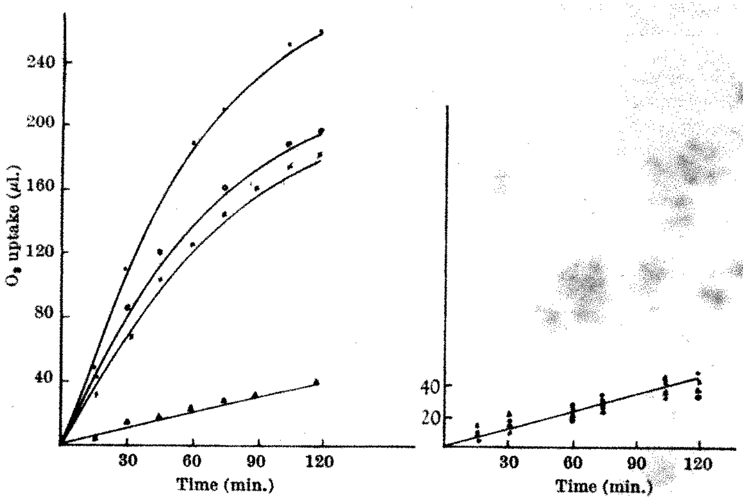

Fig. 1. Leakage of malonate into phagocytizing leukæmic cells. The effect of different concentrations of sodium malonate on the respiratory activity of human leucocytes (from a patient suffering from chronic myelogenous leukæmia) in the absence and presence of particles. Left, particles present; right, particles absent. ●, 0; ○, 1×10^{-3} *M*; ×, 1×10^{-3} *M*; ▲, 1×10^{-1} *M* malonate

Table 2. EXPECTED RELATIVE FREQUENCY OF ABNORMAL GAMETES PRODUCED BY SIMPLE MEIOTIC NON-DISJUNCTION OF THE SEX CHROMOSOMES AND THE RESULTING ZYGOTES

Stage of gametogenesis affected: First (1 deg.) or second (2 deg.) meiotic division	Relative frequency of abnormal Gametes				Zygotes				
	O	XX	XY	YY	XO	YO	XXX	XXY	XYY
Oogenesis 1 deg.	2	2	—	—	1	1	1	1	--
Spermatogenesis 1 deg.	2	—	2	—	2	—	—	2	—
Oogenesis 2 deg.	2	2	—	—	1	1	1	1	--
Spermatogenesis 2 deg.	2	1	--	1	2	—	1	—	1

studies[13,14] provided the first evidence of meiotic non-disjunction[7,15]. The $XXYY$ karyotype provided evidence of non-disjunction at the second meiotic division of spermatogenesis, and the $XXXXY$ karyotype provided evidence of non-disjunction at successive meiotic divisions, probably of oogenesis. It is probable that several or all of the possible mechanisms occur in man. These mechanisms may be further investigated by studies of colour blindness, parental age, and of the incidence of abnormal karyotypes.

Colour Blindness. Since it was pointed out[7,16] that the incidence of colour blindness in the chromosomal intersexes is consistent with meiotic non-disjunction, it has been agreed[1,16] that simple non-disjunction may occur either at the first or at the second meiotic division although the absolute or relative frequencies of these are not known. Investigations of colour blindness thus provide evidence about the frequency of non-disjunction at the first and second divisions of oogenesis and spermatogenesis and about the position of the colour blindness locus relative to the centromere[17]. An appropriate model for simple primary non-disjunction, in circumstances which may not be so very artificial, is perhaps provided by a study of recessive homozygosis in irradiated *Drosophila*[18].

Parental Age. Since the previous communication[1] was submitted further detailed data[19] have supported a maternal age effect in Klinefelter's syndrome but not in Turner's syndrome. These new results shed further light on the mechanism of meiotic non-disjunction and invite an explanation. There may, for example, be selective loss of nullo-X gametes in the polar bodies during oogenesis. It seems likely that a maternal age effect could be influenced by the relative frequency of non-disjunction, in males and females, in the first and second divisions of oogenesis and by loss, either of gametes before fertilization or of zygotes before birth. Several or all of these mechanisms may operate. It is also possible that the population sampled may not be homogeneous. Thus cases of chromatin-positive Klinefelter's syndrome may include examples with an XXY or $XXYY$ sex chromosome constitution. This would not, however, invalidate the conclusion[13] that more than one X chromosome was present. for the X chromosomes of XXY and $XXYY$ subjects may sometimes be of independent origin in the same way as the X chromosomes of the female normally are. In cases of chromatin-negative Turner's syndrome, however, more serious objections arise, for there is evidence of frequent mosaicism in such subjects[20]. This alone might account for the apparent lack of maternal age effect in Turner's syndrome, but it raises the problem of the relative infrequency of XO zygotes. This in turn might be largely explained by reduced viability of these zygotes since congenital abnormalities are commonly found in Turner's syndrome.

Incidence of Abnormal Karyotypes. As a first approximation the relative frequency of abnormal karyotypes, produced in consequence of simple primary non-disjunction, may be calculated on the assumption that non-disjunction is as frequent during the first and second divisions and as frequent during oogenesis as spermatogenesis (Table 2). Hence an observed excess of XXY zygotes would suggest that most exceptional zygotes arise in consequence of a defect of the first meiotic division of spermatogenesis and an excess of XXX zygotes would suggest a more frequent defect at the second meiotic division of spermatogenesis. Available data on the nuclear sex of infants at birth[21,22] are insufficient for a firm conclusion but suggest that XXY karyotypes may be more common than XXX karyotypes.

The probabilities for aneuploid zygotes with more than two sex chromosomes are more difficult to assess since these may arise in different ways. An added difficulty is their infrequency, which makes detection laborious, even in selected populations; but the incidence in such populations may be of value if most or all of the abnormal zygotes are included in the sample. It is therefore of value to establish the frequency of such karyotypes in oligophrenic populations and to establish, in subjects with each karyotype, the incidence of oligophrenia. The expected relative frequencies of abnormal karyotypes may be calculated (Table 3).

It seems likely that one abnormality may predispose to further abnormalities. Thus, successive non-disjunction may be more common than double non-disjunction. This supposition could be investigated by determination of the relative frequency of abnormal karyotypes in the general or selected populations and by seeking a possible maternal age effect in the subjects discovered. Such studies might also provide information about the frequency of successive non-disjunction in oogenesis and spermatogenesis.

Alternative Mechanisms during Meiosis

In all cases of heteroploidy the possibility of familial cases[23] must, of course, be considered. In familial examples the non-disjunction would be regarded as secondary if it arises in heteroploid subjects.

Other mechanisms entirely different from meiotic non-disjunction might operate. A hypohaploid gamete might be produced by simple elimination of a chromosome without simultaneous occurrence of a hyperhaploid sister gamete. Likewise a hyperhaploid gamete might be produced by simple duplication of a chromosome although colour blindness studies[13] provide evidence that this does not occur in every case.

Table 3. EXPECTED RELATIVE FREQUENCY OF ABNORMAL GAMETES WITH MORE THAN TWO SEX CHROMOSOMES PRODUCED BY SUCCESSIVE MEIOTIC NON-DISJUNCTION OF THE SEX CHROMOSOMES AND THE RESULTING ZYGOTES

	Relative frequency of abnormal Gametes					Zygotes					
	XXX	XXY	XYY	$XXXX$	$XXYY$	$XXXY$	$XXXX$	$XXYY$	$XXXXX$	$XXXYY$	$XXXXY$
Oogenesis	2	—	—	—	—	1	1	—	—	—	—
Spermatogenesis	—	1	1	—	—	1	—	1	—	—	—
Oogenesis	—	—	—	2	—	—	--	—	1	—	1
Spermatogenesis	--	—	—	—	2	—	--	1	—	1	—

Mitotic Non-Disjunction

Mechanisms of mitotic non-disjunction may be investigated in a way similar to that used for meiotic non-disjunction.

Karyotype Analysis. Karyotype analysis should provide much information about mechanisms of mitotic non-disjunction. Non-disjunction at the first mitotic division in subjects with a normal (XX, YY) or abnormal (XO, XXX, XXY) karyotype may produce various types of mosaicism (Table 4) and non-disjunction at a later mitotic division would produce, in addition, clones of cells with the same karyotype as the original zygote. It is unlikely, however, that all cell clones would be of equal viability and some might be rapidly eliminated. Several examples of mosaicism, established or suspected, have been reported. An XXX/XO mosaic[24] was apparently due to non-disjunction at an early mitotic division, perhaps the first, since no XX cells were found. In XX/XO mosaics[20] the mode of origin is less clear. They might arise from XX zygotes due either to non-disjunction at the second or subsequent division with loss of XXX cells or to simple elimination of one X chromosome. Alternatively XX/XO mosaics might arise from XO zygotes in consequence of non-disjunction at the second or later division with loss of OO cells. Similarly an XXY/XY mosaic[3] might arise from XY or XXY zygotes with loss of YO or $XXYY$ cells. The reports of viable XXX[23,24] and $XXYY$[10] subjects are perhaps evidence against the XX and XXY origin of XX/XO and XXY/XY mosaics, respectively.

Colour Blindness. A normal or increased incidence of colour blindness would be expected in mosaics derived from normal (XX or XY) zygotes, but the colour blindness might not always be regular[17]. In general, a higher incidence of colour blindness would be associated with non-disjunction during early mitotic divisions. In mosaics derived from XXY zygotes the incidence would lie between that found in normal males and females and in XX/XO mosaics derived from XO zygotes a male or higher incidence of colour blindness would be expected. The incidence of colour blindness in the fathers of colour-blind subjects with gonadal dysgenesis should also be recorded. A high incidence of colour blindness, a little more than 52 per cent, would be expected in the fathers of colour-blind mosaics derived from XX zygotes. This estimate is based on a colour blindness gene frequency of 8 per cent. Colour blindness would be present in half the fathers of simple heterozygotes and in all the fathers of double heterozygotes and of homozygotes. The frequency of colour blindness in the fathers of colour-blind mosaics derived from XO zygotes would vary with the frequency of non-disjunction in the male and female. It would be 54 per cent if these were equal, for half the fathers would pass on their colour blindness gene and 4 per cent of the others would by chance be colour blind.

Parental Age and Incidence. No maternal age effect would be expected in cases of mitotic non-disjunction unless they were derived from a heteroploid zygote. Studies to determine the relative frequencies might be of value, although the many variable factors, including selection of cases, differential viability and possible association of abnormalities, would make interpretation especially difficult.

Although the mechanisms of non-disjunction in man are complex it is possible to elucidate these mechanisms with appropriate techniques. The collection of much further data is necessary.

Table 4. Some of the Possible Mosaics which may result from Simple Non-disjunction at the First Mitotic Division

Non-disjunction at subsequent mitotic divisions would include, in addition, clones of cells with the same karyotype as the original zygote and, perhaps also, clones of cells with more complex karyotypes

Original zygote	Some possible mosaics
XX	XXX/XO
XY	XXY/YO ; XYY/XO
XO	XX/OO
XXX	$XXXX/XX$
XXY	$XX/XXYY$; XXX/XYY $XXXX/YY$; $XXXY/XY$

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CENTROMERES, SITES OF AFFINITY AND GENE LOCI ON THE CHROMOSOMES OF *SACCHAROMYCES*

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IN the course of constructing chromosome maps by tetrad analysis, two structural features may be encountered; centromeres and (less frequently) sites of affinity. The former may be used for identifying and enumerating the different chromosomes of an organism on the principle that each chromosome possesses one centromere. Sites of affinity, in turn, may be used as a distinguishing feature of certain linkage groups which have not yet been assigned to a chromosome by the centromere criterion. The procedure for locating a centromere was first established by Lindegren[1] for ordered tetrads, later adapted by Lindegren[2] to unordered tetrads[3]. The method is based on the fact that centromeres segregate reductionally at the first division. Sites of affinity, on the other hand, arise in connexion with exceptions to the rule of independent assortment of non-homologous chromosomes. Investigations of a variety of organisms[4–10] indicate that, in certain cases, regions located on different chromosomes pass to the same first division poles with high frequency. Genes located near these regions or sites may appear to be linked. In certain circumstances these sites can be located in a chromosome map.

The first chromosome maps of *Saccharomyces*[11–14] have been recently extended[15,16] and the present article concerns new relationships of centromeres, sites of affinity and gene-loci, demonstrated by hybridizing cultures from a variety of sources[14–18]. The problem of identifying genes in *Saccharomyces* has been complicated by the fact that some distinctly different mutants (which complement and re-combine) have been designated identically by different workers. A temporary device for distinguishing mutants by letters (*L* for Lindegren, *O* for Oshima) as well as by numbers, is adopted in this article; the main emphasis is on giving distinct names to distinct mutants. Table 1 lists the source and the symbols of 18 recently located mutants. New maps have been constructed which include 16 newly located genes together with 34 previously locáted genes.

Table 1. THE SOURCES AND DESIGNATIONS OF 18 DIFFERENT RECENTLY LOCATED GENES

Gene symbols					
Lindegren	Magni	Hawthorne Mortimer	Oshima	Chromosome	Phenotype
**ly*-L					Lysineless
**hi*-L					Histidineless
**su*-L					Non-fermenter sucrose
ar-37	**ar*-3	*ar*-7			Arginineless
**mt*-30					Methionineless
**va*-1					Valineless
mt-02			**mt*-2	X	Methionineless
mt-03			**mt*-3	VI	Methionineless
ar-16	**ar*-1	*ar*-6		V	Arginineless
**mt*-5				V	Methionineless
**le*-2	*le*-4	*le*-2		III	Leucineless
hi-0			**hi*	III	Histidineless
ly-0			**ly*	II	Lysineless
**mt*-55				VI	Methionineless
**ad-L*				II	Adenineless
**bz*				V	Benzimidazole sensitive
**tre*				II	Non-fermenter trehalose
ty-2		**ty*-2			Tyrosineless

* The asterisk before a symbol means that the mutant used in this work was obtained from the author whose name heads the column. The symbols finally used in the map appear in the left column

Linkage is usually indicated by a significant excess of *PD* over *NPD* tetrads, and this is measured by χ^2 on the hypothesis: *PD* = *NPD*. Tetrad distributions are represented by three consecutive numbers, thus 28–4–43 means that for a given pair of genes, 28 parental ditype, 4 non-parental ditype, and 43 tetratype tetrads were observed. For such a distribution, $\chi^2 = (28-4)^2/(28+4) = 24^2/32 = 18$, and linkage would be indicated.

Chromosome II. The gene, *ga*-1 (non-fermenter of galactose) is linked to the centromere of chromosome II[12]. The gene *ly*-2 is 45 Perkins's units[19] distal to *ga*-1[15] and *ly*-0 is also distal to *ga*-1[16]. Although the data suggest that *ly*-0 is farther from *ga*-1 than *ly*-2, the possibility that these represent the same locus cannot be excluded. Current data on linkage of *ac*-1 (actidione sensitivity) and *ga*-1 from this laboratory reveal a total tetrad distribution of 269–11–149, and in family 201, it has been proved that the centromere lies between *ac*-1 and *ga*-1, *ac*-1 being 15 stranes from the centromere[20]. The genes *tre* (trehalose fermentation) and *ad-L* (an adenineless locus) also exhibit linkage with this group (Table 2). The gene order is *ac*-1, centromere, *ga*-1, *tre*, *ad-L*, but *tre* and *ad-L* are not shown on the map because their relations with *ly*-2 have not yet been worked out.

Table 2. TETRAD DISTRIBUTIONS FOR GENES LOCATED ON CHROMOSOME II

The total tetrad distributions are drawn only from families heterozygous for *ac*-1, *ga*-1, *tre*, and *ad-L*, simultaneously. χ^2 gives the value of chi-square for the hypothesis that $PD = NPD$. $\chi^2 > 6\ 2$ indicates linkage

Gene combination	*PD*	*NPD*	*T*	χ^2
ac-1, *ga*-1	194	7	88	174·0
ac-1, *tre*	41	29	96	2 0
ac-1, *ad-L*	56	45	191	1·2
ga-1, *tre*	61	22	95	18 3
ga-1, *ad-L*	63	49	186	1·7
tre, *ad-L*	91	15	63	54 5

Chromosome III. The mating type locus and *hi*-4 are located on opposite arms of chromosone III and the gene *thr*-4 is distal to mating type[15]. A histidineless mutant (*hi*-0)[16] is also linked to the mating type locus. The tetrad distribution for *hi*-0 and mating type is 537–30–432. Complementation studies show that the loci, *hi*-4 and *hi*-0 are different. The gene *le*-2, isolated in this laboratory, is linked both to mating type and to *hi*-0 and fails to complement with Magni's *le*-4[17]. (Hawthorne and Mortimer's *le*-2 also fails to complement Magni's *le*-4 and may be identical to our *le*-2.) Tetrad distributions totalled from miscellaneous crosses show mating type and *le*-2 to be linked: 132–3–104. Tetrad distributions from hybrids heterozygous for both *hi*-0 and *le*-2 indicated that *le*-2 and *hi*-0 were linked in the order, *hi*-0, *le*-2, mating type. The tetrad distributions were 37–0–11 (*hi*-0 and *le*-2), 26–3–20 (*hi*-0 and α), and 30–0–18 (*le*-2 and α).

Centromere of chromosome III was located between *le*-2 and mating type, by data from families 200 and 201, in which the centromere-linked genes *ur*-3, and *ch*-1 could also be followed (Table 3). The gene, *le*-2 is approximately 7·4 stranes from its centromere, while mating type is approximately 22 stranes from its centromere[20]. The latter figure compares well

Table 3. DATA CONCERNING THE LOCATION OF THE CENTROMERE ON CHROMOSOME III

The value, *z*, is the normal curve parameter for the hypothesis that the relative tetratype frequency is 2/3. In each case, *T* is significantly below 2/3 the total number of tetrads

Gene combination	Tetrad distributions for centromere-linked markers			
	PD	*NPD*	*T*	*z*
le-2, alpha	98	3	75	6 77
le-2, *ga*-1	65	58	44	10·05
le-2, *ur*-3	59	65	53	10 36
ch-1, alpha	40	35	93	8 11
ch-1, *ga*-1	35	40	85	3 63
ch-1, *le*-2	36	46	82	4 53
ch-1, *ur*-3	70	3	97	2 66

Triplet	Gene—centromere distances				
	alpha	*ga*-1	*ur*-3	*le*-2	*ch*-1
alpha, *ga*-1, *ur*-3	25·3	4 3	13 6		
alpha, *ga*-1, *le*-2	23 3	6·2		10·6	
alpha, *ga*-1, *ch*-1	17 9	11·6			41 3
alpha, *ur*-3, *le*-2	26 4		12·4	7 5	
alpha, *ur*-3, *ch*-1	16 9		22 0		42 4
alpha, *le*-2, *ch*-1	23 5			10 5	35 8
ga-1, *ur*-3, *le*-2		7·4	10·5	9 5	
ga-1, *ur*-3, *ch* 1		3·2	14·6		49·7
ga-1, *le*-2, *ch* 1		11 8		5 1	41 2
ur-3, *le*-2, *ch* 1			19 0	0 9	45 2
Average	22 2	7 4	15 4	7 4	42 6

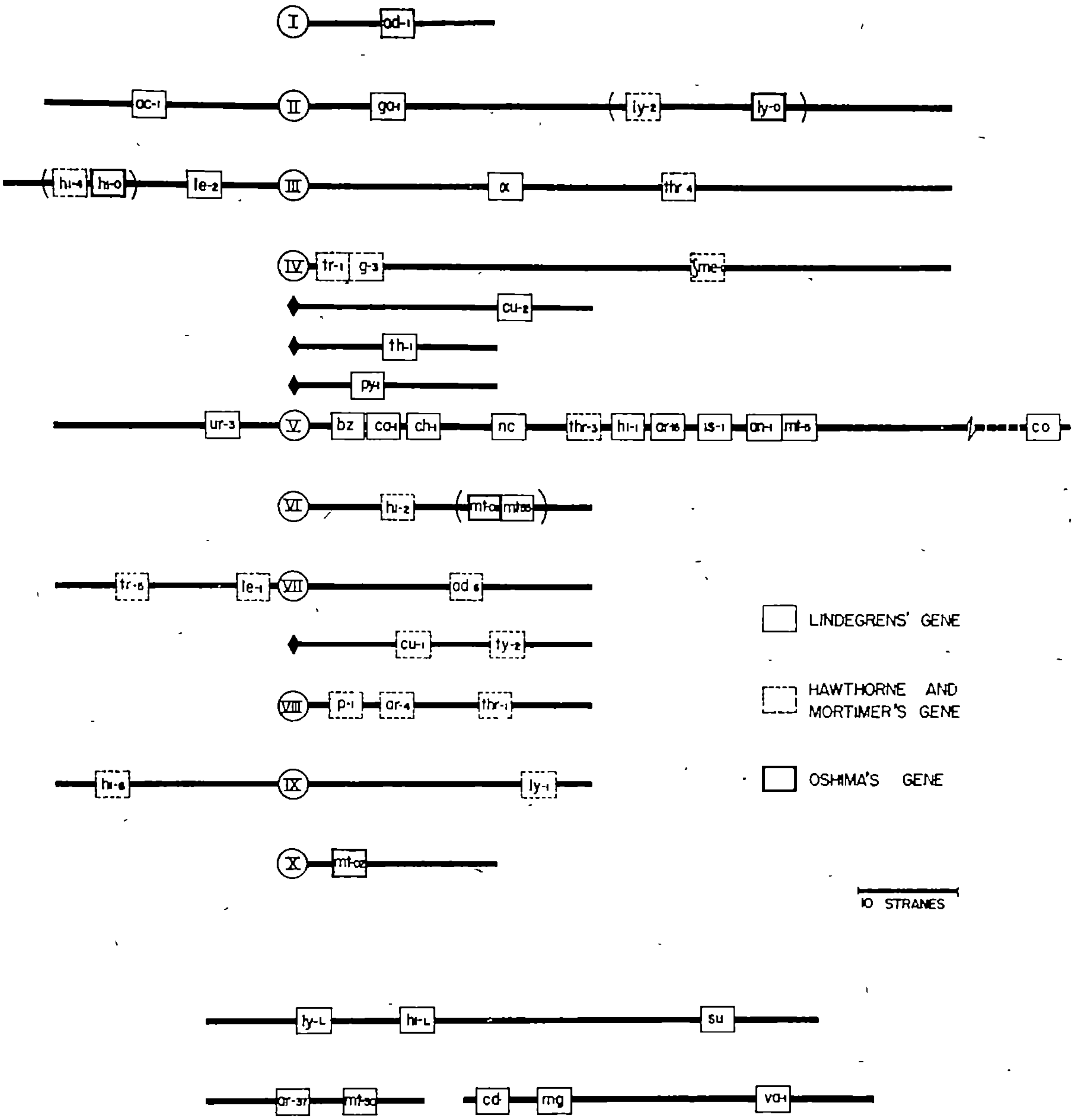
I
od-1
ac-1
II
ga-1
ly-2
ly-0
hi-4
hi-0
le-2
III
α
thr-4
IV
tr-1
g-3
me-1
cu-2
th-1
py-1
ur-3
V
bz
ca-1
ch-1
nc
thr-3
hi-1
ar-16
is-1
an-1
mt-5
co
VI
hi-2
mt-α
mt-30
tr-5
le-1
VII
od-6
cu-1
ty-2
VIII
p-1
ar-4
thr-1
hi-6
IX
ly-1
X
mt-α2
ly-L
hi-L
su
ar-37
mt-30
cd
mg
va-1
LINDEGRENS' GENE
HAWTHORNE AND MORTIMER'S GENE
OSHIMA'S GENE
10 STRANDS

Fig. 1. Chromosome maps of *Saccharomyces*. This map is based on data drawn from a number of different sources (refs. 10–16 and 20) as well as the more recent results presented in this article. The map contains 10 chromosomes, 4 chromosome segments carrying sites of affinity and 3 chromosome segments which have not yet exhibited linkage with either a centromere or a preferentially segregating site. Centromeres are depicted as round circles containing the numeral of the chromosome it characterizes. Sites of affinity (when they could be located) are represented by a black diamond. Sites on segments *A*, *B* and *C*, preferentially segregate with a point near *ch*-1 on chromosome V, while that on segment *D* preferentially segregates with a point near *ar*-4 on chromosome VII

with 19 [15], 24 [21], and 20·9–25·7 [14]. Data from family 227 show *hi*-4 distal to *le*-2 and in family 241 (*hi*-4 × *hi*-0), 12 *PD* tetrads were obtained. (One *NPD* tetrad was also obtained, but the two histidine-independent progeny grew slowly and may reflect either mutation or a slight 'leakiness' in *hi*-4. In general, crosses carrying *hi*-4 give poorer germination of spores.) Although the relative order of *hi*-0 and *hi*-4 has not been determined (if indeed they do recombine) the total gene arrangement for chromosome III is (*hi*-4, *hi*-0), *le*-2, centromere, mating type, *thr*-4.

Chromosome V. In chromosome V the sequence *ur*-3, centromere, (*ch*-1, *ca*-1), *nc*, *hi*-1, *is*-1, *an*-1 and *co* has been established[14]. Subsequent data from families 131 and 132 showed that the gene *bz* (benzimidazole-sensitive) was closely linked to *ch*-1 and *ca*-1 (*bz*-*ch* : 39–1–11, *bz*-*ca*: 49–0–16). The relative order of these loci was determined in two crosses, families 200 and 201, as centromere, *bz*, *ca*-1 and *ch*-1 [20].

The gene *ar*-16 has exhibited strong linkage with *hi*-1, and in a series of crosses heterozygous for *ar*-16, *ch*-1, *hi*-1, *is*-1 and *an*-1 the tetrad distributions given in Table 4 were obtained. The gene order indicated is *ch*-1, *hi*-1, *ar*-16, *is*-1, *an*-1, establishing the total sequence: *ur*-3, centromere, *bz*, *ca*-1, *ch*-1, *nc*, *hi*-1, *ar*-16, *is*-1, *an*-1 and *co*.

The gene *mt*-5, which controls the synthesis of methionine, is tightly linked to the genes *is*-1 and

Table 4. TETRAD DISTRIBUTIONS ACCUMULATED FROM FOUR HYBRIDS HETEROZYGOUS FOR *ch*-1, *hi*-1, *is*-1, *an*-1, AND *ar*-16

Gene combinations	*PD*	*NPD*	*T*
ch-1, *hi*-1	23	0	21
ch-1, *ar*-16	20	0	26
ch-1, *is*-1	12	0	26
ch-1, *an*-1	9	1	25
hi-1, *ar*-16	41	0	7
hi-1, *is*-1	26	0	14
hi-1, *an*-1	18	0	19
ar-16, *is*-1	26	0	15
ar-16; *an*-1	20	0	17
is-1, *an*-1	28	0	8

an-1. The tetrad distributions obtained from family 302 for the gene pair *mt*-5 and *is*-1 is 31–1–4, and for the gene pair *mt*-5 and *an*-1 is 35–0–1. These tetrad distributions suggest that the gene *mt*-5 is probably distal to *an*-1.

Chromosome VI. Tetrad data obtained by Dr. Oshima[16] showed that *mt*-03 is approximately 25 stranes from a centromere. The total tetrad distribution of *mt*-03 with *hi*-2 is 18–1–20, showing that *mt*-03 lies on chromosome VI distal to *hi*-2. Another methionineless mutant, *mt*-24 fails to complement with *mt*-03. The total tetrad distribution for *hi*-2 and *mt*-24 is 32–1–24. In a cross, *mt*-24 × *mt*-03, no methionine-independent progeny were obtained in nine tetrads, indicating that these loci are close together, if not identical. A second methionineless mutant, *mt*-55, complements with *mt*-03 (and *mt*-24), but also suggests linkage with *hi*-2 on chromosome VI (family 265, tetrad distribution 4–0–1). No methionineless-independent offspring were obtained in 20 asci from a *mt*-03 × *mt*-55 hybrid, indicating that these two methionine loci lie very close together. Thus, an overall sequence in chromosome VI is: centromere-*hi*-2-(*mt*-03, *mt*-55).

Chromosome VIII. The following order of the genes and the centromere on chromosome VIII has been proposed[15]: centromere, *p*-1, *ar*-4, *thr*-4, *cu*-1. Recent results[10] show that *cu*-1 lies on a distinct chromosome arm which segregates preferentially with chromosome VIII.

Identification of a 'new' chromosome. The genes *mt*-02 and *mt*-03 are complementary, and tetrad results obtained by Dr. Oshima[16] indicate that these loci are on two different chromosomes distinct from chromosomes I, II, III and V. The gene *mt*-02 is 7 stranes from its centromere, and *mt*-03 is 25 stranes from its centromere on chromosome VI.

Table 5 presents the total tetrad distribution of *mt*-02 with the centromere markers of chromosomes I–IX, drawn from data obtained in this laboratory. The strong *F*-distributions with *ad*-1, *ga*-1, mating type, *tr*-1, *ur*-3 and *le*-1 show conclusively that the *mt*-02 chromosome is distinct from chromosomes I, II, III, IV, V and VII.

The problem of locating *mt*-02 with regard to *hi*-2 (chromosome VI) and *ar*-4 (chromosome VIII) involves a somewhat more sophisticated argument. The gene, *hi*-2, is 18 Perkins's units from its centromere[15]. Consequently, if *mt*-02 were on chromosome VI, its distance from *hi*-2 would be either 11 or 25 stranes. (At this magnitude, Perkins's units and stranes are approximately equal.) In either case, one would expect linkage to be manifest in the tetrad distribution for *mt*-02 and *hi*-2. Assuming the distance of 25 stranes, one would expect the tetrad distribution for *mt*-02 and *hi*-2 to be 25·748–0·820–14·432 rather than 10–9–22, the observed distribution. The observed value of $PD/(PD + NPD)$ differs from the expected value with $z = 11{\cdot}32$ and $P < 10^{-10}$. Thus one must reject the hypothesis that *mt*-02 is located on chromosome VI, opposite the centromere from *hi*-2. This also entails rejection of the hypothesis that *mt*-02 is on chromosome VI at all, for if *mt*-02 were on the same arm with *hi*-2 they would lie approximately 11 stranes apart and the expected linkage would be even 'tighter' than that rejected above.

If *mt*-02 were located on chromosome VIII, it should show linkage with *ar*-4, which is also 7 Perkins's units from its centromere. The assumption that they lie on opposite sides of the centromere would place

Table 5. TETRAD DISTRIBUTIONS FOR *mt*-02 AND THE CENTROMERE-LINKED MARKERS OF CHROMOSOMES I–IX

Chromosome No.	I	II	III	IV	V	VI	VII	VIII	IX	
Gene	*ad*-1	*ga*-1	alpha	*tr*-1	*ur*-3	*hi*-2	*le*-1	*ar*-4	*hi*-6	*ly*-1
Tetrad distribution	83–17–14	41–33–19	61–78–89	52–33–4	35–39–29	10–9–22	25–28–14	38–39–53	11–14–31	19–15–45

Table 6. TETRAD DISTRIBUTION OF *ly-L* WITH GENES ON CHROMOSOME V, AND THREE GENES LINKED TO POINTS OF AFFINITY IN SEGMENTS SEGREGATING PREFERENTIALLY WITH A POINT NEAR CENTROMERE V χ^2 TESTS $PD = NPD$

Family	Genes on Chromosome V			Segments *A*	Segments *B*	Segments *C*
	bz-1	*ca*-1	*ch*-1	*cu*-2	*th*-1	*py*-1
97			1–0–5		1–0–4	0–0–6
101			1–2–0		1–1–3	2–2–0
106			9–1–12	6–4–23	10–2–34	3–6–25
110			2–6–12		1–6–10	
111			16–10–45	14–14–53	11–3–29	
111*			10–2–9			
116			25–18–76		16–13–44	
116*			3–2–13			
117			2–3–14	7–3–16	2–3–11	
21862 × 7*H*-1	4–1–3	6–1–3			2–0–0	5–1–1
124	2–1–12	4–1–21	5–1–23	5–4–12		
125			3–1–15		3–0–6	3–1–11
126				2–2–5	2–1–5	1–0–8
131	5–5–12	7–7–29	7–4–29		7–3–19	
201	21–4–45	21–6–49	19–7–45	20–9–46		19–6–49
Total	32–11–72	38–15–102	103–57–298	54–36–155	56–32–165	38–16–100
χ^2	10·256	9·981	18·225	3·600	6·545	8·963
P	< 0·15 per cent	< 0·2 per cent	< 0·05 per cent		< 1·5 per cent	< 0·3 per cent

them approximately 14 stranes apart so that the expected tetrad distribution (in 130 tetrads) would be 99·32–0·91–29·77. This differs radically from 38–39–53, with $PD/(PD + NPD)$ achieving the observed value with probability far less than 10^{-12}. If *ar*-4 and *mt*-02 were on the same side of the centromere, linkage would be nearly complete, which obviously it is not. Thus *mt*-02 does not lie on chromosome VIII.

The genes *hi*-6 and *ly*-1 lie on opposite sides of centromere of chromosome IX at 17 and 29 Perkins's units respectively[15]. Thus, if *mt*-02, which is 7 stranes from a centromere were on chromosome IX, it would have to lie between *hi*-6 and *ly*-1. Consequently the Trow[22] formula:

(1) $1 - 2f(hi\text{-}6,\ mt\text{-}02) \times 1 - 2f(mt\text{-}02,\ ly\text{-}1) = 1 - 2f(hi\text{-}6,\ ly\text{-}1)$ should hold. The symbol $f(A, B)$ denotes the frequency of recombination between genes A and B). The tetrad distribution 75–8–130 for *hi*-6 and *ly*-1 was accumulated from miscellaneous crosses (Table 10 [14]), so that $1-2f = (75-8)/(75 + 8 + 130) = 0{\cdot}315$. The two tetrad distributions listed in Table 5, 11–14–31 (*hi*-6 and *mt*-02) and 19–15–45 (*mt*-02 and *ly*-1), yield the expected fraction $(-0{\cdot}054) \times (0{\cdot}051) = 0{\cdot}002754$. Since f is normally distributed, so is $1-2f$, and the two values can be compared by the z test, with N equal to the number of tetrads. (In tetrad data, the genotypes of the spores do not appear independently. Consequently N cannot be taken as the number of cultures in a normal-curve test. Since $1-2f = a_1 - a_2$ (a_1 and a_2 are relative frequencies of PD and NPD tetrads) $PD - NPD$ is a normally distributed parameter in a sample of size $N = PD + NPD + T$.) In a sample of size 213, the proportion 0·343 differs from the expected proportion 0·002754, with $z > 70$, and with p attaining a fantastically small value. Correction for the fact that the parameters, f(*hi*-6, *mt*-02) and f(*mt*-02, *ly*-1), which were used in calculating the theoretical value, are themselves also random variables with associated variances, could not possibly raise p even within range of the 1 per cent level. Consequently, the hypothesis that *mt*-02 lies on chromosome IX must be rejected.

A methionineless gene *me*-2 [16] was placed on chromosome X largely on the basis 163/273 = 59·7 per cent second division segregation in ordered tetrads. This differs from 2/3 only at the 2 per cent level. In their data, *me*-2 exhibits 473/744 = 63·6 per cent tetratype tetrads, with the markers *ad*-1, *ga*-1, *tr*-1, *le*-1 and *p*-1, all lying within 8 units of their respective centromeres. In data obtained from this laboratory *me*-2 exhibits 82/129 = 63·6 per cent tetratype tetrads with *ga*-1, *tr*-1, *ur*-3, *le*-1 and *ar*-4 (also, all lying within 8 units of a centromere). Thus the data from ordered asci are sub-significant, and segregational data are completely negative with regard to *me*-2 being centromere linked. Furthermore, there are no data indicating that *me*-2 lies on a chromosome distinct from chromosomes I–IX, and its location on 'chromosome X' must be considered premature. However, the gene, *mt*-02, lies only 7 units from its centromere, on a chromosome known to be distinct from chromosomes I–IX. We therefore propose to designate this chromosome, chromosome X.

The chromosome segment carrying ly-L. The gene *ly-L* cannot be identified with either *ly*-0 (with which it complements) or *ly*-2, since both of the latter exhibit linkage with *ga*-1, while *ly-L* does not. The tetrad distributions of *ga*-1 with *ly*-2 [15], *ly*-0 [14] are 32–6–95 (*ga*-1 and *ly*-2), 148–44–454 (*ga*-1 and *ly*-0) and 132–150–683 (*ga*-1 and *ly-L*). However, there does appear to be some association between *ly-L* both with genes located near centromere V and genes on segments which segregate preferentially with centromere V (Table 6). This association does not necessarily prove that *ly-L* is located on chromosome V, since the significant excess of parental over non-parental ditype tetrads could result from preferential segregation of a site of affinity located somewhere near the *ly-L* gene.

This inference is supported by: (*a*) the fact that the weak linkage of the *ly-L* with chromosome V genes varies erratically from family to family since parental preferential segregation may not obtain in all families (namely, family 110); (*b*) the fact that other loci, *cu*-2, *th*-1 and possibly *py*-1, are known to lie on chromosome arms which segregate preferentially with the genes, *bz*, *ca* and *ch*-1 on chromosome V [9,22]; (*c*) the fact that the cumulative linkage effect does not appear with markers *ur*-3 and *is*-1 which lie on opposite sides of the short *bz-ca-ch* linkage group on chromosome V (Table 7), indicating preferential segregation toward points interior to the *ur*-3, *ch*-1, *is*-1 group on chromosome V.

Table 7. TETRAD DISTRIBUTIONS OF *ly-L* WITH GENES LOCATED ON OPPOSITE ARMS OF CHROMOSOME V

Hybrid No.	*ly-L*, *is*-1	*ly-L*, *ur*-3
88	6–5–18	4–4–21
89	10–2–13	0–5–21
91	7–10–26	8–10–25
97	3–4–16	3–4–18
106	5–9–36	5–6–38
111		17–18–66
115		2–2–10
116		20–19–99
117		2–2–23
124	8–5–23	5–5–27
125	4–6–9	
126	1–3–5	
127		0–1–4
128		1–6–28
131	10–9–24	4–4–35
133	0–1–4	
Y-1	17–32–96	22–26–89
133*a*	2–1–4	
133*b*		3–1–5
*Y*2	3–3–12	
133*c*	2–1–7	
133*d*		0–1–4
200	16–19–61	10–15–71
201	16–11–48	17–9–58
201*a*	5–2–9	4–2–10
201*b*	2–1–4	
203	2–1–8	
203*a*	1–1–4	2–6–9
204	7–6–17	4–4–19
208		2–0–5
209	1–1–3	
215		0–0–8
231	2–1–1	0–1–3
Total	130–134–448	135–146–696
	$\chi^2 = 0{\cdot}061$	$\chi^2 = 0{\cdot}431$

The tetrad distributions for the genes *ly-L*, *hi-L* and *su* are as follows below:

Hybrid	Gene combination *hi-L*, *ly-L*	*hi-L*, *su*	*ly-L*, *su*
28150 × *N*2 (*hi*)	7–0–9		
30393 × 22464	4–0–3		
30393 × 29442	4–0–1		
200	69–1–22	29–6–60	22–5–68
201	44–2–34	27–5–50	17–2–57
211		6–2–9	
212	13–0–10		
215	5–0–3		
216			15–0–29
32308 × 24310 (*an*)	4–0–3		
239	65–1–22	27–7–61	19–7–62
Total	215–4–107	80–20–180	73–14–216
	0 660–0 012–0 328	0 308–0 069–0 623	0 241–0 046–0 7128

The tetratype frequencies suggest the gene order *ly-L*, *hi-L*, *su* since *ly-L* and *su* exhibit the highest tetratype frequencies. Gene order was determined in the three triply heterozygous families 200, 201 and 239, by the three tests for gene order[9]. The three-point data for the three families are as follows:

	Hybrid 200	Hybrid 201	Hybrid 239	Total
	ly-L + *su* / + *hi-L* +	*ly-L* + *su* / + *hi-L* +	*ly-L hi-L* + / + + *su*	
Non-exchanges	17	12	17	46
Singles				
Region I	11	13	4	28
Region II	48	28	45	121
Doubles				
4-*st*, region I	1	0	0	1
4-*st*, region II	2	1	3	6
2-*st*	3	5	2	10
3-*st*	6	12	7	25
4-*st*	2	1	4	7
Triples				
4-*st* *R*I, *sR*II	0	2	1	3
4-*st* *R*II, *sR*I	3	2	5	10
	93	76	88	257

A chromosome segment. The gene *ty*-2 controls the synthesis of tyrosine, and is also associated with a phenylalanine requirement. (Three other cases are known in yeast in which a mutant locus is associated with a multiple biochemical requirement: *is*-2 (isoleucineless, valineless), *po*-1 ('polyaromatic': tyrosine, phenylalanine and anthranilic acid requirements), and *glt*-1 (glutamicless, lactate negative). Linkage between *ty*-2 and *cu*-1 was evident in several families, although the amount of crossing-over varies considerably between families. The total tetrad distribution is 25–6–43. The segment preferentially segregates with chromosome VIII. The points of highest affinity lie near *cu*-1 on the segment distal to *ar*-4 on chromosome VIII [19].

Linkage groups in two segments not located near centromeres or sites of affinity. The mutant *mt*-30 complements with all the other methionineless cultures (*me*-2, *mt*-02, *mt*-03, *mt*-55 and *mt*-5) and exhibits linkage with *ar*-37; the total tetrad distribution from two hybrids was 26–0–8. A valineless mutant, *va*-1, exhibits linkage with *mg* (α-methyl glucoside fermentation). The total distribution accumulated from six hybrids is 22–7–66. The gene *cd*, controlling resistance to 0·01–0·02 per cent cadmium[23], lies about 25 stranes from *mg*. The total tetrad distribution for *cd-mg* is 77–8–58, but the order of these genes with respect to *va*-1 is not known.

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HIGH BACKGROUND RADIATION ON A SMALL ISLAND

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THERE is, at this time, a great deal of interest in the effects of exposure to a high background radiation in animal and plant life. Various interested organizations claim to be searching for groups of people, suitable for comparative studies, living under identical conditions—one group exposed to radiation and the other not exposed. This article is intended to indicate the value of a full-scale biological survey of Chilwa Island in Nyasaland by an organization capable of dealing with the problem.

Geology

Chilwa Island is situated near the south-western shore of Lake Chilwa, twenty miles east of Zomba, the administrative capital of Nyasaland. The island, which measures about two miles across, is the largest and most northerly of an irregular chain of volcanic carbonatite necks adjacent to the eastern border of southern Nyasaland with Portuguese East Africa

The superior resistance to tropical weathering of the carbonatites on Chilwa Island, relative to the older Basement Complex Rocks, have led to the formation of a steep ring-like feature. This topographic feature has been breached by erosion on its western side and a natural amphitheatre has been formed which bounds a flat arena of alluvium and eluvium of terra rossa type.

A geological survey of Chilwa Island in 1952 revealed marked radioactive anomalies at three localities (Garson and W. Campbell Smith, 1958). These were: (1) the floor of the Chirima arena in the area of Chirima Village; (2) the summit plateau; (3) dyke-like carbonatitic bodies on slopes above the Chirima arena.

The primary area of interest in this article is the Chirima arena because the other two areas are uninhabited and covered for much of the year by dense vegetation. The arena has an area of approximately 120 acres. Gamma-ray intensity is highest in an elongated zone, roughly parallel to the northern and southern margin of the arena. Channel samples collected from this area by D. Ostle and J. Taylor of the Atomic Energy Division (1954) indicate an equivalent urania content of between 0·020 and 0·031 per cent, confirming the isorad map produced during the 1952 investigation. It is clear that the radioactive anomaly in the arena is due to the accumulation of radioactive debris derived from the radioactive dykes on the carbonatite slopes. These dykes contain up to 2·14 per cent thoria.

Laboratory investigations at the Atomic Energy Division were directed towards the identification of the radioactive minerals, and the determination of the phase status of the radioelements. The source of the radioactivity in the rocks was found to be mainly in patches of goethite and to a minor extent in tiny grains of pyrochlore.

It was also found that thorium was predominant over uranium in a soil sample, and that most of the γ- and β-radiation must be attributed to the presence of disintegration products of original radioelements which remained fixed in the soils after the latter were leached away. These disintegration products were believed to be decay products of uranium, ionium and radium, which, having no source of replenishment, disintegrated further to elements of shorter half-life periods. This is further indicated by the fact that there is an observed decrease in the degree of radioactive equilibrium as bed-rock is approached. In view of the relationship between the high radiometric anomaly in the Chirima arena and physiological factors described here it is important that future research should establish the exact nature of these short half-life elements.

Further analyses of samples from the Chirima arena are being carried out to determine the presence of cæsium-137/strontium-90 and iodine-131.

Pilot Survey

Following the report of the geological survey of Chilwa Island carried out in 1952 by M. S. Garson and W. Campbell Smith, it was decided to investigate the effects of this high background radiation on the health of the population. The background radiation was estimated to be approximately thirty times normal. Accordingly a small team, consisting of Mr. R. A. Clift, a Government medical laboratory technician, a medical assistant to act as an interpreter and I, spent three days on Chilwa Island in November, 1954, carrying out a preliminary survey.

The geography of the island has already been mentioned, but a few words should be included here about the distribution of the population. There are approximately 1,000 inhabitants living in 21 villages spread out around the flat perimeter of the island. There are no villages on the peaks. Each village has about 45–50 men, women and children governed by a village headman. A census of the people is said to have been held in 1948. Everyone on the island was vaccinated against smallpox in 1951. The island is small and infested with malaria-carrying mosquitoes.

Fishing is the main occupation of the islanders although some tend cattle which have been brought over from the mainland.

The lake provides bilharzia-infected water for all domestic purposes.

Two villages lie within the area known as the Chirima Arena. That nearest the centre of the arena was chosen as the test village. Chief Nkumbira's village, which lies outside the area of radioactivity, was chosen as the control village.

The total white cell counts and hæmoglobin estimations were used as indicators. As the hæmoglobin estimations were of doubtful value they will be excluded from this report.

Capillary blood from the finger was used for all the estimations.

All the persons in the test series except three had lived in the arena since birth. The three exceptions had lived there for 2, 12 and 17 years respectively. None of the control series had lived in the Chirima Arena.

Table 1. DISTRIBUTION OF WHITE CELL COUNTS

Age years	Test series Males	Test series Females	Control series Males	Control series Females
0–5	0	0	0	0
5–10	0	6	3	0
10–15	1	2	7	0
15–20	5	1	2	1
20+	14	9	12	3
Total	20	18	24	4

A comparison of the means of white cell counts from the two complete series. Table 2 shows frequency distribution (test series).

Table 2

1 White blood cells (×1,000)	2 Frequency	3 Difference in units	4 2×3	3×4
1–	1	−5	−5	25
2–	2	−4	−8	32
3–	5	−3	−15	45
4–	4	−2	−8	16
5–	9	−1	−9	9
6–	5	0	0	0
7–	3	+1	+3	3
8–	1	+2	+2	4
9–	2	+3	+6	18
10–	2	+4	+8	32
11–	2	+5	+10	50
12–	1	+6	+6	36
13–	1	+7	+7	49
	38		−3	319/38 = 8·39

$$\text{Mean} = 6·5 - \frac{3}{38} = 6·42$$

$$S.D = \sqrt{8·39 - (0·08)^2} = \sqrt{8·384} = 2·89$$

Table 3 shows frequency distribution (control series).

Comparison of test and control series

Mean of test series = 6·42

Mean of control series = 7·96

Difference of means = 1·54 (1)

Standard of error of (1)

$$= \sqrt{\frac{(2·7)^2}{38} + \frac{(2·7)^2}{28}} = \sqrt{\frac{7·3}{38} + \frac{7·3}{28}}$$

$$= \sqrt{0·19 + 0·26} = \sqrt{0·45}$$

$$= 0·67 \qquad (2)$$

$$\text{Ratio } (1) : (2) = \frac{1·54}{0·67} = 2·3$$

This ratio is well above the level of significance, suggesting that the observed difference of means might occur by chance once in 50 tests.

Test Village

The village headman was very co-operative and called all the people together. Each person's name, age, years of residence on the island, and physical complaints were recorded under the direction of the medical assistant. It was not possible to examine specimens of urine as there was insufficient time. Nevertheless, on the evidence available, it appeared

Table 3

1 White blood cells (×1,000)	2 Frequency	3 Difference in units	4 2×3	3×4
5–	6	−3	−18	54
6–	4	−2	−8	16
7–	6	−1	−6	6
8–	4	0	0	0
9–	4	+1	+4	4
10–	2	+2	+4	8
11–	1	+3	+3	9
12–	0	+4	0	0
13–	0	+5	0	0
14–	1	+6	+6	36
	28		−15	133/28=4·75

$$\text{Mean} = 8{\cdot}5 - \frac{15}{28} = 7{\cdot}96$$

$$S.D. = \sqrt{4{\cdot}75 - (0{\cdot}53)^2} = \sqrt{4{\cdot}47} = 2{\cdot}11$$

that many of the inhabitants suffered from bilharziasis.

The women were questioned about the number of pregnancies, abortions, still-births and neonatal deaths among them. It is difficult to decide from such a brief survey whether the abortion and still-birth-rates were higher on the island than on the mainland.

From 14 women of child-bearing age there was a history of 12 abortions, 3 still-births, 16 neonatal deaths and 41 living children. Out of a total of 72 pregnancies only a little more than half of them resulted in a living child who had survived for one year. Only one case of twins was reported. There were no cases of maternal death due to confinement.

There were three interesting cases of skin disease of an unusual type. The three young men were about twenty-one years old. The skin showed large watery blisters which, according to the patients, "had appeared quite suddenly" and for no apparent reason. The word "suddenly" as used by Africans in this context really means spontaneously. Itchiness was complained of in each case. The skin lesion resembled very closely the skin reactions following intensive deep X-ray therapy. No cases of this condition occurred in villagers living outside the arena. The blood pictures of these young men were also of interest. All three had white blood cell counts between 2,000 and 3,000 per mm.^{3}.

The polymorph count in each case was markedly decreased whereas the lymphocyte count was increased. The hæmoglobin estimations averaged 80 per cent Sahli.

No cases of tropical ulcers were seen or reported for the previous fifteen years. There was one epileptic and one mentally defective youth in the village. The village headman could not recall any cases of murder or suicide in his village. There was an unusually high number of graves in the area of the arena.

Control Village

Co-operation was not good here mainly because Chief Nkumbira's religious principles were in conflict with anything to do with medical science. This resulted in many of the women refusing to be examined. Only six women could be induced to give a history of their pregnancies. The figures given were 8 living children, 5 neonatal deaths, 1 still-birth and 1 abortion. These totals, although small, give practically the same proportion of living children surviving to one year as in the test village. No cases of twins could be recalled. There had been one maternal death due to confinement in the last 15 years.

Again no cases of tropical ulcers were seen or recalled over the past fifteen years. There were no epileptics or mental defectives in this village. There had been one case of suicide many years ago. No murders had been committed in living memory. There were no cases of low white cell counts or unusual skin lesions among the inhabitants.

Discussion

On the whole the health of the people in both villages was very good and above the average as compared with the mainland. The complete absence of cases of tropical ulcers is very striking. This was also noted by the Government medical officer who carried out the smallpox vaccination campaign in 1951.

As the people were not desirous of being examined for venereal disease no importance can be attached to the high rate of unsuccessful pregnancies until the women can be properly examined, especially for syphilis. All that can be said is that difficulties during labour must be very uncommon since only one maternal death occurred as the result of a confinement within the past fifteen years, which is in sharp contrast to obstetrical experience on the mainland.

The average white cell counts obtained from the two villages in the survey differ by a figure much larger than that to be expected from chance. This difference may be due to one of two causes. Either the average white blood cell count for the control group is unduly high or the average count for the test group is low. An examination of the figures suggests the latter. Factors reducing the white cell count are relatively uncommon. With the high incidence of parasitic infection one would expect the white blood cell counts to be raised in both the test village and the control villages. As no selection, based on clinical features, was used in the samples the conclusion was that the cases of leucopænia in the test village could be due to exposure to radiation. No other obvious clinical cause for the leucopenia was found.

Recommendation

It is felt that the results of this brief survey merit a fuller investigation by a team qualified to undertake such a task. One suggestion is that the islanders be persuaded to wear photographic film badges which would give an accurate assessment of the degree of exposure. This is a cheap and simple method of investigation. Bone from the bodies in the numerous graves in the area of the arena could be examined for radioactive isotopes.

The effects of radiation on vegetation and wild life require to be investigated. One geologist considers that he has seen a mutant of the plant *Euphorbia candelabrae* in the radioactive arena.

The amount of radioactive material absorbed into the lake water and hence into the fish might provide useful results. The island probably presents a unique field for the investigation of the long-term biological effects of radiation.

Acknowledgments

I wish to express my thanks to the director of medical services, Nyasaland, Dr. P. R. Stephens, for permission to publish this article. I am also indebted to the following for their kind help: Dr. M. S. Garson for geological data and Mr. R. A. Clift for the tables of white cell counts and other useful data obtained during our survey of Chilwa Island.

LETTERS TO THE EDITOR

PHYSICS

Water Waves and Hamilton's Method

OCEAN waves approaching a beach may be discussed by the method of geometrical optics; the frequency having been assigned, the phase velocity is determined by the depth; the rays are determined by Fermat's principle, and the wave crests are the orthogonal trajectories of the system of rays[1]. But it may not be generally realized that Hamilton's optical method, suitably generalized, can be used to treat a much wider class of problems of water waves. The method is essentially Huygens' construction put into mathematical form.

We start with an equation connecting phase velocity W and wave-length λ:

$$W^2 = \frac{\lambda}{2\pi} \tanh \frac{2\pi h}{\lambda} \left[1 + \frac{T}{g\rho}\left(\frac{2\pi}{\lambda}\right)^2 \right]$$

where h is depth, ρ density, and T surface tension. Let y_1, y_2 be wave-numbers so that $(2\pi/\lambda)^2 = y_1^2 + y_2^2$ ($= y^2$, say), and y_3 circular frequency $(2\pi W/\lambda)$; then we have the equation:

$$\Omega \equiv y_3^2 - \tanh hy \ (gy + y^3 T/\rho) = 0 \qquad (1)$$

This we may call the 'Hamilton equation'; regarded as a surface in y-space, it is the analogue of what Hamilton called the "surface of components of normal slowness". Let x_1, x_2 be space co-ordinates, x_3 the time and ψ the phase. Replacing y_r by $\partial\psi/\partial x_r$ in equation (1) ($r = 1, 2, 3$), we get for ψ a partial differential equation which is the analogue of the so-called Hamilton–Jacobi equation. The solution of wave-problems is merely a matter of solving it. This is done by first finding the rays[2] which satisfy the canonical equations $\mathrm{d}x_r/\mathrm{d}w = \partial\Omega/\partial y_r$, $\mathrm{d}y_r/\mathrm{d}w = -\partial\Omega/\partial x_r$, w being a parameter. Then:

$$\psi(x) - \psi(x') = \int_{x'}^{x} y_r \, \mathrm{d}x_r$$

the integral being taken along the ray joining the events x'_r and x_r, with the summation convention. This phase difference is essentially Hamilton's principal function.

As a simple example take $h = \infty$, $T = 0$; the Hamilton equation is then $y_3^2 - gy = 0$, and the rays are $x_1 - x'_1 = -wgy_1/y$, $x_2 - x'_2 = -wgy_2/y$, $x_3 - x'_3 = 2wy_3$, $y_r =$ constant. The phase difference is $\psi(x) - \psi(x') = g(x_3 - x'_3)^2/4\xi$, where $\xi^2 = (x_1 - x'_1)^2 + (x_2 - x'_2)^2$. From this we obtain by simple algebra the familiar Kelvin ship-wave pattern, putting $\psi = 0$ at the prow, for which $x'_1 = Ux'_3$, $x'_2 = 0$, and eliminating x'_r by a condition of stationary phase.

Just as the ordinary method of geometrical optics permits approximate solution of a class of problems far wider than those for which Maxwell's equations can be solved, so the above Hamiltonian method can be used to discuss a very wide range of hydrodynamical problems including those for which h and T vary with position, and even with time. The Hamilton equation can be modified to deal with tidal currents. The most fundamental results in geometrical optics occur in reflexion or refraction at a surface of discontinuity; analogues arise in hydrodynamics at a sharp change in depth, or surface tension (for example, at the edge of an oil-slick), or tidal current. The method deals only with phase; conclusions about amplitude can only be inferred indirectly.

Note added in proof. It may be useful to have a word, analogous to 'photon', 'phonon' and 'graviton', for a fictitious particle which travels with the ray (or group) velocity and carries energy: we suggest the name 'hydron'.

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A New Method for the Determination of Plasma Electron Temperature and Density from Thomson Scattering of an Optical Maser Beam

THE Thomson scattering of light by free electrons has not yet been observed in a laboratory plasma—the cross-section for the process is small and until recently no suitable source of light has been available. The advent of a very bright monochromatic light source in the form of the optical maser has markedly changed the situation, however, and in this communication a method is proposed for measuring the temperature and the relative density of electrons in a plasma from the Doppler broadening and the intensity of light scattered from an optical maser beam.

Ruby optical masers already constructed have delivered pulses of 10^4 W. of parallel monochromatic light at 6943 Å. lasting 200 μsec.[1]. Multiple reflexions of such a beam between a mirror placed at the far side of a plasma and the highly reflecting front surface of the optical maser crystal could enhance the beam power by a factor of ten or more, because attenuation by the plasma is slight. Despite the very small cross-section for Thomson scattering ($6{\cdot}65 \times 10^{-25}$ cm.^{2} per electron), with an effective beam-power of 10^5 W. it becomes feasible to detect light scattered from a moderate number of electrons. For example, even at the rather low plasma electron density of 10^{10} cm.$^{-3}$, in 200 μsec. about $4{\cdot}7 \times 10^5$ photons would be scattered from 1-cm. length of beam, and if 1/20 of these were received by a photomultiplier of 4 per cent quantum efficiency a signal-to-noise ratio of more than 30 would be obtained, provided contributions from particles other than electrons may be neglected. The scattering cross-sections at 6943 Å. for most other particles are even smaller, though for neutral atoms of the alkali metals they are comparable.

It will be possible to distinguish between the scattered light and the self-luminosity of the plasma, provided the latter is not overwhelmingly bright, because light scattered through 90° is fully plane-polarized perpendicular to the scattering plane, whereas the self-luminosity of the plasma will generally be unpolarized, or nearly so. Thus the difference in power between components of the light reaching the detector polarized perpendicular to and parallel to the scattering plane will be due to the Thomson scattered light. A polarizing beam splitter and matched photomultiplier channels could conveniently be used for the analysis. To conserve light it would probably be advantageous to use an optical maser crystal cut so as to produce a plane polarized output beam, and orientated so that the plane of polarization is perpendicular to the scattering plane.

The double Doppler effect due to thermal motion of the electrons will give the scattered light a Gaussian wave-length distribution the half-intensity breadth $\delta\lambda_e$ of which depends on the temperature of the electrons T_e, the primary wave-length λ and the scattering angle[2]. If multiple reflexions are used to enhance the optical maser beam-power a scattering angle of 90° is desirable, in which case $\delta\lambda_e = 4{\cdot}32 \times 10^{-5}\lambda T_e^{\frac{1}{2}}$. Thus, for example, when $T_e = 2{\cdot}5 \times 10^5$ ° K., if $\lambda = 6943$ Å. then $\delta\lambda_e = 150$ Å. The electron temperature can be determined from measurements of the relative intensity of the scattered light at different wave-lengths. The Doppler broadening of light scattered by all other particles will be very much smaller, being inversely proportional to the square root of the mass, so if a narrow central region of the wave-length distribution is excluded their contribution to the scattered light will be eliminated.

An electron drift velocity in the scattering plane may be detected because in general it will produce a displacement of the maximum in the intensity distribution away from the optical maser wave-length. The magnitude of the displacement will depend on the drift direction.

Absolute measurements of the electron density, from the ratio between the effective beam-power and the scattered light intensity, would be very difficult. Variations in the relative density could be observed, however, and if a plasma of known density was available a direct calibration could be obtained.

It would be necessary to take great care to keep to a minimum the amount of stray light entering the detecting system from the primary beam. A little could be tolerated, because unlike the light scattered by the electrons it would lie entirely within the narrow wave-length region containing the primary beam—a region which need not be used in determining the wave-length distribution of the Thomson scattered light. Its polarization would be uncertain.

A lower limit to the useful temperature-range is set at about 10^4 ° K. by the breadth of the ruby optical maser output line (~ 1 Å.) and its displacement as a result of crystal heating during optical pumping ($\sim 0{\cdot}05$ Å. ° C.$^{-1}$) (ref. 3), which also make the smallest detectable drift velocity in the scattering plane about 2×10^7 cm. sec.$^{-1}$. Because symmetrical observations of the wave-length distribution of scattered light are desirable, the highest temperature measurable in a hydrogen plasma will be about 10^7 ° K., owing to the almost inevitable presence of strong H_α light at 6563 Å. from the cool outer regions of the plasma.

The density-range over which the method can be used will depend on many factors. Assuming (*inter alia*) that the optical maser beam has an effective power of 10^5 W. and a cross-section of 10^{-2} cm.^{2}, and that the light scattered from 1-cm. length of the beam in a plasma 30 cm. deep is examined with a resolving time of 10^{-4} sec., a lower density limit of about 5×10^{11} cm.$^{-3}$ (due to photomultiplier noise) has been estimated and an upper limit, for a hydrogen plasma, the self-luminous radiation of which in the region of 7000 Å. consists mainly of bremsstrahlung, of about 10^{14} cm.$^{-3}$. Both these limits will be pushed back proportionally if the optical maser power is increased, provided the output pulse length is not shortened unduly.

The method proposed here will not disturb the plasma, and as it will be suitable for pure hydrogen or deuterium plasmas it should be valuable in thermonuclear research. It will provide better space resolution than any existing optical method. The time and space resolution attainable, and the working density-range, are limited only by the optical maser beam power and energy available, which may be expected to be improved in future.

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A Fast Response Total-Radiation Detector

A PYROELECTRIC thermal detector having a high-frequency response of 5 μsec. has been made and has been used to measure the total-radiation loss from a high-temperature gas discharge. This device, which can easily be improved to give a time-constant of better than 1 μsec., should be compared with the more conventional bolometer types having, at best, a response of about 50 μsec.[1]. It consists of a piece of spontaneously polarized ferroelectric material (in this case barium titanate), having electrodes on opposite faces (Fig. 1). The electrode on one face of the ferroelectric is a film of conducting black used to absorb the pulse of incident radiation (and hence the device has a wide spectral response).

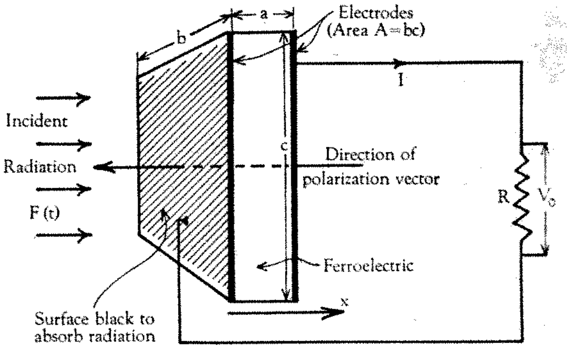

Fig. 1. Pyroelectric unit with electrodes on faces

Solution of the heat conduction equations by the method of Laplace transforms for such an arrangement shows that if a radiation flux $F(t)$ is absorbed at the surface electrode, then:

$$F(t) = \rho S \frac{d}{dt}(a\bar{\theta}) \tag{1}$$

where ρ = density of ferroelectric, S = specific heat, a = thickness of ferroelectric and $a\bar{\theta} = \int_0^a \theta(x)\,dx$, where $\theta(x)$ is the rise in temperature at a thickness x.

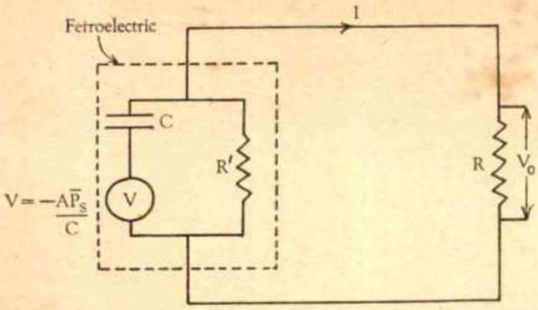

Fig. 2. Equivalent circuit of pyroelectric unit. C, Bulk capacitance of ferroelectric; R^1, leakage resistance of ferroelectric; $\bar{P}_s$, average polarization in the X direction

Equation (1) holds provided that no heat is lost from the ferroelectric. This implies that: (1) The thermal mass per unit area of the electrode M_T should be negligible with respect to the ferroelectric being heated or $M_T < \rho S(\varkappa\tau_1)^{\frac{1}{2}}$, where τ_1 is the minimum resolution time and $\varkappa$ is the thermal diffusivity of the material. (In general, τ_1 can be made less than the other time constants involved.) (2) There should be negligible heat loss from the rear surface of the ferroelectric or $a \gg (\varkappa\tau_2)^{\frac{1}{2}}$, where τ_2 is the maximum length of the radiation pulse.

For short radiation pulses, if the device is mounted in vacuum re-radiation is small, as is any heat loss down the leads due to conduction, if their cross-sections are small.

When the radiation flux is incident, it causes a change in temperature of the barium titanate and hence a change in spontaneous polarization[2]. (This process can be very rapid since dielectric relaxation, associated with the polarization, does not set in until frequencies in excess of 100 Mc./s.) If the absorbing black is efficient, any photo-induced processes will be small. The unit is polarized in the direction between the electrodes, and if the electrodes are connected to a resistance, R, the change in spontaneous polarization P_s manifests itself as a voltage developed across the electrodes. It can be shown[3] that the equivalent circuit is given as in Fig. 2. Now, if $\lambda = (\partial P_s/\partial\theta)$ is the rate of change of spontaneous polarization with temperature at constant stress (almost a constant in the temperature-range of 20°–100° C.), then $\bar{P}_s = \lambda\bar{\theta} = \frac{\int_0^t F(\tau)\mathrm{d}\tau}{a\rho S}$, so that the output for a radiation flux $F(t)$ is given by:

$$V_0(t) = \frac{4\pi\lambda}{\rho S\varepsilon}\exp\left\{-t\left(\frac{1}{R}+\frac{1}{R^1}\right)\Big/C\right\}\int_0^t \exp\left\{\tau\left(\frac{1}{R}+\frac{1}{R^1}\right)\Big/C\right\} F(\tau)\mathrm{d}\tau \quad (2)$$

where ε = dielectric constant of barium titanate; $C = \varepsilon A/4\pi a$ is the bulk capacitance of the barium titanate with electrodes of area A: R^1 = leakage resistance of the barium titanate.

Since the ferroelectric is also piezoelectric, there will be thermal stresses set up due to non-uniform heating, and so in order for equation (2) to apply it is necessary that the ferroelectric is in mechanical equilibrium, with no net resultant body forces on it (in fact supported from rear so that it is free to expand). This is only true when the frequencies associated with the radiation pulse are less than the mechanical resonant frequencies of the ferroelectric, for when they are comparable, the thermal expansion of the ferroelectric excites a mechanical resonance (and hence there is no longer mechanical equilibrium), so that an oscillatory output is superimposed on that of equation (2)—the mean of these oscillations over the frequency of the resonance in fact gives equation (2), but, by the averaging process, the frequency response is effectively limited to $1/f_r$, where f_r is the frequency of the lowest excited resonance.

Since λ changes rapidly close to the Curie temperature at about 110° C., where the spontaneous polarization disappears, the surface temperature should not exceed this value. This places a restriction on the maximum input energy. From the solution of the conduction equation (3) this implies that:

$$F(t) < \theta_{\text{max.}}\left(\frac{\pi\varkappa}{4}\right)^{\frac{1}{2}}\rho S t^{-\frac{1}{2}} \quad (3)$$

but for $t = 1$ msec., this gives $F(t) < 3$ kW. cm.$^{-2}$, so that the fluxes involved by this limitation are very high.

For RC large compared with τ_2, $V_0(t)$ is proportional to $\int^t F(\tau)\mathrm{d}\tau$ and is independent of the dimensions of the ferroelectric. For R large, the device is essentially capacitive, and the random noise associated with it is small. In order to obtain an output proportional to the incident flux the output proportional to $\int^t F(\tau)\mathrm{d}\tau$ is differentiated electronically with a time constant τ (so that the flux $F(t)$ is average of the energy arriving during time τ).

In fact, for a single crystal of barium titanate:

$$V_0 \simeq 980\int^t F(\tau)\mathrm{d}\tau.\text{V. (for } F(t) \text{ in W. cm.}^{-2})$$

For a time constant of 5 µsec., taking typical electronic noise the minimum detectable flux is about 100 µW. cm.$^{-2}$, but with longer time constants the pyroelectric detector can be very sensitive, especially when operated close to the Curie point[4].

Experimental work carried out confirms equation (2) and indicates that reproducible results may be obtained if sufficient time (~20 sec.) is allowed for the temperature of the detector to return to ambient between radiation pulses. It also shows that the upper frequency response is limited by the excitation of mechanical resonances by thermal expansion, rather than by any thermal time constant. Fig. 3 shows the radiation flux obtained with a 5 µsec. time constant from a high-temperature gas discharge[5]. When an equivalent spectral region was used identical results for the flux profile were obtained with photomultipliers; however, since a standard source was not available, the device has not been directly calibrated. The dimensions of the ferroelectric (which in this case

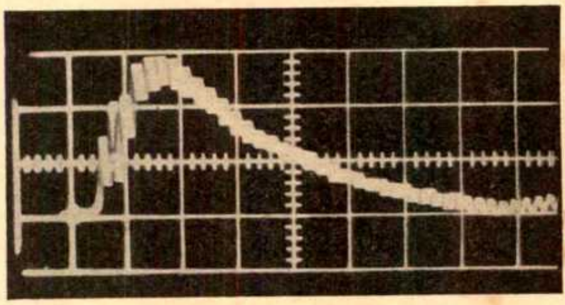

Fig. 3. Radiation flux $F(t)$ from a pinched discharge. Time base, 20 µsec./large division, and time constant, 5 µsec. (Note the superimposed oscillations at 230 kc./s. due to a mechanical resonance)

was a block of ceramic) were 1·0 cm. × 0·5 cm. × 0·125 cm. with radiation falling on the 1·0 cm. × 0·5 cm. face, and a resistance of R = 100 MΩ gave an RC time constant of 0·5 sec. (that is, $\gg\tau_2$). The oscillation at 230 kc./s. seen in Fig. 3 corresponds to a resonant mode of the 1 cm. length of the ferroelectric and, in this case, limits the high-frequency time constant to about 5 μsec. Since equation (2) is independent of the dimensions of the unit provided that the conditions on equation (1) hold, the size of the ferroelectric may be reduced so that resonant frequencies may be increased to give time constants of better than 1 μsec. When ceramic barium titanate is used, other electrode arrangements are possible, for example, electrodes on the edges hold some advantages[3]. These results will be published in detail elsewhere.

This type of detector, besides having a fast response, is very simple to use and manufacture, since it requires no complicated current supply and there is negligible Joule heating. Outputs proportional to both $F(t)$ and $\int^{t} F(\tau)d\tau$ can be obtained. Since the ferroelectric is also piezoelectric, care has to be taken to avoid acoustic pick-up (by placing in vacuum), but it should be a useful instrument where large, short-duration energy fluxes are available, in particular, since (due to the surface black) it has a uniform spectral response, for measurement of radiation losses in controlled thermonuclear research, or in measurement of radiation from explosions, etc.

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An Anisotropy Effect in Diffusion under Electric Field

TRACER and self-diffusion investigations under electric field of Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺ ions in fused alkali nitrates[1], as also in aqueous-agar gel media[2], revealed an asymmetry in their spatial distribution following migration: corresponding data for diffusion in the absence of field showed no asymmetry. Present initially as a narrow zone, 2–5 mm. wide, at the origin, the band migrates 30–50 cm. in the field direction, depending on the intensity and the duration of the applied field; the actual displacement of the centre is a measure of the ionic mobility. A plot of the ion concentration as a function of the distance from origin is sensibly Gaussian with a pronounced skew, the arm in the field direction being more precipitous (Fig. 1).

The asymmetric spread implies an anisotropy effect in the Brownian displacements, and hence, unequal diffusion-rates D_1 and D_2 in and against the field direction, the values being in order of magnitude:

$$D_2 > D_1 > D_0$$

where D_0 corresponds to symmetric diffusion in the absence of field. The relative difference in the diffusion coefficients $(D_2 - D_1)/D_0$ depends on the nature of the ion and its atmosphere. This increases with the intensity and the duration of the field applied and decreases with rise of temperature.

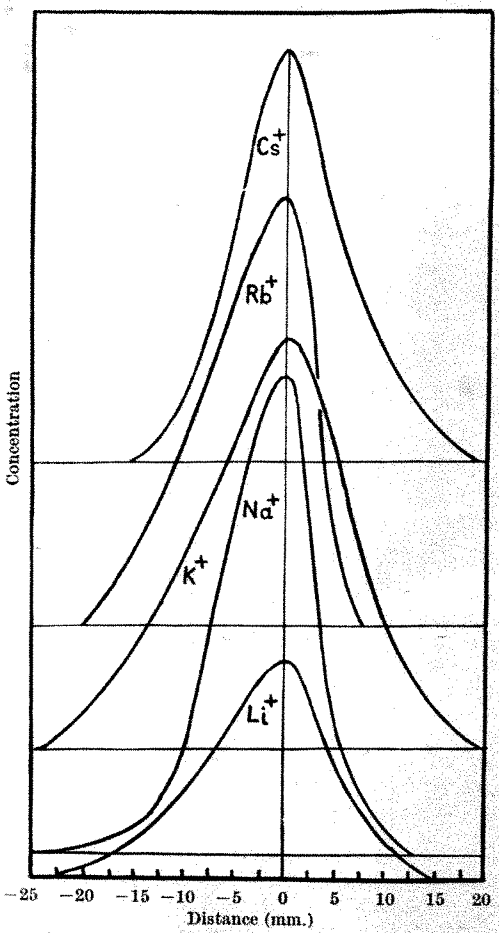

Fig. 1. Concentration distribution of alkali ions following diffusion in fused sodium nitrate at 350° under electric field of 7–10 V./cm., replotted with the zero of the concentration axis displaced to avoid overlapping

The theory of electrodiffusion due to Simnad[3], Lamm[4], Mysels[5,6] and Kauman[7] anticipates an accelerated diffusion, given by:

$$D_e = D_0 + U^2E^2/2k$$

where U is the product of ionic mobility and charge, E the intensity of the field and k a rate constant. The finding that D_0 the diffusion coefficient in the absence of field is invariably less than either D_1 or D_2 is in agreement with this deduction. The theory, however, applies mainly to electrodiffusion of a 'front' of a column of uniform initial concentration where Brownian displacements in a direction reverse to the field are indistinguishable, being behind the 'front'. This is unlike the present work, where the spread of the initial 'zone' is measured in both directions and found to be unequal. This necessitates a further correction, ± δ to the electrodiffusion term D_e, operative in the two directions, namely:

$$D_1 = D_e - \delta$$
$$D_2 = D_e + \delta$$

Though Mysels has considered the case of diffusion from a plane surface[6], the treatment does not include the asymmetry effect reported here.

A simple interpretation of the correction ± δ suggests unequal relaxation times for the completion of the atmosphere around the ion, diffusing in and against the field direction, out of the ionic residua, and that the proportion of these residua is different for ions of same sign but diffusing in opposite directions with respect to the field. It is significant that the asymmetry is reversible with the field direction, and is absent under alternating fields[2]. The idea of unequal time-lags for an ion of a given sign in occupying 'holes' available in and against the field direction is distinct from the difference in the 'free-state' average lives of ions of opposite signs implied in earlier theory.

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Yield Stress exerted on a Body immersed in a Bingham Fluid

Boardman and Whitmore[1] have described experiments in which the yield stress in a suspension is measured from the force exerted on a rectangular body which is immersed in the suspension and which has come to rest. They find that the force on the body due to the yield stress depends on the orientation of the body, which it should not, and that this dependence can be expressed by assuming a horizontal-face yield stress about five times the vertical-face yield stress.

From my experience of the flow of suspensions[2,3] I would assume that the boundary over which the yield stress acts is not the surface of the body, but that there is a large quantity of 'solid' suspension above and below the rectangular body acting as part of the body, and that the yield stress is operative over the surface of this much greater area. The reason that this extra 'solid' part of the immersed body exists is probably because the suspension has both a dynamic and a static yield value[2,3]. If the static yield value exceeds the dynamic yield value (with my suspensions of titanium oxide in paraffin it was about 50 per cent greater) and as some of the suspension above and below the rectangular body could be imagined to be brought into action only by shear forces exerted by flowing suspension, then it is clear that a certain amount of the suspension will remain 'solid' in contact with the body.

From the results of Boardman and Whitmore it is apparent that the effective area of the 'solid' suspension above the immersed body is about five times the area in which it is in contact. It can also be assumed that the dynamic yield value is about 29 dynes cm.$^{-2}$, the value found for vertical faces. This can be expected to be less than the value found by assuming a Bingham relationship, owing to the curvature of the shearing-stress/rate of shear curve near the rate of shear axis[3].

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LIKE Dr. Rae we too thought that the shear was probably taking place over an envelope of fluid surrounding the immersed body and we carried out some simple visualization experiments in order to detect the shell[1]. The indications were that although an appreciable decay in the rate of shearing occurred near the body there was no sharp envelope. This may have been because our static yield stresses were always less than the dynamic values, even when allowance had been made for curvature of the shearing stress/rate of shear curve near the rate of shear axis[2].

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METALLURGY

Growth of Aluminium Whiskers by Vapour Condensation

WHISKERS of volatile metals such as zinc[1,2] and cadmium[2,3] have been grown by vapour condensation methods; but whiskers of other metals have mostly been produced by chemical reduction[4,5]. Aluminium presents particular problems because its compounds are resistant to reduction and because the vapour pressure at its melting point is very small (by extrapolation $1{\cdot}0 \times 10^{-43}$ mm. mercury). At higher temperatures where there is an appreciable vapour pressure the metal is extremely reactive, and even contact with alumina leads to the formation of a sub-oxide. The work reported here shows that it is nevertheless possible to produce aluminium whiskers by a vapour condensation method using high supersaturations.

A growth-chamber of recrystallized alumina, consisting of a 2-c.c. cylindrical crucible inverted on a dish, was contained in a graphite susceptor and supported inside a demountable silica envelope. In the chamber was placed a 0·3-gm. pellet of zone-refined aluminium (resistivity ratio $R_{293^\circ\text{K.}}/R_{4{\cdot}2^\circ\text{K.}} > 11{,}000$); the envelope was evacuated and pumped continuously. High-frequency heating was applied to the susceptor from a work-coil outside the envelope, thus maintaining the lower part of the growth chamber at a temperature of about 1,250° C. for 1 hr. At equilibrium there was a vertical temperature gradient of about 600° C./cm. in the chamber. Although whiskers have been grown at argon pres-

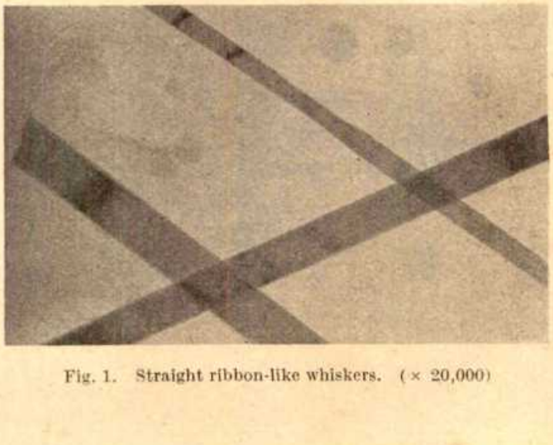

Fig. 1. Straight ribbon-like whiskers. (× 20,000)

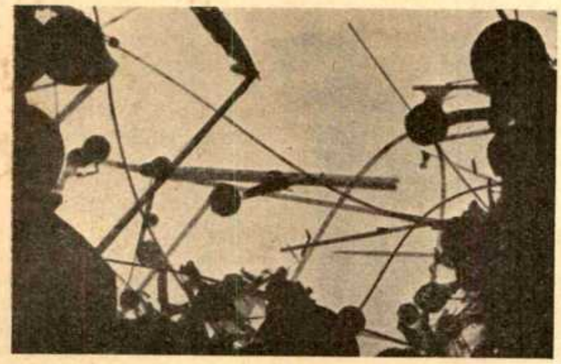

Fig. 2. Coagulated group of whiskers, many with attached balls. (× 4,000)

sures of about 10^{-1} mm. mercury, uncontaminated whiskers have so far been grown only when an evacuated sealed growth-chamber was used. This result led to the particular design of chamber adopted. When molten, the aluminium itself acts as a liquid seal between the crucible rim and dish; it seems likely that the first material evaporated getters any residual gases in the chamber.

At a position where the temperature was favourable, whiskers and ferns grew on the wall of the crucible. Whiskers 300μ in length and up to 20μ in diameter have been found, but many are sufficiently thin to be transparent in the electron microscope (Fig. 1); electron diffraction has shown that they are single crystals.

Each whisker grows with a ball at its tip (Fig. 2), and these balls are believed to be an essential feature of the growth process. Branched whiskers with many balls are quite common, but these tend to be brittle; straight whiskers are very elastic. The whiskers necessarily grow under conditions of extreme supersaturation; the growth mechanism and their properties are being studied and detailed results will be reported later.

I thank J. H. Burrow of the University of Bristol for constructing the silica-ware, and G. Blankenburgs for operating the electron microscope.

Note added in proof. It has just been found that other workers[6] have mentioned the growth of aluminium whiskers by vapour condensation at low pressures in a field emission tube.

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CRYSTALLOGRAPHY

Epitaxial Growth of Condensed Aromatic Polycyclic Compounds

The epitaxial growth of thin crystalline films vacuum evaporated on a surface of single crystal is a very common phenomenon nowadays. However, the materials used so far have been mostly restricted only to metals or inorganic compounds. Recently, being concerned with the semiconductor behaviour, vacuum-evaporated films of various kinds of condensed aromatic polycyclic compounds have been often dealt with by many investigators[1]. In the course of electron microscopy and diffraction study of vacuum-evaporated films of those organic materials including metal phthalocyanine, anthanthrone, indanthrone, flavanthrone, pyranthrone, violanthrone and iso-violanthrone, we have found that the epitaxial growth also preferably occurred when those materials were condensed on to a cleavage surface of muscovite crystal, the temperature of which had been raised to an appropriate extent.

In the work reported here, about 2 mgm. of each dye-stuff, which had been originally very fine powder, was evaporated vertically on to a fresh cleavage surface of muscovite (1 in.^{2}) very slowly from a small crucible of fused quartz heated by an electrical furnace in an evaporator, the vacuum of which was about 7×10^{-5} mm. mercury. The substrate crystal had been preheated up to the temperature just below the sublimation point of each compound, which was mostly in a range of 300–400° C. At much lower temperature, the epitaxial growth seemed not to be preferred and the films were composed of small particles, the size of which was about 300 Å. or much less in diameter, quite similar to that condensed on a glass surface. The film thus prepared could be easily detached from the substrate crystal and floated on a clean surface of pure water without any apparent destruction of the film structure as a whole. The specimen for electron microscopy and diffraction study was prepared by placing the floating film on a specimen grid covered with a supporting film of vacuum-evaporated carbon in the ordinary way.

Figs. 1 and 2 show the electron micrographs of condensed films of platinum phthalocyanine and copper phthalocyanine, respectively, together with the corresponding electron diffraction patterns. The film structure of both compounds is not uniform

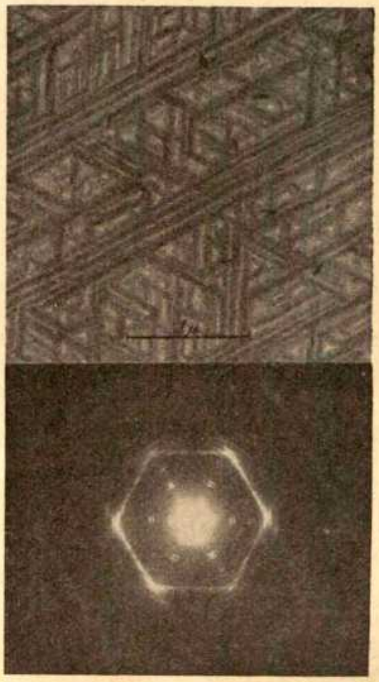

Fig. 1. Vacuum-evaporated film of platinum phthalocyanine on muscovite

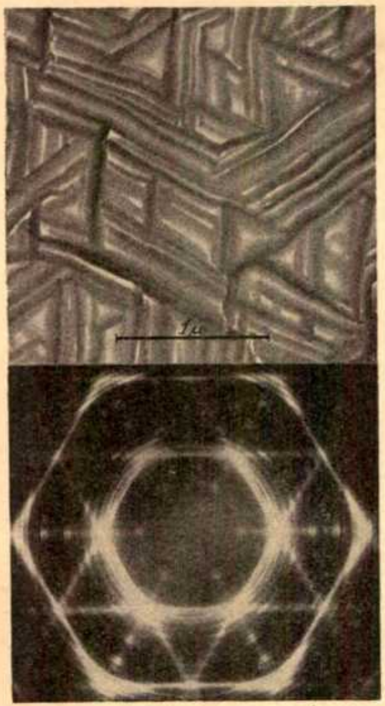

Fig. 2. Vacuum-evaporated film of copper phthalocyanine on muscovite

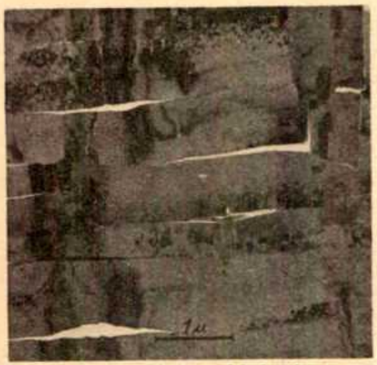

Fig. 3. Vacuum-evaporated film of indanthrone on muscovite

but takes the form of labyrinthine networks composed of long and narrow crystal lamellæ of varying lengths and widths. In the case of platinum phthalocyanine, the elemental crystal strip is rather straight, and at several places very slender ones run parallel in the same direction, while in the case of copper phthalocyanine, it is somewhat twisted. The crossing angle of the elemental crystal strip is mainly 120° in both cases; but in some cases it is locally about 75° for the film of copper phthalocyanine. This can be also confirmed by each electron diffraction pattern, which also evidently indicates the existence of preferred orientation of these organic crystal films.

The diffraction patterns are quite similar to each other, being composed of the three superimposed identical fibre patterns, the meridians of which meet one another at an angle of 120°. The fibre periods along the meridian are 3·77 Å. and 3·79 Å. for copper and platinum phthalocyanines respectively. Furthermore, two series of diffraction spots co-exist on each equator, suggesting that the original films both contain two sorts of lamellæ which differ in crystal habit though their longer axes run in parallel. This is similar to that of the stable β-phase of copper phthalocyanine assuming the 'raft structure' as has been reported in previous papers[2]. These series of reflexions on the equator correspond to the interplanar spacings of 12·99 Å. and 12·09 Å. for copper phthalocyanine and 13·13 Å. and 11·97 Å. for platinum phthalocyanine. Analysis of overall diffraction spots for copper phthalocyanine has revealed that the crystal film assumes the unstable α-phase of its polymorphic modifications. The resemblance of the diffraction pattern to platinum phthalocyanine suggests the similar phase, the existence of which has not so far been reported.

So far as the relative lattice orientation of both films to the substrate crystal is concerned, the equator of one of the elemental fibre patterns is fixed to be in parallel to the *b*-axis of muscovite.

Other condensed aromatic polycyclic compounds also assume preferred orientation on the surface of muscovite when prepared by the same method. Most of them assume one-directional orientation, except zinc phthalocyanine, which shows two-directional orientation. As for the shape of elemental crystal composing the film, pyranthrone, flavanthrone and indanthrone give rise to straight, long and narrow crystals, whereas violanthrone and iso-violanthrone make somewhat wavily twisted ones. Nickel, tin and zinc phthalocyanines contain very small crystal flakes having varying contrasts and indeterminate forms. Particularly in the case of indanthrone, elemental long and narrow crystals become very large and closely contact continuously to each other, showing a tendency to make up a thin single crystalline film as a whole, the electron micrograph of which is reproduced in Fig. 3.

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GEOLOGY

Recent Dolomite in the Persian Gulf

A STUDY of recent carbonates in the Qatar area, Persian Gulf, is at present being made by the Koninklijke/Shell Exploratie en Produktie Laboratorium in the Netherlands, in association with V. C. Illing and Partners of London. This work has the work of Houbolt[1] as a background, and is concentrating particularly on the near-shore and intertidal sediments.

In lagoons around the Qatar Peninsula, which are especially numerous on the west side, salinities rise to 45–55 parts per thousand even when the tidal range is 3 ft. or more. On the lower parts of the tidal flats, carbonate sediment generally consists of about 80 per cent aragonite. The proportion of aragonite rises to 95 per cent or more on some of the algal flats just below the level of diurnal high-tide, where salinities of nearly 100 parts per thousand were recorded. This high concentration of aragonite, evidently the product of calcium carbonate precipitation out of

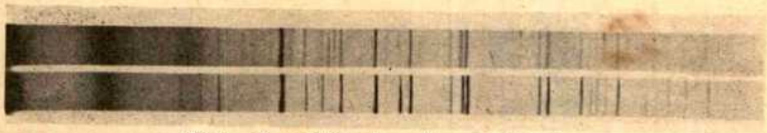

Fig. 1. X-ray diffraction patterns of dolomites

Sample	Upper line Recent dolomite from tidal flat, Qatar, with traces gypsum, quartz	Lower line Dolomite No. 88 U.S. Bureau of Standards
Cell dimensions of dolomite (Å.) a_0	4·82	4·807
Cell dimensions of dolomite (Å.) a_0	6·11	16·004
Approximate molecular composition	$Ca_{0·54}Mg_{0·46}CO_3$	$Ca_{0·50}Mg_{0·50}CO_3$

Photographs (taken on Guinier–de Wolff focusing camera II using copper radiation) and calculations by D. H. Porrenga, Koninklijke/Shell Exploratie en Produktie Laboratorium

sea-water left in and on the tidal flat between tides, marks the beginning of the evaporitic environment.

Sediment on the upper parts of the broad tidal flats, between diurnal and spring or storm high tides, has been found to consist largely of dolomite and gypsum. In the lowest part of this zone, aragonite in the carbonate fraction progressively decreases landwards and dolomite appears and proportionately increases to about 60 per cent, when the sediment may contain several per cent of gypsum. Higher up, aragonite and calcite decrease sharply, to such an extent that the sediment may consist virtually of dolomite and gypsum. Salinity of the water in this sediment is of the order of 275 parts per thousand. In the highest part of the intertidal zone dolomite becomes subordinate and the sediment consists very largely of gypsum. All these tidal flat sediments contain, in addition, a variable proportion of wind-blown material (largely quartz silt) and halite (almost certainly dissolved again with each high tide).

It is apparent that the dolomite and gypsum have both been derived from sea-water left behind in the sediment on the upper part of the tidal flats and concentrated by evaporation during the time between spring or storm high tides, although it has not yet been established to what extent the dolomite is primary and to what extent it is a penecontemporaneous replacement of pre-existing carbonate. Laboratory experiments over many years have consistently failed to produce true dolomite by evaporation of sea-water at atmospheric pressure, so that any occurrence of recent dolomite is of considerable interest. What is of particular importance in this case is not only that it is found associated with evaporitic minerals at the end of a normal and apparently often-repeated marine carbonate cycle, but also that the scale on which it occurs is obviously of geological significance.

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Origin of Ignimbrites

F. J. FITCH[1] has criticized a new hypothesis proposed by me[2] on the origin of ignimbrites of Pliocene to Pleistocene Age, from the North Island of New Zealand. The new petrogenetic concept is based on the presence of two contrasting glasses and their textural relationship, which suggest liquid immiscibility. It is emphasized that the presence of the two glasses can be observed in fresh ignimbrites only because both glasses undergo early selective crystallization. It is because of this crystallization of the glassy constituents that liquid immiscibility has not been recognized earlier. When both glasses have crystallized the ignimbrite contains no glass at all, although two glasses were present immediately on solidification of the rock. Many ignimbrites, particularly ancient ones, therefore, do not afford any evidence concerning the presence of two contrasting glasses and their textural relationship.

Fitch[1] argues the problem of two contrasting glasses on the evidence he obtained in studying Ordovician ignimbrites from North Wales, though he discusses also ignimbrites from New Zealand. His arguments to a great extent appear to be based on insufficient petrographic evidence. This is clearly demonstrated by his statement " . . . only rare quartz phenocrysts preserved within xenolithic obsidian lumps show that intricate resorption outline regarded by Steiner as evidence for gentle flow . . .". The fact is that in New Zealand ignimbrites quartz phenocrysts are commonly abundant, and that in some ignimbrites they are absent. In numerous ignimbrite sheets they occur in the groundmass made up entirely of glass shards and mesostasis. No obsidian xenoliths are present in these rocks. It is these quartz phenocrysts occurring in the groundmass that have been taken by me as supporting evidence of emplacement by flow.

In fresh pulverulites the groundmass consists of glass shards or stream-lined shreds and the intervening glassy mesostasis of different refractive index and colour. One should not confuse these two contrasting glassy phases with the multi-coloured shards and shreds which are present in many ignimbrites and have the same refractive index. If angular or rounded xenoliths of various volcanic and sedimentary rocks are present they show invariably sharp boundaries, and no re-melting or resorption is noticeable. Occasionally, pumiceous xenoliths, always well rounded and more or less flattened, can be observed. These pumiceous xenoliths also show no re-melting or resorption, but they are usually partly or completely crystallized. Thus, so far as the pulverulites are concerned, there is obviously no need for invoking xenolithic origin for any of the two contrasting glasses.

So far as fresh lenticulites are concerned, it is important to distinguish between the rounded pumiceous xenoliths and the common lenticles and shreds, both having characteristically ramifying ends. These ramifying lenticles and shreds are not accidental but primary constituents of these rocks. The lenticles, shreds and the shards, all made up of the same glass, are enclosed by a second glassy phase, that is, the mesostasis. They make up the bulk of the rock. The ramifying lenticles and the larger shreds show gradational separation into glass shards and mesostasis, and a chilled globular emulsion can commonly be seen in the lenticles. The emulsion consists of small immiscible globules of one glass suspended in a glassy base of different refractive index and colour, and should not be confused with the granular mesostasis. The globular emulsion forms also inclusions in plagioclase phenocrysts, and evidently indicates that the magmatic liquid, caught up by the growing crystal, separated, like the bulk of the residual liquid, into two immiscible liquids.

The occurrence of immiscible globules is considered to be a significant evidence of liquid immiscibility. The occurrence of such a chilled emulsion has been recently described by Hjelmquist[3].

No xenoliths identical with or even slightly resembling the mesostasis have been noticed in any of the ignimbrites from New Zealand studied by me, although I examined several hundred ignimbrite samples from numerous localities covering the whole Taupo volcanic zone. There is no evidence that the ignimbrite magma resorbed xenoliths of older ignimbrites and tuffs. Thus, Fitch's criticism opposing the operation of liquid immiscibility is in disagreement with the petrographic evidence afforded by fresh lenticulites and ignimbrites in general. Moreover, to ascribe the origin of a common volcanic rock type, such as ignimbrite, to decomposition of accidental xenoliths, which of necessity would be required to form the bulk of the rock, appears to be highly improbable, if not impossible.

It is significant that none of the countless witnessed nuée ardente eruptions has ever produced an ignimbrite and that the deposits produced by witnessed nuée ardente eruptions differ essentially from fresh ignimbrites. The inconsistencies of the nuée ardente hypothesis[2] suggest that the mode of emplacement of ignimbrites is of fundamentally different type from that of the nuée ardente. On the other hand, the operation of liquid immiscibility explains satisfactorily the textural features of the ignimbrites, and their mode and mechanism of emplacement.

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[1] Fitch, F. J., *Nature*, **190**, 995 (1961).

[2] Steiner, A., *N.Z. Geol. Surv. Bull.*, n.s., 68 (1960).

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THE hypothesis that ignimbrite sheets are formed as a result of the eruption of a special kind of acid magma, which develops two immiscible liquid fractions on eruption, is strongly defended by Steiner. He rejects the objections raised in my criticism of his work[1], and repeats much of his original argument[2]. Steiner's hypothesis rests on a fundamentally different interpretation of the textural relationships seen in ignimbrites from that made by the majority of petrologists. Having proved that two contrasting glasses are present as recognizable phases in one ignimbrite, Steiner then assumes that the detailed textural pattern of all ignimbrites can be explained on the basis of this observation. Statistically convincing proof of the assertion that two contrasting phases are present in all ignimbrites is not given, and the volume difficulties inherent in his hypothesis are not considered.

The vast majority of the phenocrysts seen in ignimbrites are broken and abraded, whatever their composition, and Steiner's denial of this fact must throw doubt on his whole philosophy of petrographic interpretation. Without pursuing these differences, however, it is obvious that valid petrological hypothesis cannot be based on petrographic evidence alone, and, if they are to be accepted, Steiner's views on the origin of ignimbrites must withstand a field examination of the rocks. Again we find that the majority of field geologists have arrived at conclusions, regarding the origin of ignimbrites, fundamentally different from those suggested by Steiner's petrographic interpretation. The ash-flow hypothesis is now almost universally accepted on the basis of overwhelming field evidence, amply catalogued by such workers as Enlows[3], Martin[4], Ross and Smith[5], and many others.

One fact alone will illustrate the difference between Steiner's interpretation and those of other petrologists. He asserts that the lenticles and shreds having characteristically ramifying ends, common in many ignimbrite specimens, have a different origin from the rounded pumiceous inclusions seen in other examples. Though this may be so in some rare instances, field evidence indicates that in the vast majority of cases the lenticles are undoubtedly flattened and welded pumice lumps identical in origin to the rounded unwelded pumice lumps present in the unwelded parts of the same ignimbrite sheet. The transition from rounded to flattened pumice lumps in the same sheet can be observed in the field, and to deny it must only reveal a failure to make a thorough field examination of the rocks investigated.

The field evidence undoubtedly supports the hypothesis of collapse and welding for the production of the internal structures and textures of ignimbrites (see the references quoted by Ross and Smith[5]). In the face of the field evidence it is just not possible to accept Steiner's interpretation of the evidence available. The possible alternative origins for the contrasting glass phases seen in some ignimbrites, put forward in my criticism of Steiner, may not be applicable to all cases, but they suggest a more likely explanation of the facts than does his hypothesis. In this connexion, work on the presence of contrasting glass fractions in ignimbrites by Williams[6] is of interest.

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Relationships of Fauna and Substratum in the Palæoecology of the Chalk and the Chalk Rock

AN investigation of the palæoecology of the Chalk Rock of south-central England (Oxfordshire–Hertfordshire) has led me to believe that the most important difference between the Chalk Rock and normal Chalk environments was in the nature of the substratum.

The normal Chalk environment was presumably of soft-bottom type, with the bottom consisting of unlithified chalk-mud. The Chalk Rock, in contrast, marks hard-bottom conditions. Its general lithological features in the area examined, where a single main rock band is typical, are related to three occurrences: (*a*) penecontemporaneous lithification of chalk sediment; (*b*) erosion, with production of irregular surfaces and of pebbles of lithified sediment (so-called 'nodules'); (*c*) phosphatic and glauconitic impregnation of exposed erosion surfaces and pebbles, presumably hardening them further. Lithification of sediment before erosion is implied by its ability to form pebbles, and by the occurrence of reworked fossils of which, for example, molluscan examples include sharp internal moulds with the shell lost. An encrusting fauna (for example, serpulid and spirorbid worms, cyclostome and cheilostome Bryozoa, *Spondylus latus* (J. Sowerby), *Ostrea* sp.) found on erosional surfaces and pebbles proves their status as actual features of the bottom. At maximum departure from normal Chalk conditions, the bottom was a hard current-scoured surface, with vertical relief of up to 15 cm. or more due to small-scale erosional pocketing. Later, erosional hollows were filled with new sediment, resulting in close association of both hard and soft sub-strata.

The effect of a change to hard-bottom conditions on members of the normal Chalk benthos will have varied according to their habits in relation to substratum Chalk benthos generally comprises forms adapted to two distinct types of sub-stratum, in summary as follows:

Group A. Soft-substratum elements, living on or in the general chalk sediment: (*a*) surface group: (i) active, including asteroids, some echinoids (for example, *Cidaris*), some lamellibranchs (for example, *Plagiostoma, Syncyclonema*), most gastropods, most non-planktonic foraminifera, plus (ii) sedentary: rhizophytous or lophophytous sponges (for example, *Leptophragma, Guettardiscyphia, Porochonia, Oncolpia, Ventriculites; Rossella*), some other forms, for example, *Spondylus spinosus* (J. Sowerby); (*b*) sub-surface group: burrowing echinoids (for example, *Micraster, Holaster*), some lamellibranchs (for example, *Nucula, Nuculana*), *Dentalium*, some gastropods (‘*Natica*’, *Aporrhais*).

Group B. Hard-substratum elements, cemented or attached in other ways to hard skeletal bodies (for example, echinoid tests, brachiopod or lamellibranch valves, calcareous or siliceous sponge frameworks): (i) cemented: adherent Foraminifera (for example, *Bullopora*), basiphytous sponges (for example, *Farrea, Eurete*), corals (for example, *Parasmilia*), encrusting Bryozoa (for example, *Berenicea*), serpulid and spirorbid Annelida, *Crania*, cemented lamellibranchs (for example, *Ostrea, Dimyodon, Spondylus latus*); and (ii) attached organically: articulate brachiopods (for example, *Cretirhynchia, Gibbithyris*), byssally attached lamellibranchs (for example, *Arca, Septifer*).

Benthos from the normal Chalk consists typically of members of both main faunal facies. Replacement of the normal soft bottom by an altogether hard one will presumably have led to disappearance of most of the soft-bottom benthos, and certainly of sedentary and burrowing forms which could only inhabit soft sediment. But hard-substratum elements, in contrast, could have spread to the bottom itself; in practice, its colonization by cementing forms at least is confirmed by their occurrence on erosional surfaces and pebbles. Abundance of soft-bottom elements in the nodular top of the rock is likely to mark their reappearance during filling of erosional hollows by new sediment. Rhizophytous sponges, the scaphopod *Dentalium*, and probably the echinoid *Micraster*, are examples which can only have lived when such sediment was available.

This communication is an outline of part of the results of an investigation carried out at University College, London, during 1951–54, when I held a research studentship from the Nature Conservancy, under supervision by Dr. T. Barnard. I thank Prof. S. E. Hollingworth for providing facilities.

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RADIATION CHEMISTRY

Polymer Production in the Radiolysis of Liquid Cyclohexane

THE radiolysis of cyclohexane gives cyclohexene and bicyclohexyl as the major liquid products[1], the G values of which decrease with dose at low dose-rates or high doses[2]. The formation of a ‘polymer’ (defined here as a substance containing three or more cyclohexane units) has been reported at a low dose-rate and high dose[3], but several other investigations, carried out over a wide range of dose-rate and dose conditions, indicate that no products above C_{12} hydrocarbons are formed[1,3–5].

We have irradiated liquid cyclohexane at ambient temperature (about 20° C.) with cobalt-60γ at a dose-rate of $1{\cdot}2 \times 10^{17}$ eV./min./gm. After being analysed for cyclohexene and bicyclohexyl (by gas liquid chromatography), samples were evaporated to constant weight in a vacuum desiccator using a water pump. This procedure removed cyclohexane and the more volatile products and the residue contained less than 2 per cent by weight of bicyclohexyl. The yields of the products together with the sum of the three yields (total) are plotted against absorbed dose in Fig. 1. The experimental points are omitted for the sake of clarity. The variation of the overall $G(C_6H_{12} \longrightarrow \text{polymer})$ with dose is shown in Fig. 2.

The samples of polymer (usually 50–100 mgm.) were redissolved in 1 ml. cyclohexane and analysed on a 1 m. silicone grease column at 200° C.: these gave two major peaks and at least five minor ones which, from their elution times, were assumed to be

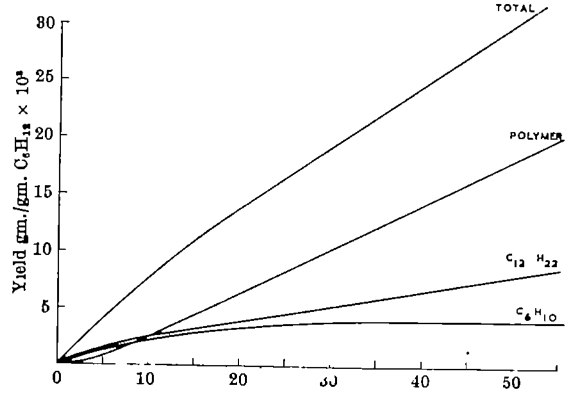

Fig. 1. Product yields versus dose

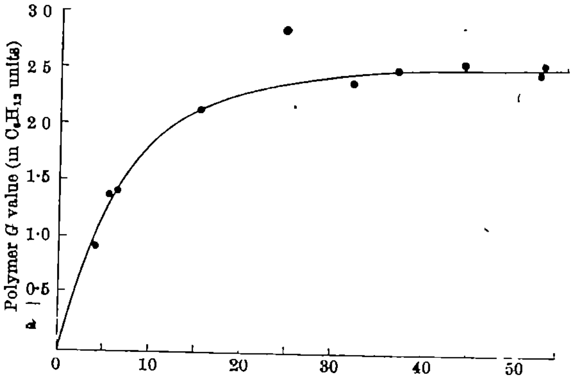

Fig. 2. Polymer G value versus dose

C_{18} hydrocarbons. The variation with dose of the percentage by weight of the C_{18} fraction in the polymer is shown in Table 1. Analysis on the same column at 324° C. gave a diffuse shoulder where C_{24} hydrocarbons eluted. A mass spectrum of the polymer formed at the highest dose indicated the presence of hydrocarbons up to C_{30} (the maximum of the mass range scanned) and the cracking pattern was characteristic of cyclohexyl ring compounds. Although the polymer seemed quite stable in cyclohexane solution, if left exposed to the air for several days, it did not completely redissolve in cyclohexane, giving a white precipitate (cf. ref. 3). These observations indicate that the polymer formed on radiolysis of liquid cyclohexane is a secondary product consisting of a complex mixture of six membered ring hydrocarbons, the molecular weight of which increases with dose.

Table 1. FRACTION OF C_{18} IN POLYMER

Dose eV./gm. × 10^{-20}	Percentage by weight of C_{18} in polymer
5·4	23·7
32·1	14·2
44·4	9·5
52·7	10·8

The following conclusions can be drawn from Fig. 1. The G values of cyclohexene and bicyclohexyl decrease with dose up to an 'equilibrium' dose beyond which the net $G(C_6H_{10}) = 0$ and $G(C_{12}H_{22})$ maintains a constant value. G(polymer) increases from zero at zero dose (Fig. 2) to a constant value. These facts can be explained by the reaction scheme

$$C_6H_{11} + C_6H_{11} \longrightarrow C_6H_{10} + C_6H_{12} \quad (1)$$

$$C_6H_{11} + C_6H_{11} \longrightarrow C_{12}H_{22} \quad (2)$$

$$C_6H_{11} + C_6H_{12} \longrightarrow C_6H_{12} + C_6H_{11} \quad (3)$$

$$C_6H_{11} + C_6H_{10} \longrightarrow R_1 \quad (4)$$

$$R_1 \quad + C_6H_{10} \longrightarrow R_2 \quad (5)$$

Reactions (1) and (2) have been postulated by Dewhurst[1], reaction (3) should be important at this dose-rate from the work of Schuler and Muccini[6], and reaction (4) has been suggested by Dyne[2]. Further addition of radical R_1 to a cyclohexene molecule has been postulated to account for the C_{30} hydrocarbons found in the polymer. It is envisaged that the polymer is formed by ultimate disproportionation and combination of radicals such as R_1 and R_2. These radicals may also react in a manner analogous to reaction (3), but not exclusively so, since this would form a fully saturated polymer, which does not seem to be the case. It should be noted that reactions of radicals such as R_1 and R_2 may give rise to all secondary products, not just those found in the polymer. Thus cyclohexyl cyclohexene, characterized as a secondary product by Dyne[7], could arise from disproportionation of two R_1 radicals.

Irradiations with a pulsed X-ray beam of instantaneous dose-rate = 4·2 × 10^{20} eV./min./gm. gave $G(C_6H_{10})$ and $G(C_{12}H_{22})$ both independent of dose over the limited range studied (up to 5 × 10^{20} eV./gm.) and no polymer formation. It can be shown kinetically that this is due to the preponderance of reactions (1) and (2) over reactions (3) and (4), in contrast to the low dose-rate irradiation. This is in agreement with the dose-rate effect noted in refs. 2 and 6.

Initial values of $G(C_6H_{10})$ and $G(C_{12}H_{22})$[7] give a composite value of G = 7·2 expressed in cyclohexane units, whereas the straight-line portion of the total yield plot in Fig. 1 gives G = 3·9. Thus there is a decrease of about a half in the total decomposition of cyclohexane (based on the yields of these three products) on going from zero dose to about 1·5 × 10^{21} eV./gm. From the shapes of the curves in Fig. 1 it seems likely that cyclohexene is the product responsible for this decrease. It cannot be attributed to a scavenging effect of cyclohexene since we have already taken account of this in the polymer yield. It could be explained as a quenching of excited cyclohexane molecules by cyclohexene, with very little chemical decomposition of the resulting cyclohexene molecule.

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Effect of γ-rays on Thymine in De-aerated Aqueous Solutions

IT is well known that X-irradiation of the pyrimidine bases in aqueous solutions in the presence of oxygen causes peroxidation at the position of the 4–5 double bond[1,2]. It has been established, however, that in the absence of oxygen this reaction does not take place, and that the destruction of the bases is considerably reduced. This communication is concerned with the identification of the products formed under these latter conditions. γ-Irradiated de-aerated solutions of thymine subjected to ion exchange column chromatography ('Dowex 50 H^+') showed two peaks, the second of which has been identified as unreacted thymine (Fig. 1*B*) and the first presumed to contain the products of radiolysis (Fig. 1*A*).

The fractions in section *A* were collected, lyophilized and subjected to descending paper chromatography. The solvent system consisted of propanol/water (10 : 3). Subsequent detection with ultraviolet light (2537 Å.) showed a single spot I (R_F = 0·46) which on elution had an ultra-violet absorption spectrum (at different *p*H's) identical with that of hydroxymethyluracil (HMU)[3] (Table 1). When mixed with an authentic sample of HMU the product is inseparable by paper chromatography in several solvents or by ion exchange column chromatography. It is therefore suggested that one of the products resulting from γ-irradiation of thymine in de-aerated aqueous solution is hydroxymethyluracil (I) G = 0·1 (Table 2).

Since addition of radicals to the 4–5 double bond would render the compounds non-ultra-violet absorbing, Fink's technique[4] was used to identify such products. The paper chromatogram was sprayed with 0·5 *N* sodium hydroxide, then with a solution of paradimethylaminobenzaldehyde, which reacts to form a coloured complex with dihydropyrimidine compounds. Four coloured spots were thus obtained on the chromatogram of which three have been

Table 1. ULTRA-VIOLET ABSORPTION RESULTS

	*p*H 1		*p*H 12	
	Maximum (mμ)	Minimum (mμ)	Maximum (mμ)	Minimum (mμ)
HMU	261	231	285	245
I	261	233	286	247

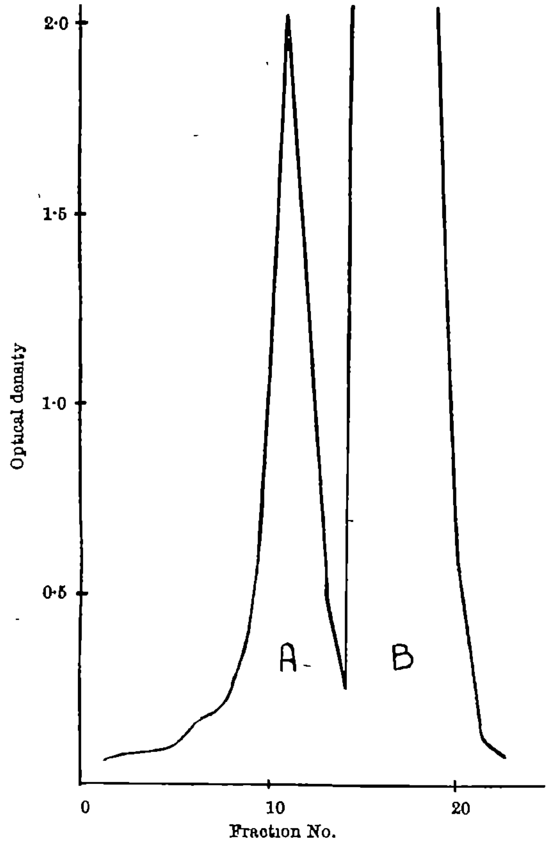


Fig. 1. Ion exchange chromatography of irradiated thymine solution 10^{-3} *M*, in water, 100 ml., irradiated in absence of oxygen at room temperature. Dose 250 kr. Chromatography on 'Dowex $50H^+$', 65 cm., elution with 0 5 *N* hydrochloric acid, at room temperature, fraction volume 3 5 ml./11 min., optical density at 260 mμ

positively identified as *cis* and *trans* thymine glycol (II and III) and dihydrothymine (IV) respectively.

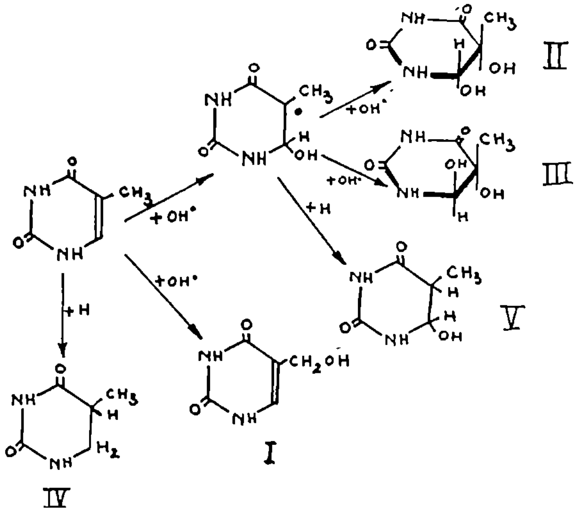
It is interesting to note that the spots of the two glycols showed a particular characteristic coloration which changes rapidly from yellow to pink and finally becomes blue. In general, most compounds of this type produce yellow spots. The chromatographic behaviour of II and III were identical with that of authentic *cis* and *trans* thymine glycol respectively (Table 2). (*Cis* thymine glycol was prepared by isomerization of the *trans* compound by heating at 100° C. for 4 hr.[5].)

Of the other two spots, which were of much weaker intensity, one was identified chromatographically with dihydrothymine. Under the conditions of this column separation this product was not completely separated from the unreacted thymine. This also occurred in control experiments on ion exchange separation of thymine and dihydrothymine.

Table 2. R_F Compounds on Paper Chromatograms

	n-Propanol/ water, 10 : 3	*n*-Butanol/ water, 86 : 13	Isopropanol/ water/conc. HCl, 65 : 18·3 : 16·7	Isobutyric acid/conc. NH_4OH/ water, 100 : 1·12 : 52	*n*-Butanol/ acetic acid/ water, 2 : 1 : 1
HMU	0 46	0·23	—	0·46	—
I	0 46	0·22	—	0·45	—
Glycol (*cis*)	0 44	0·20	0·72	—	0·45
Glycol (*trans*)	0 54	0·28	0 73	—	0·45
II	0·42	0·19	0·72	—	0 44
III	0·53	0·27	0·72	—	0 46
Dihydrothymine	0 68	—	—	—	0·67
IV	0 67	—	—	—	0·66
V	0 58	—	—	—	—
Thymine	0 70	0 47	—	0 73	—

The observed products can be explained by the following degradation of the thymine in de-aerated aqueous solutions (G = 0·65).



From the chromatographic behaviour (R_F = 0·58) it is suggested that the fourth non-ultra-violet absorbing product is V, but it has not yet been possible to synthesize this compound for actual comparison.

I thank Miss J. Conlay and Dr. R. Latarjet for their interest throughout this work, which has been supported by Euratom and by the Commissariat à l'Energie Atomique.

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CHEMISTRY

Determination of Stability Constants in Molten Alkali Metal Nitrate Solvents

Most determinations of stability constants in alkali metal nitrate solvents have concerned the halide complexes of silver[1]. Recently the cryoscopic[2], solubility[3], polarographic[4], galvanostatic[5], spectroscopic[6] and e.m.f.[7] techniques have been used for investigating the formation of the halide complexes of various metals (for example, cadmium) in these solvents. A silver–silver bromide electrode has been used to study the bromo-complexes of cadmium and lead[7]. An attempt[8] to use the silver–silver chloride electrode in a similar way to study chloro-complexes was not successful.

The measurement of e.m.f.'s of suitable electrochemical cells should be the most precise and widely applicable technique for the determination of stability constants in these systems. In many cases it is necessary and desirable to determine free metal ion concentrations. Pure metal electrodes cannot be used because of the strong oxidizing properties of the solvents, except in the cases of the noble metals. The high temperatures involved (usually > ~ 200° C.) restrict the use of amalgam electrodes to the low-melting point solvents[9].

Various possible metal-ion sensitive electrodes have been tested in this work. That, finally, found to be satisfactory was an electrode of the third kind, involving palladium metal (which was not oxidized by the solvent) and a melt saturated with the stable sparingly soluble palladium monoxide and the oxide of the metal ion to be studied (for example, CdO). The experimental details of the electrode will appear elsewhere.

In the dilute solutions investigated in this work changes of e.m.f. of the cell:

$$\text{Pd} \mid \text{PdO, CdO, Cd}^{2+} - \text{NaNO}_3 - \text{KNO}_3 \mid$$
$$\text{NaNO}_3 - \text{KNO}_3 - \text{Ag}^+ \mid \text{Ag}$$

when the concentration of Cd^{2+} is varied, will be given by:

$$\Delta E = \frac{RT}{2F}\,\Delta \ln\,[\text{Cd}^{2+}]$$

$$\text{because } [\text{Cd}^{2+}] = k[\text{Pd}^{2+}]$$

where $[Cd^{2+}]$ and $[Pd^{2+}]$ are concentrations, k is the ratio of the oxide solubility products and the dotted line represents a thin 'Pyrex' glass diaphragm. Plots of e.m.f. versus $\log_{10}[Cd^{2+}]$ in the $NaNO_3$–KNO_3 (1 mole : 1 mole) solvent at 254° C. are shown in Fig. 1. The electrode satisfied the usual criteria of reversibility. Although the plots of e.m.f. versus $\log_{10}[Cd^{2+}]$ were always straight lines the slopes varied from ~ 54 to ~ 71 mV. This variation was probably due to the conjugate equilibrium $2O^{2-}-4e_0 \rightleftharpoons O_2$, involving traces of adsorbed oxygen on the palladium electrode. In practice it was more convenient to do a three-point metal ion calibration before each titration than rigorously to exclude oxygen from the system. The validity of this procedure is evidenced by the reproducibility of $\log_{10}\alpha_0/\log_{10}[X]$ plots (see later) obtained by using electrodes with different metal ion concentration responses.

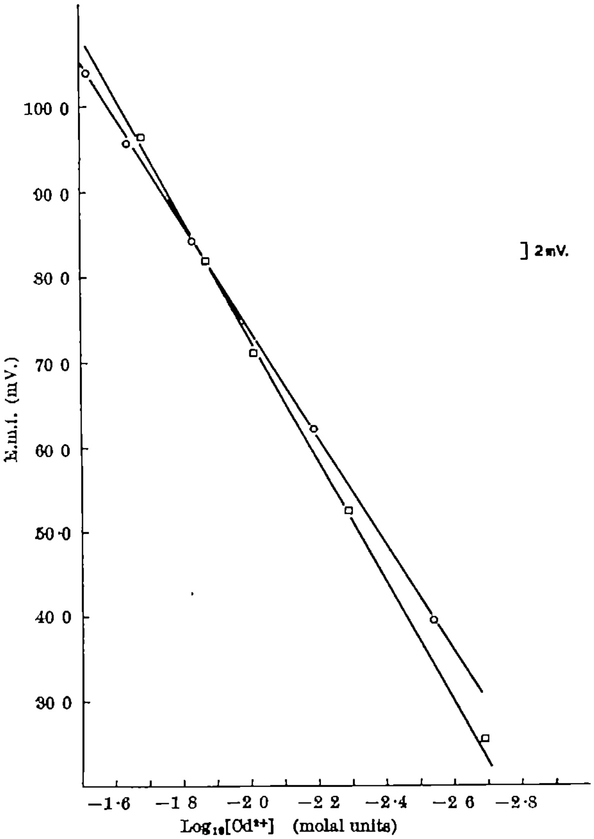

Fig. 1. E.m.f. versus $\log_{10}[Cd^{2+}]$. $NaNO_3$—KNO_3 (1 mole : 1 mole) solvent. T = 254° C. Run 37 ○; slope = 63·0 mV.; run 39 □; slope = 71·2 mV. (2·303 $RT/2F$ = 52·4 mV. at 254° C.)

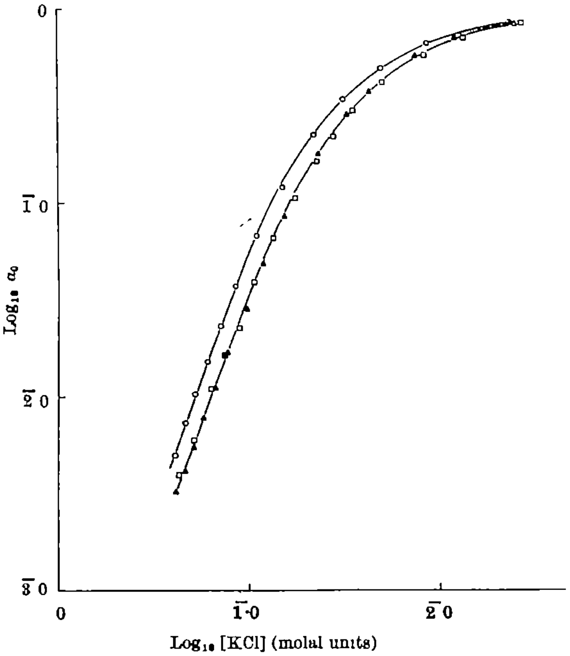

Fig. 2. $\log_{10}\alpha_0$ versus $\log_{10}$[KCl]. $NaNO_3$—KNO_3 (1 mole : 1 mole) solvent. T = 254° C. Run 49 ⊡, $[Cd(NO_3)_2]$ = 0·9892 × 10^{-2} mole kgm.$^{-1}$ ($\Delta E/\Delta \log_{10}[Cd^{2+}]$ = 58·0 mV.). Run 52 △, $[Cd(NO_3)_2]$ = 0 9896 × 10^{-2} mole kgm.$^{-1}$ ($\Delta E/\Delta \log_{10}[Cd^{2+}]$ = 54·8 mV.). Run 54 ○, $[Cd(NO_3)_2]$ = 1·961 × 10^{-2} mole kgm.$^{-1}$ ($\Delta E/\Delta \log_{10}[Cd^{2+}]$ = 57·8 mV.)

The data which are required to determine the stability constants of the chloro-complexes of cadmium in molten $NaNO_3$–KNO_3 at 254° C. have been obtained by measuring the variations of e.m.f. (to 0·1 mV.) during the titration of Cd^{2+} with Cl^- ions. Typical plots of $\log_{10}\alpha_0$ (where $\alpha_0 = [Cd^{2+}]/[Cd(NO_3)_2]$) versus $\log_{10}$[KCl] are shown in Fig. 2. A preliminary analysis by Leden's method[10] indicated that the stability constants were in agreement with the previously published data[5], which because of the limitations of the technique were necessarily less precise. These data will be further analysed using an electronic computer.

When the metal ion concentration was > ~ 2 × 10^{-3} mole kgm.$^{-1}$ the concentrations of free chloride ions were determined experimentally by using a silver–silver chloride electrode in the form of a coiled silver wire and a melt saturated with silver chloride. The high exchange current density of the silver–silver ion system in molten nitrate solvents[11] ensures the reversibility of the electrode down to low silver ion concentrations. The difficulties associated with the silver–silver electrode in the form of a coated wire[8] were not encountered. The rather large solubility product of silver chloride[12], however, restricts the applicability of this electrode.

Various complex forming mixtures are being studied and further details will be published later. I thank Dr. J. E. Prue for useful discussion and the

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Isotope Separation by Electrodiffusion

AN enhanced diffusion process occurs when an electric field is superimposed on a diffusion experiment which is being carried out with a system composed of chemically interacting electrolyte species[1,2]. This phenomenon, known as electrodiffusion, resembles ordinary diffusion in its phenomenological manifestations. The process can be described mathematically by a differential equation entirely similar to Fick's second law:

$$\frac{\partial c}{\partial t} = \text{div}, (D_e \text{ grad}, c) \qquad (1)$$

where c, t and D_e represent respectively the concentration, time and the electrodiffusion coefficient, the characteristic parameter of the process.

In general D_e can be expressed as a function of the electrophoretic migration velocities, the concentrations of the various ions, the frequency of the electric field, ν, and the rate constants of the chemical reactions which occur between the various ions. In the particular case where the chemical reaction is a monomolecular interconversion of one ionic species only ($A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$), the value of D_e is given by[3]:

$$D_e = \frac{E^2(u_A - u_B)^2 k_1 k_2}{2(k_1 + k_2)[\omega^2 + (k_1 + k_2{}^2)]} \qquad (2a)$$

or, introducing the relaxation time of the chemical reaction, [4]$\tau = 1/(k_1 + k_2)$,

$$D_e = \frac{E^2(u_A - u_B)^2 k_1 k_2 \tau^2}{2(1 + \omega^2\tau^2)} \qquad (2b)$$

where $\omega = 2\pi\nu$, and the symbols A, B, u_A, u_B, k_1, k_2, E represent the two forms of the ionic species, their electrophoretic mobilities, the rate constants of the forward and backward reactions and the average potential gradient, respectively.

The purpose of the present communication is to suggest that, in principle, electrodiffusion can be used to achieve separation of isotopes by exploitation of the kinetic isotope effect.

Thus, let A and A' represent two isotopic ions, and let us assume that A and A' react with the functional groups of an ion-exchange or ion-chelating resin forming the immobile complexes B and B':

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \qquad (3a)$$

and:

$$A' \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} B' \qquad (3b)$$

It is obvious then that if any one of the two following inequalities holds:

$$k_1 \neq k_1' \qquad (4a)$$

or

$$k_2 \neq k_2' \qquad (4b)$$

the values of the electrodiffusion coefficients calculated for reactions (3a) and (3b) will not be equal:

$$D_e \neq D_e' \qquad (5)$$

If an electrodiffusion experiment, therefore, is carried out on an ion-exchange or ion-chelating column, one half of which is saturated initially with a mixture of A and A', a separation of the two isotopes will occur. If the degree of separation α is given by:

$$\alpha = \frac{[A'][A_0]}{[A_0'][A]} \qquad (6)$$

(where $[A_0]$, $[A_0']$, $[A]$ and $[A']$ denote the initial concentrations of A and A' at points $x = 0$ and the concentrations after the elapse of time t, at points $x = x'$, respectively) its value can be calculated by solving equation (1) using the suitable boundary conditions. The solutions of equation (1) for the above experimental arrangements are:

$$[A] = \frac{[A_0]}{2}\left(1 - erf\,\frac{x'}{2(D_e t)^{1/2}}\right) \qquad (7a)$$

and

$$[A'] = \frac{[A_0']}{2}\left(1 - erf\,\frac{x'}{2(D_e' t)^{1/2}}\right) \qquad (7b)$$

By means of equations (2a), (2b), (6) and (7) the optimum separation conditions can be calculated for known values of k_1/k_1', and k_2/k_2'; in practice, however, the best conditions for separation will depend on additional factors as well (for example, ordinary diffusion, heat convection, etc.). The influence of ordinary diffusion can be easily taken into account by substituting in equations (7a) and (7b) D_e and D_e' by $(D_e + D_1)$ and $(D_e' + D_2)$ respectively, where D_1 and D_2 denote the respective diffusion coefficients of A and A'.

In Fig. 1 the value of α is plotted as a function of E for various frequencies assuming that $k_1 = k_2 = 1$ sec.$^{-1}$, $k_1' = k_2' = 1{\cdot}1$ sec.$^{-1}$, $\tau = 0{\cdot}5$ sec., $\tau' = 0{\cdot}45$ sec., $u_A = u_B' = 10^{-4}$ cm.^{2}/sec./V., $u_B = u_B' = 0$, $t = 1{,}000$ sec., $x' = 1$ cm., $D_1 = D_2 = 10^{-5}$ cm.^{2}/sec.

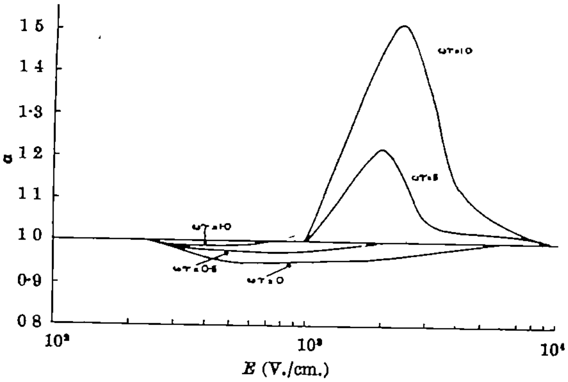

Fig. 1

The results of the computations for this particular case indicate that the separation is enhanced by the use of alternating currents, particularly at high applied voltage, and with frequencies higher than the reciprocals of the relaxation times of the reactions. Direct current also achieves separation; however, experimental considerations (for example, the necessity of using very long columns and the probable blurring effect of electrophoretic anomalies due to conductivity and pH changes in the boundary region[5]) weigh against its use.

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Existence of Gaseous Sulphides of the Transition Elements: Dissociation Energy of Gaseous MnS

It is of interest to obtain data on the lattice energy, ΔH_0^0[at.], of solids, and dissociation energies, D_0^0, of gaseous molecules of whole groups of compounds, such as homonuclear molecules[1], oxides[2], sulphides[3], etc., and to study the variation of these properties, or the ratio of these magnitudes, $\Delta H_0^0[\text{at.}]/D_0^0 = \alpha$, as a function of the electronic structure of the constituting atoms. An interesting case is that of the sulphides of transition elements.

The simplest discussion of the mechanism of vaporization of solid sulphides [MeS] can be based on three processes:

$$[\text{MeS}] \rightleftharpoons \text{Me} + 1/2\ \text{S}_2 \qquad (1)$$

$$[\text{MeS}] \rightleftharpoons [\text{Me}] + 1/2\ \text{S}_2 \qquad (2)$$

$$[\text{MeS}] \rightleftharpoons \text{MeS} \qquad (3)$$

(Condensed phases, solid and liquid, are marked by square brackets, gases without brackets.) Complications that occur when instead of reaction (2) stoichiometric compounds such as $[\text{Me}_2\text{S}]$ or non-stoichiometric solids $[\text{Me}_x\text{S}_y]$ are formed are not discussed here in detail. Though association of S_2 is not discussed, dissociation is considered.

Which of the processes (1) or (2) predominates is seen immediately by considering the equilibrium:

$$\text{S}_2 + 3[\text{Me}] \rightleftharpoons 2[\text{MeS}] + \text{Me} \qquad (4)$$

and the magnitude

$$A = 3\Delta H_0^0[\text{vap.Me}] + D_0^0(\text{S}_2) - 4\Delta H_0^0[\text{at.MeS}] + T\Delta fef\{2[\text{MeS}] + \text{Me} - \text{S}_2 - 3[\text{Me}]\} \qquad (5)$$

where ΔH_0^0[vap.Me] is the heat of vaporization of one atom-gm. [Me], ΔH_0^0[at.MeS] the heat necessary to transform one half molecule-gm. [MeS] in the constituting gaseous atoms, $D_0^0(\text{S}_2) = 100$ kcal. (ref. 3) the dissociation energy of S_2 and Δfef the difference in free energy functions of the substances given in the braces {}.

If $A > 0$ process (2) predominates:

$$-RT\ln p(\text{Me})/p(\text{S}_2) = A = \Delta H_0^0 + T\Delta fef \qquad (6)$$

For the first row of transition element sulphides[4,5] $\Delta fef \approx -5$ e.u.; the dominant term is the enthalpy difference which from FeS to CuS lies in the narrow limits $\Delta H_0^0(6) \approx +24 \pm 3$ kcal. (well within error limits), that is, $p(\text{Me})/p(\text{S}_2) \approx 10^{-4}$ at 1,000° K. and 10^{-2} at 2,000° K. In this case the relation

$$-RT\ln p(\text{S}_2)/p(\text{MeS}) = D_0^0(\text{MeS}) + 2\Delta H_0^0[\text{at.MeS}] - D_0^0(\text{S}_2) - 2\ \Delta H_0^0[\text{vap.Me}] + T\ \Delta fef\{2[\text{Me}] + \text{S}_2 - \text{MeS} - [\text{MeS}]\} \qquad (7)$$

is obtained from the equilbrium:

$$\text{MeS} + [\text{MeS}] \rightleftharpoons \text{S}_2 + 2\ [\text{Me}] \qquad (8)$$

Δfef is again small, about + 3 e.u.; from a comparison of oxides and sulphides one obtains $D_0^0(\text{MeS}) \approx$ 60–70 kcal. and $\Delta H_0^0(7) \approx -40$ to -50 kcal. for FeS, CoS, NiS, CuS. Thus $p(\text{MeS})/p(\text{S}_2)$ increases with increasing temperature but even at 2,000° K. reaches only 10^{-3}–10^{-4}. This is not easily reconciled with Hsiao and Schlechten's[6] results indicating 20–54 per cent Fe and Co transfer in the vaporization of FeS and CoS; these results would mean that $D_0^0(\text{MeS}) \approx$ 100 kcal. An explanation might be given in the terms of more complicated gaseous sulphides such as Fe_2S, Fe_2S_3 or gaseous polymers. Finally $-RT\ln p(\text{S})/p(\text{S}_2) \approx D_0^0(\text{S}_2) + \Delta H_0^0[\text{vap.Me}] - 2\Delta H_0^0[\text{at.MeS}]$ leads to $p(\text{S}_2) \approx 10$ to $100\ p(\text{S})$. If [Me] reacts with [MeS] to give stoichiometric or non-stoichiometric compounds $p(\text{S}_2)/p(\text{Me})$ is even larger.

In the first row of transition elements MnS is the only clear case of $A < 0$ (Equation 5), with $\Delta H_0^0 = -64$; at the end of this row for ZnS and GaS $\Delta H_0^0 = -99$ and -59 kcal. respectively. For the oxides ΔH_0^0 is strongly negative with the exception of CuO (+6 kcal.), where, however, probably the actual process is $[\text{CuO}] \rightleftharpoons 1/2\ [\text{Cu}_2\text{O}] + 1/2\ \text{O}_2$. For negative ΔH_0^0, that is, process (1), the ratio $p(\text{S}_2)/p(\text{MeS})$ is obtained from the equilibrium:

$$\text{MeS} \rightleftharpoons 1/3\ [\text{MeS}] + 1/3\ \text{S}_2 + 2/3\ \text{Me} \qquad (9)$$

and

$$-RT\ln p(\text{S}_2)/p(\text{MeS}) = D_0^0(\text{MeS}) - 2/3\ \Delta H_0^0[\text{at.MeS}] - 1/3\ D_0^0(\text{S}_2) + 2/3RT\ln 2 + T\Delta fef\{1/3[\text{MeS}] + 1/3\ \text{S}_2 + 2/3\ \text{Me} - \text{MeS}\} \qquad (10)$$

For MnS an estimate similar to that above gives $-\log p(\text{S}_2)/p(\text{MeS}) \approx -6{,}500/T + 1{\cdot}5$. Using the mass spectrometric vaporization technique described previously[7] experiments were carried out with MnS between 1,800° and 1,900° K.: at 1,850° K. $\log p(\text{S}_2)/\log p(\text{MnS}) = 2{\cdot}1$. From equation (10) $D_0^0(\text{MnS}) = 65 \pm 5$ kcal. was obtained. For this third law method free-energy functions were calculated from $r_e = 2{\cdot}1\ A$ and $\omega_e = 540$ cm.$^{-1}$; the data from ref. 5 were taken for Mn and S_2. Further $\Delta H_{298}^0[\text{MnS}] = 49{\cdot}0 \pm 0{\cdot}5$ kcal.[8,9,4] $S_{298}^0[\text{MnS}] = 18{\cdot}7 \pm 0{\cdot}3$ e.u. (ref. 10) and $C_p[\text{MnS}] = 11{\cdot}4 + 1{\cdot}8 \times 10^{-3}T$ (ref. 11) yield $\log p(\text{Mn}) = -3{\cdot}00 \pm 0{\cdot}35$; pressure calibrations by complete evaporation of weighed samples yield $-3{\cdot}44$. Also $-RT\ \ln p(\text{S})/p(\text{S}_2) \approx 2/3\{D(\text{S}_2) - \Delta H_0^0[\text{at.MnS}]\}$ is in agreement with experiment.

For VS, TiS, CrS there are no data: however, $\Delta H_0^0[\text{at.MeO}] - \Delta H_0^0[\text{at. MeS}] = 16{\cdot}5 \pm 2$ kcal. for Ca, Mn, Co, Ni; assuming this difference to be constant ΔH_0^0 of equation (6) is for these three sulphides -70 ± 10 kcal. Assuming $D_0^0(\text{MeO}) - D_0^0(\text{MeS}) \approx 30$ kcal. as for oxides and sulphides of groups II^A, IV^B and Mn one obtains: $\log p(\text{S}_2)/$

$p(\text{MeS}) \approx -3{,}300/T + 1 \cdot 5$ and $p(\text{S}) \approx 10p(\text{S}_2)$: a favourable situation for observing gaseous sulphides.

For the two other rows of transition elements it seems difficult to make predictions; for the rare earths, however, one would expect to find gaseous sulphides since ΔH°_{0}[vap.Me] is relatively low and D°_{0}(MeO) high[12].

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Reactions of Gaseous Hydroxyl Radicals

Very little is known of the rates of reaction of hydroxyl radicals. The most extensive work is that of Avramenko and Lorentso[1], but that has been critically reviewed by Steacie[2]. In the present work the homogeneous decomposition of hydrogen peroxide was used as a source of hydroxyl radicals[3], and the relative rates at which hydroxyl radicals react with methane, formaldehyde and carbon monoxide were estimated. The method was checked by independent experiments wherein methane was oxidized thermally at 500° C. to formaldehyde, carbon monoxide and carbon dioxide.

When, using helium as a carrier gas, hydrogen peroxide was decomposed in the presence of methane and carbon monoxide, the competing reactions were:

$$\text{CH}_4 + \text{OH} = \text{CH}_3 + \text{H}_2\text{O} \tag{1}$$

and

$$\text{CO} + \text{OH} = \text{CO}_2 + \text{H} \tag{2}$$

In the presence of small amounts of oxygen, hydrogen atoms were removed by the chain ending reaction

$$\text{H} + \text{O}_2 + \text{M} = \text{HO}_2 + \text{M} \tag{3}$$

whereas methyl-radicals were converted to ethane and a little formaldehyde. Under these conditions:

$$\frac{k_1}{k_2} = \frac{2\text{C}_2\text{H}_6 + \text{CH}_2\text{O}}{\text{CO}_2} \times \frac{\text{CO}}{\text{CH}_4}$$

The CO/CH$_4$ ratio was varied by a factor of 50 yet the values of k_1/k_2 remained constant. Even at temperatures as low as 400° C. where heterogeneous decomposition of hydrogen peroxide predominates, it was found that carbon monoxide and methane were homogeneously oxidized according to reactions 1 and 2.

When methane and oxygen were added to the gas stream most of the methyl radicals formed reacted with oxygen to give formaldehyde, which reacted further according to the reactions:

$$\text{OH} + \text{CH}_2\text{O} = \text{HCO} + \text{H}_2\text{O} \tag{4}$$

$$\text{HCO} + \text{O}_2 = \text{CO} + \text{HO}_2 \tag{5}$$

The results are detailed below.

	400° C.	450° C.	525° C.	600° C.	650° C.
k_1/k_2	0·83	1·10	2 1	2·7	8·4
k_4/k_1	—	—	33	27	22

In the oxidation of methane it was found that the relative rates of oxidation of methane and formaldehyde were proportional to their relative concentrations when surface oxidations were eliminated. Under these conditions, throughout reactions involving a considerable range of mixture strengths, reactions 4 and 1 seemed to predominate in the oxidation. A value of 35 ± 10 was obtained for k_4/k_1 at 500° C.

The variation of k_1/k_2 with temperature gives 7 kcal./mole for $E_1 - E_2$, which is consistent with the value of 10–30 for k_1/k_2 determined at about 1,800° K. by Westenberg and Fristrom[4]. It explains why less carbon dioxide is formed in the oxidation of rich methane/oxygen mixtures at higher temperatures.

The ratio of the steric factors P_1/P_2 must be about 100. This can be understood if the hydroxyl radical has to approach along the axis of the carbon monoxide molecule to give the linear carbon dioxide molecule.

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Synthesis of a Protected Octapeptide Analogue of Hypertensin II

In order to investigate whether the basicity of the amino-acid residue at position 2 in hypertensin II has any strong bearing on its biological activity or affects the balance of oxytocic and pressor properties of this hormone, the histidine^{1}–hypertensin analogue[2] was synthesized in this laboratory. I used the procedure for histidyl peptides[1], which also leads to the synthesis of the im. benzyl analogue. The latter might spread light on the biological significance of the imidazole ring of histidine.

In an earlier communication[2] the synthesis of a protected heptapeptide, carbobenzoxy-im. benzyl-L-histidyl-L-valyl-L-tyrosyl-L-iso-leucyl-im. benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester, and its corresponding benzyl ester was reported. I now wish to record the synthesis of a protected octapeptide with the amino-acid sequence asp.his.val.tyr.ileu.his.pro.phe.

In view of the sensitivity of the aspartyl bond[3] and in order also to diminish the danger of racemization, I found it safer to increase the peptide chain of the heptapeptide by *N*-terminal addition of the aspartyl residue. Therefore, the heptapeptide methyl ester was prepared again by coupling carbobenzoxy-L-proline with L-phenylalanine methyl ester by means of the ethylene phosphorochloridate method to give an oily dipeptide ester, which failed to crystallize. The oily product was converted into L-prolyl-L-phenylalanine methyl ester, m.p. 158°–159°, and used for the preparation of L-isoleucyl-im. benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide[1]. The tetrapeptide, on condensation with the tripeptide, carbobenzoxy-im. benzyl-L-histidyl-L-valyl-L-tyrosine[1], via the carbodi-imide method, afforded the heptapeptide methyl ester, which finally converted into the octapeptide by two methods. At first, the heptapeptide methyl ester was decarbobenzoxylated with hydrogen bromide in acetic acid and then precipitated by ether product, which is very hygroscopic, coupled with β-benzyl carbobenzoxy-L-aspartate[4] by the mixed-anhydride procedure[5]. Since the coupling of β-benzyl carbobenzoxy-L-aspartate with amino-acids and peptides via the mixed-anhydride procedure proceeds with low yield, the aspartate derivative was used in 100 per cent excess. After coupling, the solvent (tetrahydrofuran) was evaporated to dryness and on adding ether the residue solidified (amorphous). The octapeptide methyl ester obtained (60 per cent yield) was saponified with the equivalent amount of sodium hydroxide, diluted with water and acidified with acetic acid. The octapeptide acid was obtained in solid form and after two purifications from ethanol-ether showed $[\alpha]_D^{22}$ + 38° (*c*, 0·5 in methanol) (calculated for $C_{72}H_{84}N_{12}O_{14}$: C, 64·4; H, 6·3; N, 12·5; found: C, 63·8; H, 6·5; N, 12·1). Paper chromatography of a hydrolysed sample revealed the presence of aspartic acid, valine, tyrosine, isoleucine, proline, phenylalanine and im. benzyl-histidine.

Alternatively, the heptapeptide methyl ester was selectively decarbobenzoxylated by catalytic hydrogenation in dimethylformamide[1] and the product, without isolation, coupled with β-benzyl carbobenzoxy-L-aspartate by the carbodi-imide method. The octapeptide ester was obtained in 50 per cent yield and converted to the acid by saponification. The solid product showed identical optical rotation to that prepared by the mixed-anhydride method. Enzymic experiments are in progress to establish the optical purity of the obtained octapeptide acid. In this connexion it might be of interest to note that carbobenzoxy-L-isoleucyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine, incubated with chymotrypsin for 24 hr., failed to give a ninhydrin-positive compound, when the incubation mixture was checked by paper chromatography.

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BIOCHEMISTRY

Effect of Tobacco Smoke Condensate on the Aerial Oxidation of Cysteine

THE recent publication by Lange[1] on the inhibition of certain enzymes by tobacco smoke condensate has prompted the publication of an examination of the catalysed aerial oxidation of cysteine.

The oxidation of thiols to disulphides is known to be catalysed by free radicals[2]. A detailed mechanism for the catalysed aerial oxidation of cysteine has been proposed by Rydon[3], who showed that the intermediate thioperoxy radicals would afterwards react at an unionized thiol group:

$$\alpha\text{–S}^- + X\cdot \longrightarrow X^- + \alpha\text{–S}\cdot$$
$$\alpha\text{–S}\cdot + O_2 \longrightarrow \alpha\text{–S—}O_2\cdot$$
$$\alpha\text{–S—}O_2\cdot + \text{HS–}w \longrightarrow \alpha\text{–S—S–}w + HO_2\cdot$$
$$\alpha\text{–S}^- + HO_2\cdot \longrightarrow \alpha\text{–S}\cdot + HO_2^-$$

The presence of free radicals in tobacco smoke has been reported[4] and is thought to cause the rapid darkening of tobacco smoke condensate on prolonged standing at room temperature, even in the absence of oxygen.

Little direct chemical evidence is available for the presence of peroxides in fresh tobacco smoke condensate[1], although the autoxidation of long chain dienes, for example, neophytadiene, has been observed in neutral extracts from fresh tobacco smoke condensate[5].

The rate of oxidation of cysteine to cystine at *p*H 8·5 has been examined in the presence of varying amounts of tobacco smoke condensate. A constant diffused air flow was maintained into the aqueous solution of cysteine while cigarettes were smoked directly into the solution (Fig. 1, curves 1 and 2). Curves 3 and 4 show the effect of pre-condensing the smoke and allowing an ageing period before adding the tar to the cysteine solution and commencing aeration.

Curve 5 shows the negligible effect of pre-inhaled tobacco smoke on the oxidation time, supporting Lange's observations that, in this context, the active principle of the tobacco smoke was readily adsorbed on the surface of the lung. Little immediate loss of cysteine occurred when excess tobacco smoke condensate was added to the cysteine solution in absence of air. This evidence indicates that the oxidation mechanism occurs predominantly through the form-

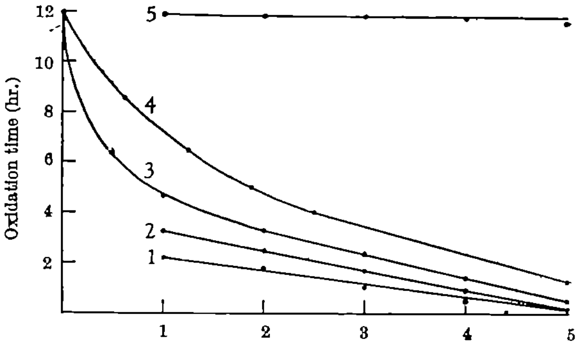

Fig. 1. Effect of tobacco smoke and condensate on the aerial oxidation of cysteine. Curves: 1, Dark-coloured tobacco smoked directly into cysteine solution; 2, light-coloured tobacco smoked into cysteine solution; 3, pre-formed condensate aged 1 month; 4, pre-formed condensate aged 12 hr.; 5, pre-inhaled smoke from light coloured tobacco blown into cysteine solution

ation of thioperoxy radicals. Substantial inhibition of the free radical catalysed oxidation was achieved by the addition of alkyl substituted phenols, for example, thymol, to the reaction mixture.

The proposed mechanism involving thioperoxy radicals does not preclude an *in vivo* mechanism via the unstable mercapturic acid derivatives of the higher polycyclic hydrocarbons[6] formed from the free radicals of the tobacco smoke and cysteine.

The supposition that smokers of dark, well-fermented varieties of tobacco, such as the Eastern European peoples, are less likely to contract forms of lung cancer has been largely disproved[7]. In the present experiments the oxidation time using well-fermented tobacco having an alkaline mainstream smoke was significantly smaller than that of lighter coloured tobacco having an acid mainstream smoke (curves 1 and 2).

The results of this work provide evidence in support of the presence of trapped free radicals in tobacco smoke and of their ability to participate as radical chain propagators in biochemical systems.

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An Improved Method for the Separation and Purification of Chlorophyll a

THE procedure[1,2] which has been used for the separation of chlorophyll *a* has involved two awkward steps: (1) the transfer of the chlorophylls from the acetone extract of the plant to a hydrocarbon phase; (2) the adsorption of chlorophylls on talc-like materials prior to the sugar chromatography. The chlorophyll *a* band on the sugar column invariably has a xanthophyll on its leading edge due to the failure of step (1) to complete the xanthophyll separation and a fair amount of pheophytin introduced by step (2). The xanthophyll contaminant causes an enhanced apparent intensity value of the blue band of the chlorophyll *a* spectrum previously reported. Heretofore it has been very difficult to obtain crystalline chlorophyll using these procedures.

In our procedure the initial acetone extract from the plant material is passed through a column containing polyethylene powder where the chlorophylls are completely separated from the xanthophylls, carotenes, pheophytins and lipids. The chlorophylls are then transferred to iso-octane and chromatographed on sugar. The chlorophyll *a* is eluted with 0·5 per cent *n*-propanol in iso-octane and allowed to crystallize out in a refrigerator. The microcrystals have a well-defined powder X-ray pattern and decreased intensity of the blue band. The ratio of the blue band to the red band in our preparation is 1·19 compared with 1·31 of Benitez and Smith[3]. The spectrum of the chlorophyll *a* crystals (using the method of Shibata *et al.*[4]) has a maximum at 740 mμ compared with 735 mμ reported[4].

The procedure for separating chlorophyll *a* is as follows: 200 gm. of spinach is washed and dried on blotting paper, then extracted with 500 ml. of absolute reagent grade acetone. The acetone is diluted with water to give an 80 per cent water–acetone solution. The aqueous extract is applied to a glass column of 5 cm. diameter dry packed with 1,000 ml. of powdered polyethylene ('Dow', melt index (*MI*) < 2) which has been washed with 70 per cent aqueous acetone. After the pigments are adsorbed the column is developed with 70 per cent aqueous acetone with the complete elution of the xanthophylls. The order of the pigments from the bottom of the column is xanthophylls, chlorophyll *b*, chlorophyll *a*, pheophytins and carotenes. The chlorophylls are eluted with 85 per cent aqueous acetone. The chlorophylls can be separated into chlorophyll *a* and chlorophyll *b* at this stage by using polyethylene of low melt index (*MI* 0·044)[6]. However, the separation of the chlorophylls at this point is not essential, as they are readily separated by sugar chromatography, which reverses the order of adsorption of chlorophylls, the sugar chromatography bringing about a further improvement in purity. To the acetone–water solu-

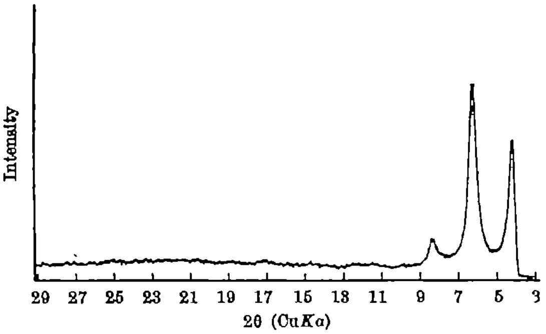

Fig. 1. X-ray pattern of chlorophyll *a* crystals

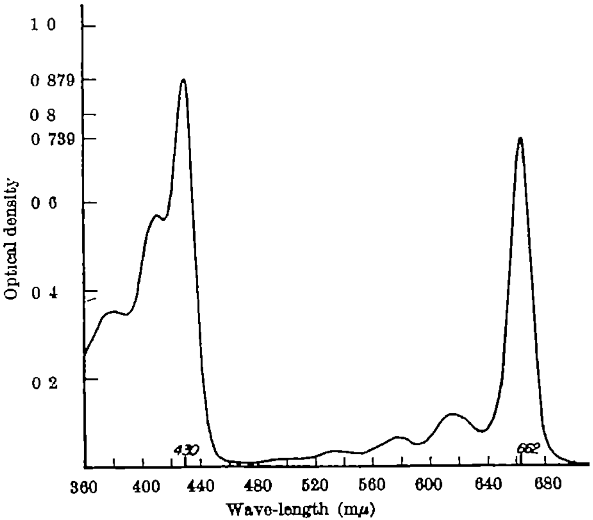

Fig. 2. Absorption spectrum of chlorophyll *a*. Solvent: diethyl ether

tion of the chlorophylls is added 200 ml. of iso-octane (Phillips pure grade) in a separatory funnel and the acetone carefully washed out with water. The iso-octane is dried with anhydrous sodium sulphate and applied to a glass column of 5 cm. diameter dry packed with 1,000 c.c. of powdered confectioners' sugar which has been washed with 250 ml. of iso-octane. The column is developed with 0·5 per cent *n*-propanol in iso-octane and the chlorophyll *a* eluted. The chlorophyll *a* eluate is allowed to remain in a refrigerator overnight when microcrystalline chlorophyll *a* can be collected by filtration or centrifugation.

The yield of chlorophyll *a* is 40 mgm. The X-ray pattern is shown in Fig. 1, and the optical density versus wave-length is shown in Fig. 2.

We are now not only in a position to examine the properties of very pure materials in solution, but also of ordered arrays which previously had been difficult if not impossible to obtain.

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5-Hydroxytryptamine in the Sub-cellular Particles of Rabbit Brain

In the course of experiments on the sub-cellular distribution of substance *P* in the rabbit brain[1], we have confirmed Whittaker's finding[2] that the rat fundus stimulating action of his "synaptic vesicle fraction" was higher than that of the mitochondrial or supernatant fraction. As pointed out by him, however, the total amount of brain 5-hydroxytryptamine (5HT) thus estimated was about several times as much as that reported by previous workers[3]. This should be attributable to his omitting extracting 5HT by acetone. Using the acetone extraction[3] and Vane's rat fundus preparation[4], therefore, we attempted to compare the sub-cellular distribution of brain 5HT with and without acetone treatment. Methods of preparation of the rabbit brain homogenates and of sub-cellular fraction were as described previously[1]. As illustrated in Fig. 1, the amount of 5HT estimated is lowest in the vesicle fraction, while that in the mitochondrial fraction is relatively high. It is apparent at a glance that the 5HT distribution obtained by omitting acetone treatment as in Whittaker's experiment is quite similar to that of substance *P*[1,2]. In fact, it was found that the rat's fundus was considerably sensitive to substance *P*, and the 5HT equivalence of acetone-insoluble aliquot of each fraction measured with the fundus preparation ran parallel to their substance *P* content (Fig. 1).

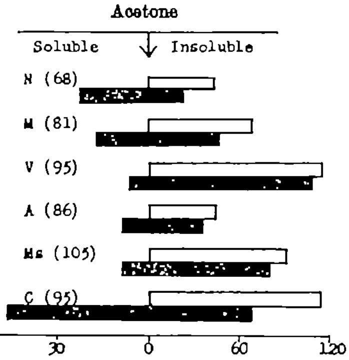

Fig. 1. White, without acetone extraction; black, acetone-soluble (left) and acetone-insoluble (right). Abscissæ: mμgm. 5-HT equivalence; 1 μgm. 5-HT corresponding to about 570 unit substance *P* equivalence. *N*, Nuclear; *M*, mitochondrial; *V*, synaptic vesicular; *A*, amorphous; *Ms*, microsomal, *C*, cytoplasmic supernatant fraction. The values in parentheses represent percentage of recovery of (5-HT substance *P*) activity after acetone treatment with reference to the 5HT equivalence without acetone treatment

Our finding that 5HT of the brain was present rather in the heavier granules than the lighter ones seems to resemble Baker's results on 5HT in the intestinal mucosa[5]. Using the centrifugal fractionation in the density gradient similar to Baker's[5] or Blaschko's[6], therefore, the distribution of 5HT in the combined sub-cellular fraction of mitochondria and microsomes was examined. As shown in Fig. 2, the 5HT of the brain was higher in the heavier particles as in the intestinal mucosa[5], while the fundus-stimulating activity of the acetone-insoluble aliquot, that is, substance *P* activity, was higher in the lighter ones. In view of these findings, it might be said that the 5HT of the brain would play a less significant part in transmission in the central nervous system, if the central chemical transmitter existed in the so-called 'synaptic vesicle fraction'. In connexion with the latter view, however, many questions still remain[7].

We have been able to confirm also that such a particulate 5HT in the brain was liberated (66–99 per cent) by raising the temperature (60° C.), lowering the *p*H (3–4), hypotonicity (0·03 *M* sucrose) and treatment with ether or acetone. Potassium ion (80–160 m*M* potassium chloride) also effected release of 5HT (47–62 per cent at 4° C.), but sodium, calcium or magnesium chloride did not (0–20 per cent). Two hours after adding reserpine (10 ~ 50 μgm./ml.) into the particulate suspension at 4° C., only 20 per cent of 5HT was transferred from the particles into the supernatant. In contrast to the bound acetylcholine, the particulate 5HT was almost completely inactivated on incubation with crude monoamine oxidase prepared from pig serum by Blaschko's method[8].

During these observations, we found that the particulate 5HT was not so unstable as described by

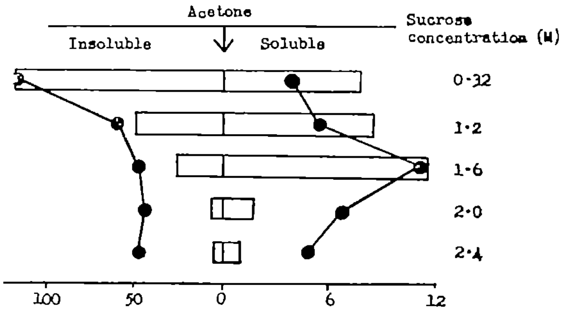

Fig. 2. Solid circles, the activity–protein ratio. Abscissæ: the same as in Fig. 1. The scale for the activity–protein ratio was arbitrarily chosen, readily to compare with the distribution of 5HT activity

Whittaker[2]. Using the 5HT solution (50 ngm./ml. in 0·3 *M* sucrose) as the substrate, therefore, we investigated in which fraction the 5HT-destroying activity was found and confirmed that only the microsomal fraction had such an activity of some significance. But it was not enhanced by ether treatment as suggested by Whittaker, while the substance *P*-destroying activity of this fraction[1] was largely activated by ether[9]. The former activity was inhibited by iproniazid, but the latter was not. This also indicates that the particulate 5HT activity of brain homogenates observed by Whittaker was mainly due to the particulate substance *P*. In the microsomal fraction copper was found, and 5HT inactivation seems to be attributable to contained cæruloplasmin.

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Effect of a Concentrate of Rice Polishings on Serum Lipoproteins

Oncley *et al.*[1] have shown that in man about 75 per cent of the plasma cholesterol is associated with the β-lipoprotein, the remaining 25 per cent being associated with the α-lipoprotein. Examination of serum lipoproteins of patients suffering from hypercholesteræmia has revealed a moderate depression of α-lipoprotein and elevation of β-lipoprotein[2]. Bossak *et al.*[3] reported serum lipoproteins in various animal species and showed that, with ingestion of cholesterol, β-lipoprotein increased with a simultaneous depression of α-lipoprotein.

It has recently been shown that a concentrate of rice polishings can prevent the development of fat, fatty liver, and cholesterol fatty liver[4,5]. The lipotropic action of the concentrate has been attributed to choline and other lipotropic substances present in this concentrate. Since a concentrate of rice polishings reduced hepatic lipids[4], it was thought worthwhile to investigate the effect of this concentrate on serum lipoproteins of albino rats kept on cholesterol-supplemented diet. The experiment was performed on 24 albino rats. The animals were divided into four groups of six each. The animals of Group I were kept on a basal diet containing casein 20 per cent, sucrose 65 per cent, groundnut oil 10 per cent, salt mixture 4 per cent and vitaminized sucrose 1 per cent. The animals of Group II were given the above basal diet supplemented with 0·5 per cent cholesterol. The animals of Group III were given the basal diet supplemented with 0·5 per cent cholesterol + 20 c.c. rice polishing concentrate/100 gm. of the diet. The animals of Group IV were given basal diet supplemented with 0·5 per cent cholesterol + 30 c.c. rice polishing concentrate/100 gm. of the diet. The animals were killed after 12 weeks of experiment. The different sera were subjected to paper electrophoretic separation. The technique utilized was essentially similar to that used by Flynn and deMayo[6].

The paper strips were stained with sudan black *B* according to the method of Jencks and Durrum[7], cut uniformly into 5-mm. sections and eluted with chloroform and methanol mixture 2 : 1, and readings were recorded at 590 mμ in a photoelectric colorimeter. Readings obtained were calculated and expressed as percentage of the total α- and β-lipoproteins. The results are shown in Table 1.

Table 1. Average Percentage Distribution of α- and β-Lipoproteins in the Sera of Albino Rats kept on High Cholesterol Diet and treated with Rice Polishing Concentrate (R.P.C.)

Group	No. of animals	Diet	Experimental period in weeks	Average percentage distribution of α- and β-lipoproteins α	β	β/α ratio
I (normal)	6	Basal diet	12	45·0	55·0	1·22
II	6	Basal diet + 0·5 per cent cholesterol	12	16·0	84·0	5·25
III	6	Basal diet + 0·5 per cent cholesterol + 20 c.c. R.P.C./100 gm. diet	12	34·2	65·8	1·92
IV	6	Basal diet + 0·5 per cent cholesterol + 30 c.c. R.P.C./100 gm. diet	12	44·0	56·0	1·27

A concentrate of rice polishings is a very good source of the B vitamins. This concentrate has also been found to contain essential amino-acids[8]. It is likely that B vitamins, especially choline, inositol, nicotinic acid and essential amino-acids, may possibly influence the physiological levels of lipoproteins[9-11].

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Origin of the Lactate Dehydrogenase Isoenzyme Pattern found in the Serum of Patients having Primary Muscular Dystrophy

The electrophoretic pattern of lactate dehydrogenase (LDH) isoenzymes has repeatedly been determined in human serum for specific detection of tissue necrosis. Indeed, it is generally accepted that the necrotic tissue impresses its LDH pattern on the basic distribution of LDH isoenzymes as found in the serum. Thus in hepatitis the electrophoretically slowest serum LDH (LDH5) (for nomenclature see ref. 1) markedly increases as it constitutes the dominant fraction of liver LDH. In myocardial infarction especially LDH1 (the most rapid fraction) rises as it corresponds to the dominant LDH isoenzyme of human heart muscle.

This being accepted, the results obtained on the serum of patients having primary muscular dystrophy become rather challenging. On this material total LDH activity is found to be considerably increased[2] although plain necrosis seems absent from the diseased muscle. However, it was demonstrated[3,4] that the release of enzymatic protein from dystrophic muscle is much more intense than from normal controls. If this observation elucidates the high total LDH-level found in the serum of those cases, another difficulty remains. One expects that the serum pattern of LDH isoenzymes should present a shift towards the slowest fractions since in human skeletal muscle LDH5 strongly dominates[5,6]. Richterich *et al.*[7] found the increase concerned mainly with LDH1 and LDH2. Our investigations confirm these observations, but we found that LDH3 also participates in that increase. Different explanations can be proposed: (1) selective release of LDH isoenzymes from diseased muscle; (2) selective elimination of these isoenzymes; (3) changes in the LDH pattern of the affected muscle. We have obtained experimental evidence which strongly supports the third hypothesis.

Using agar-gel electrophoresis for separation of LDH isoenzymes and a histochemical procedure for their subsequent detection, we examined the LDH isoenzyme pattern in biopsy specimens of normal and dystrophic human muscle. Biopsy specimens of about 10 mgm. fresh weight were homogenized in twice their volume of distilled water and centrifuged for 10 min. at 10,000*g*. For the separation we used a small-scale method of agar-gel electrophoresis[8,9] needing sample volumes of only 1·5 μl. Electrophoresis was effected at a field strength of 35 V./cm. and at the constant temperature of + 12° C. The agar concentration was 1 per cent in a barbital buffer of *p*H 8·4 and ionic strength 0·05.

Zones of LDH activity were revealed by a staining reaction based on the reduction of nitro-blue tetrazolium into a purple insoluble formazan. We kept to the original Van der Helm procedure[10]; but the substrates were mixed with an agar solution (final agar concentration 1 per cent), poured on top of the electrophoresis strip and left for setting. This double agar plate was then incubated for 60 min. at 37° C., fixed in 2 per cent acetic acid, dried into a clear film and finally scanned at the wave-length of 546 mμ. Percentages between the zones of LDH activity were then calculated in the conventional way.

We thus analysed muscle extracts from 5 patients having primary muscular dystrophy and compared them with 5 normal controls. Results are presented in Table 1. In all pathological samples LDH5, which constitutes the dominant LDH of normal skeletal muscle, was found to be markedly decreased. It may even become the weakest fraction. Thus in dystrophic muscle the percentage of LDH1, LDH2 and LDH3 increases at the expense of LDH4 and LDH5. This shift is clear-cut and is revealed even by simple visual estimation of the stained zones. These modifications, apart from providing an explanation for the serum LDH pattern, may also constitute an important feature in the biochemical detection of muscular dystrophy.

Table 1. PERCENTAGE BETWEEN LDH ISOENZYMES IN MUSCLE EXTRACTS FROM NORMAL INDIVIDUALS AND FROM PATIENTS HAVING MUSCULAR DYSTROPHY (AGAR-GEL ELECTROPHORESIS)

Normal skeletal muscle		LDH1	LDH2	LDH3	LDH4	LDH5
Case	1	3·7	11·8	19·4	26·0	38·6
	2	5·8	14·9	20·4	25·5	33·4
	3	12·2	20·4	21·5	19·6	26·3
	4	13·4	17·7	18·2	24·7	26·0
	5	5·1	15·7	18·3	24·8	36·6
Skeletal muscle presenting primary dystrophy						
Patient	*a*	22·1	29·2	27·5	15·4	5·8
	b	26·6	30·9	24·2	14·9	3·3
	c	25·2	26·7	20·1	19·6	8·4
	d	20·1	24·6	21·9	19·4	14·1
	e	15·3	22·7	22·9	20·1	18·9

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Proteins of Central Nervous Origin Present in Cerebrospinal Fluid

WHILE the cerebrospinal fluid from healthy persons has a relatively poor protein pattern, the number of demonstrable protein fractions may be high in patients with neurological or some mental diseases. More than 30 different proteins can thus be demonstrated in the cerebrospinal fluid by micro-immuno-electrophoretic technique[1]. All these proteins were identified with anti-human serum during the precipitation phase of the immuno-electrophoresis. These proteins are therefore of plasma origin or at least immunologically identical with plasma proteins.

Formerly the cerebrospinal fluid was regarded as a serum transudate only reproducing the serum protein distribution in a diluted form. This concept has now been revised, especially since electrophoresis has revealed different distributions of the proteins in the cerebrospinal fluid and serum, respectively. Furthermore, an intrathecal production of proteins has been supported by investigations with radioactive proteins[2].

In an attempt to elucidate the problem of an intrathecal production, the cerebrospinal fluid was analysed by micro-immuno-electrophoresis in which anti-human serum was replaced during the precipitation phase by a polyvalent anti-cerebrospinal fluid serum. This was prepared by immunization of rabbits with a pooled concentrate of cerebrospinal fluid proteins mainly from patients with cerebral tumours.

33 samples of cerebrospinal fluid from patients with neurological and mental disorders and from some healthy persons were examined. In addition to the proteins which were demonstrated in the cerebrospinal fluid by means of anti-human serum we were able to identify 3 hitherto unknown fractions with the aid of anti-cerebrospinal fluid serum. All 3 fractions are situated in the β_2- and γ-globulin field. We have given these fractions the preliminary symbols $C\beta_{2\cdot 1}$-, $C\beta_{2\cdot 2}$- and $C\gamma$-globulin (Fig. 1). The fractions could also be demonstrated separately after absorp-

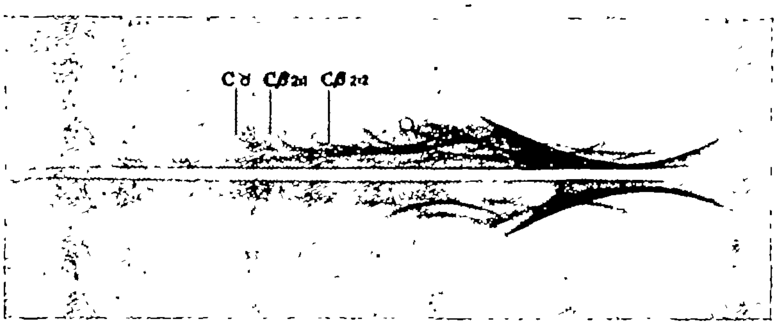

Fig. 1. An original micro-immuno-electrophoretic slide showing all the three specific cerebrospinal fluid proteins, namely, two β_2-globulins ($C\beta$-2 : 1 and $C\beta$-2 . 2) and one γ-globulin fraction ($C\gamma$), precipitated with the aid of anti-cerebrospinal fluid serum in one and the same sample (above). Serum from the same patient studied with anti-cerebrospinal fluid serum. The three above-mentioned fractions cannot be demonstrated (below)

tion of the anti-cerebrospinal fluid serum with normal serum.

The fraction $C\beta_{2\ 1}$ was found in 11 out of 33 samples from patients with multiple sclerosis, polyneuropathy, cerebral or spinal cord tumour, virus meningitis, paranoid psychosis, and severe anxiety state. $C\beta_{2:2}$ was observed in 7 samples from patients with multiple sclerosis, polyneuropathy, cerebral or spinal cord tumour, and virus meningitis. $C\gamma$-globulin was demonstrated in 2 samples from patients with polyneuropathy and cerebral tumour, respectively. These 3 fractions were never seen in the cerebrospinal fluid from patients without neurological or mental disorders. In none of the cases with one or more of these 3 fractions in the cerebrospinal fluid was any corresponding fraction found in the serum from the patients.

It thus appears that proteins of other origin than serum can be found in the cerebrospinal fluid of patients with neurological and mental disorders. As these 3 fractions were not demonstrable in serum, they do not seem to migrate in retrograde direction over to the blood.

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Incorporation *in vitro* of Carbon-14 from NaH^{14}CO$_3$ and 2^{14}C-Glycine into DNA of Red Cells of the Duck

The exchange or renewal reactions of deoxyribonucleic acid (DNA) in non-dividing cells have been proved by many investigators[1]. But the DNA does not seem to be in a dynamic state in erythrocytes of birds[2]. Indeed, Allfrey and Mirsky did not find incorporation of nitrogen-15 from ^{15}N-glycine into DNA of chicken red cells *in vitro*. On the contrary, studying the fixation of carbon-14 from NaH^{14}CO$_3$ by duck red cells *in vitro* we found that DNA was radioactive[3]. Both carbon dioxide and glycine are precursors of DNA[1].

Allfrey and Mirsky found that ^{15}N-glycine is taken up into hæmoglobin by reticulocytes, and the nitrogen-15 enrichment of globin was found to be 0·020 after 23 hr. of incubation. The error is ± 0·001. As the nitrogen-15 enrichment of DNA was found to be 0·000, we can deduce that the incorporation of nitrogen is at least 20 times lower in DNA than in hæmoglobin.

In our measurements 50 ml. of duck blood were incubated with 200 μc. of NaH^{14}CO$_3$. We found that the specific activity of globin was 154 dis./min./mgm. of C and that of DNA was 83 (error < 5 per cent). Because the fixation of carbon-14 from NaH^{14}CO$_3$ into globin takes place through the dicarboxylic acid cycle followed by transaminations of ketoacids[4] and the ^{14}CO$_2$ is directly used for purine and pyrimidine synthesis, our results cannot contradict those of Allfrey and Mirsky.

Further work has proved the existence of a dynamic state of DNA in the erythrocytes using both NaH^{14}CO$_3$ and 2^{14}C-glycine.

Samples of 100 ml. of heparinized duck (*Cairina muschata* L.) blood, to which were added 30 mgm. of sodium-benzyl-penicillin and 35 mgm. of dihydrostreptomycin sulphate, were incubated in vessels slowly rotating (25 cycles/min.) for 20 hr. at 38·5° C. in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. 300 mgm. of glucose and 10 μc. of 2^{14}C-glycine (exp. 1) or 0·5 mc. of NaH^{14}CO$_3$ were also added. After the incubation the red cells were collected by centrifugation and washed several times with saline, then hæmolysed by freezing in liquid air bath and thawing twice.

The hæmolysate was then centrifuged at 2,500*g* and hæmoglobin was prepared from supernatant solution. The sediments, ghosts and nuclei were washed four times with saline. They were homogenized and extracted by stirring for 6 hr. with 20 vol. of 10 per cent sodium chloride at 85° C. The suspension was centrifuged at 10,000*g* for 1 hr. and the clear extract was poured into 6 vol. of distilled water. Then the fibrous nucleoprotein precipitated was collected and from that DNA was prepared according to Allfrey and Mirsky[2]. The DNA purity was proved by infra-red absorption spectroscopy. The spectrum obtained is shown in Fig. 1 and is very similar to those reported by Sutherland[5].

The deoxyribose content of DNA was determined by the diphenylamine reaction according to Barton[6], and it was found to correspond to deoxyribose of pure DNA (purchased from the California Corporation for Biochemical Research, Los Angeles, California).

The DNA and globin specific activities were measured by the gas phase counting method[3].

In Table 1 the ratios between DNA and globin ^{14}C specific activities are reported. We can see that this ratio is of the order of 1 per cent using 2^{14}C-glycine and of the order of 40 per cent with ^{14}CO$_2$. These results agree with those found by Allfrey and Mirsky using ^{15}N-glycine and with our previous measurements with NaH^{14}CO$_3$. Indeed, owing to experimental error and low efficiency of their measurements

Table 1

Exp. No.	Labelled precursor	DNA*	Globin*†	DNA/globin
1*a*	2 ^{14}C-glycine	14·2	360	0·04
1*b*		7·2	570	0 01
2*a*	NaH^{14}CO$_3$	13·6	37	0·37
2*b*		83	154	0·54

* ^{14}C specific activity in disintegrations/min./mgm. of C.
† Error, 5 per cent.

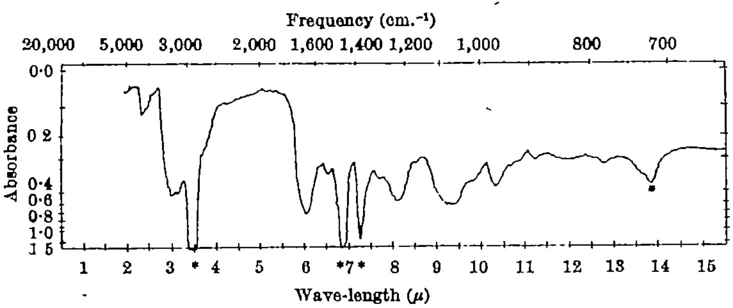

Fig. 1. Infra-red spectrum of DNA in silicon oil, Perkin-Elmer spectrometer model 221 with sodium chloride prism. Picks with asterisks are due to silicon oil

Allfrey and Mirsky could not detect so low a nitrogen-15 incorporation into DNA.

The higher value of ratio between the specific activities of DNA and globin using as labelled precursor $^{14}CO_2$ instead of 2^{14}C-glycine can be explained by the following:

(1) Carbon dioxide is a direct precursor of DNA, but incorporation of carbon-14 from CO_2 into globin takes place indirectly via dicarboxylic acids cycle and keto-acids transaminations.

(2) Glycine is a direct precursor of both DNA and hæmoglobin.

The fixation of carbon-14 into DNA is higher by a factor of 10 using labelled glycine than using labelled carbon dioxide. Probably it could be due to the different amounts of unlabelled carbon dioxide and glycine present in the system.

Finally, in our opinion, it can be concluded that the turnover of exchange or renewal reactions for DNA in duck erythrocytes *in vitro* is much lower than in that for hæmoglobin, but incorporation of carbon-14 represents a test for a dynamic state of DNA also.

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Occurrence of Salicyluric Acid in Milk

HIPPURIC acid was isolated from milk by Karabinos and Dittmer nearly twenty years ago[1], but approximately 7 mgm. per cent of the non-protein nitrogen in milk is still unaccounted for[2]. Using ion exchange chromatography, several nitrogenous constituents, other than amino-acids, have been identified in milk very recently by Schwartz and Pallansch[3]. In the present communication evidence that cow's milk contains the glycine conjugate of salicylic acid is presented.

Salicyluric acid was detected during attempts to decrease the development of oxidized flavours in milk by increasing the level of anti-oxidants or sequestrants of dietary origin. Since all rations fed to dairy cattle contain flavonoids, which are non-toxic and possess anti-oxidant properties, a large supplementary dose of quercetin (150 gm) was given in three doses by drenching to a dairy cow. Daily milk and urine samples were collected before and after the quercetin supplement, and examined for the presence of quercetin or its degradation products. The results were negative in that no quercetin metabolites were detected in either the milk or urine. However, a substance now identified as salicyluric acid was detected in both the milk and urine whether quercetin was fed or not.

The paper chromatographic technique applied to the milk was the same as that used earlier to detect the metabolites of quercetin in rat urine[4]. Briefly, this involved extraction of milk with petroleum ether to remove lipids, followed by extraction of aromatic acids from the acidified milk (pH 2–3) with ether. Non-fat milk powders were first extracted in a Soxhlet apparatus, with petroleum ether followed by acetone. Aliquots of the ether or acetone extracts of milk or urine were then migrated in 2 dimensions on 17-in. sheets of Whatman No. 1 filter paper. The chromatograms were developed in the first direction overnight in the bottom phase of a chloroform/acetic acid/water mixture (2 : 1 : 1) and in the second direction by a 3-hr. migration in 20 per cent potassium chloride. The air-dry chromatograms were examined under short-wave ultra-violet light for the presence of fluorescent and absorbing areas. Finally, each chromatogram was sprayed with diazotized sulphanilic acid (DSA) and 20 per cent sodium carbonate in order to produce colours with phenolic substances.

The major aromatic acids detected in cow's urine were salicyluric, hippuric, and salicylic. The chromatographic properties of these acids are shown in Table 1. Three additional observations were confirmatory as to the identity of salicyluric acid. First, the area on the chromatogram suspected of being salicyluric acid was cut out and subjected to hydrolysis for 3 hr. by refluxing with 20 per cent hydrochloric acid. The paper chromatogram of the ether extract of the hydrolysate contained almost all the salicyluric acid unchanged, indicating great stability to acid hydrolysis under these conditions; which is also true of authentic salicyluric acid. Second, an area corresponding to salicylic acid was detected on the same chromatogram, indicating that a small but significant amount of the salicyluric acid was converted to salicylic acid with a different set of R_F values (Table 1). Third, a positive test of the salicyluric acid spot on the paper chromatograms of milk extracts resulted when the procedure described by Gaffney *et al.*[5] for the determination of hippuric acid in urine was applied. This involves a reaction between the glycine moiety of salicyluric acid with p-dimethylaminobenzaldehyde to produce a yellow colour (azlactone).

Both hippuric acid and salicyluric acid, but not salicylic acid, were detected in fresh milk, evaporated milk, and non-fat powdered milk. These results are confirmatory of hippuric acid in milk[1]; but salicyluric acid has not to our knowledge been reported previously.

Table 1. CHROMATOGRAPHIC BEHAVIOUR OF AROMATIC ACIDS

Compound	R_F values $CHCl_3$	 KCl	Behaviour under ultra-violet	Colour with $DSA\text{-}Na_2CO_3$
Salicyluric acid	0·40	0·72	blue fluorescence	yellow
Salicylic acid	0·90	0·65	blue fluorescence	yellow
Hippuric acid	0·58	0 93	absorbs	colourless

The amount of salicyluric acid in milk was determined by comparison of the spot size of suitable aliquots of the ether extracts of acidified milk with the spot size of known amounts of salicyluric acid (1–5 µgm.) on two-dimensional chromatograms. A sample of fresh milk from a local store contained approximately 160 µgm. of salicyluric acid per litre, a commercial sample of evaporated milk (about 27 per cent total solids) contained about 400 µgm./l., and two commercial non-fat powdered milk samples contained between 200 and 300 µgm. salicyluric acid per 100 gm. of the dry powder.

The most reasonable explanation for the origin of salicyluric acid in milk is that small amounts are able to pass through the mammary gland from the blood. Most of the salicylic acid ingested in the ration apparently is converted to salicyluric acid by glycine conjugation, followed by excretion in the urine. The possibility that the presence of salicyluric acid in milk indicates contamination by urine can be ruled out because we were able to detect salicyluric acid in milk that was obtained by hand milking and known to be uncontaminated by urine.

Reference to a company or product by name does not imply approval or recommendation of the product by the Department of Agriculture to the exclusion of others that may also be suitable.

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Structure of Mucoprotein isolated from Normal and Tumoral Cartilage

CERTAIN experimental evidence has been reported by different authors on the variations existing in some properties of complex polysaccharides isolated from normal tissues and from tumours.

With regard to cartilage it has previously been reported[1] that in the course of experimental carcinogenesis differences can be detected in the composition of mucopolysaccharides. On the other hand we have observed[2] that molecular properties of mucoprotein isolated from normal cartilage and from chondroma are almost similar and the only change of the molecule is restricted to the chemical structure as evidenced by a higher content of the protein component in the chondroma mucoprotein.

Recently results reported by Muir[3] suggest that the mucopolysaccharide isolated from cartilage is a complex consisting largely of chondroitin sulphate cemented by a small amount of peptide or protein. The complex is depolymerized to some extent by proteolytic enzymes, and the kinetics of depolymerization seems to be specific for each complex polysaccharide under digestion.

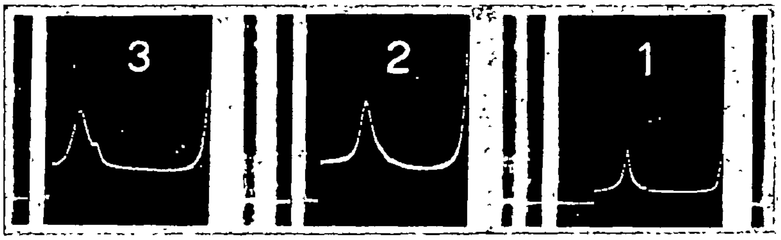

Fig. 1. Ultracentrifuge patterns of cartilage mucoprotein. 1, Undigested mucoprotein, 2, mucoprotein digested with trypsin; 3, mucoprotein digested with papain. Photographs have been taken 120 min. after reaching full speed (47,660 r.p.m.)

In this communication the action of proteolytic enzymes on normal and tumoral cartilage mucoproteins is reported in order to elucidate further the differences existing between the compounds under investigation.

It has been observed that crystalline proteolytic enzymes do not affect the viscosity of mucoprotein solutions (Table 1). Also papain is poorly effective on both compounds, while in Muir's experiments a reduction of 80 per cent in viscosity was observed.

Table 1. PERCENTAGE REDUCTION OF VISCOSITY OF NORMAL (1) AND TUMOUR (2) CARTILAGE MUCOPROTEINS AFTER DIGESTION WITH PROTEOLYTIC ENZYMES

	(1)	(2)
Papain	8	7
Trypsin	6	5
Chymotrypsin	–	–
Carboxypeptidase	3	3

Since it is difficult to believe that the structure of Muir's and my preparations were different, a series of experiments was carried out to find out if differences have occurred in some other molecular properties. In Fig. 1 sedimentation diagrams of mucoprotein isolated from normal cartilage are shown before and after treatment with proteolytic enzymes. It is noteworthy that proteolytic enzymes are able to modify the normal sedimentation coefficient of mucoprotein (Table 2).

Table 2. ACTION OF PROTEOLYTIC ENZYMES ON SEDIMENTATION COEFFICIENT OF MUCOPROTEIN ISOLATED FROM NORMAL CARTILAGE

Undigested	$S_{20,w}$ = 5·4
Digested with:	
Papain	5·4 (main component)
	6·7 (aggregates)
Trypsin	4·8 (asymmetric peak)
Chymotrypsin	5·3
Carboxypeptidase	5·2

It was of interest also to note that mucoprotein isolated from chondroma behaved in a similar manner.

The results reported here indicate that the molecular structure of mucoproteins which I have isolated is different from the chondroitin sulphate complex described by Muir, and give stronger support to the hypothesis of the similarity of structure of normal and tumoral mucoproteins.

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PHYSIOLOGY

Action of 3-Hydroxytyramine and some Tryptamine Derivatives on Spinal Neurones

PHARMACOLOGICAL studies on the depression of spinal reflexes produced by the topical application of comparatively strong solutions of 3-hydroxytyramine to the exposed spinal cord have led to the conclusion that this catecholamine could be an inhibitory transmitter acting on spinal motoneurones[1]. Recently, it has also been suggested that derivatives of tryptamine may be transmitters within the spinal cord[2]. When applied electrophoretically, adrenaline and nor-adrenaline have no action on spinal neurones[3] although these catecholamines, 3-hydroxytyramine and many derivatives of tryptamine depress the synaptic excitation of lateral geniculate neurones[4]. These agents are not, however, inhibitory transmitters in this portion of the visual pathway.

In the present experiments, 3-hydroxytyramine (Grade *A*, California Corp. Biochemical Research), tryptamine, α-methyl-tryptamine and ergometrine were applied electrophoretically to various types of spinal neurones located in the lumbar segments of cats lightly anæsthetized with pentobarbital sodium. The compounds were passed as cations from the drug-containing barrels of 5-barrel electrodes, the central barrels of which were used for recording extracellular potentials[3]. The neurones were identified by their responses to volleys in dorsal and ventral root fibres[3]. None of these substances affected the responses of spinal interneurones even though they were applied by electrophoretic currents as large as 150–200 × 10^{-9} amp. It is assumed that extracellular concentrations of electrophoretically applied compounds are proportional to the currents which are used, and usually currents of 50–100 × 10^{-9} amp. are sufficient to demonstrate the activity of neuronal excitants and depressants[3–5]. 3-Hydroxytyramine failed to influence the firing of interneurones and Renshaw cells and was without action on motoneurones, even after the commercial product had been recrystallized[6]. In the case of motoneurones the compound was passed into the environment of cells from which extracellular focal potentials, both antidromic and orthodromic, were being recorded. The application of γ-amino-*n*-butyric acid from another barrel of the electrode reduced both types of response[5], yet 3-hydroxytyramine, passed with larger electrophoretic currents, had no effect.

These observations indicate that this catecholamine is unlikely to be an inhibitory transmitter in this region of the central nervous system. The term 'inhibition' was applied to the depression of reflexes produced by topical application of the compound because the depression was prevented by strychnine[1]. When administered intravenously this alkaloid diminished the magnitude of inhibitory postsynaptic potentials[7]; but it is surprising that the doses which were used in the recent investigation[1] were so effective in abolishing the action of topically applied 3-hydroxytyramine. It is conceivable that the catecholamine was converted to an inhibitory transmitter during its diffusion through spinal tissue. Another possibility to be considered is that 3-hydroxytyramine bears some structural similarity to the transmitter. If this be so, the comparatively large amounts of the catecholamine reaching motoneuronal receptor sites after topical application may be sufficient to operate the inhibitory synapses, whereas the concentrations attained electrophoretically may be inadequate.

The tryptamine derivatives and ergometrine, a powerful depressant of lateral geniculate synapses[4], did not affect the responses of interneurones, although L-glutamic acid had its usual excitatory action when passed from other barrels of the electrodes[3]. It is therefore unlikely that tryptamine receptors are of great significance with respect to the operation of synaptic transmitters within the spinal cord.

I wish to acknowledge gifts of recrystallized 3-hydroxytyramine hydrochloride (Dr. H. McLennan, University of British Columbia) and of α-methyl-tryptamine methanesulphonate (Dr. A. Hofmann, Sandoz, Ltd.).

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Sodium Chloride Sensitivity and Electrochemical Effects in a Lorenzinian Ampulla

IN the course of experiments on effects of polarizing currents on Lorenzinian ampullæ, in which current pulses were applied to the receptor with electrodes filled with sodium chloride, we found accidentally that the receptor is sensitive to very small amounts of sodium and chlorine ions. This prompted us to examine the chemosensitivity of the ampulla.

Single ampullæ of *Mustelus canis* were set up in a flow chamber as illustrated in the inset of Fig. 1. Large ampullæ, about 1 cm. long and 0·5 mm. in diameter, were chosen for this purpose. The ampulla pore (*P*), which *in situ* opens into the sea-water, was superfused at constant flow-rate with sea-waters of different composition. A selector device allowed switching from one sea-water type to another with no appreciable change in flow-rate. The ampulla bulb (*B*), which contains the receptor nerve ending (*R*), and the emerging axon were in mineral oil. Generator and action potentials were led off single dissected axons (*A*) near the point of their emergence from the bulb.

At constant temperature[1,2], pressure[3,4], and flow-rate, the receptor produces a background discharge of impulses of fairly constant frequency. This frequency is altered when the sodium chloride content of the perfusing sea-water is changed. An increase in concentration of sodium chloride above that of normal sea-water causes an increase in frequency; a decrease in sodium chloride causes the opposite effect (Fig. 1*a*).

The effect is not caused by an unspecific osmotic pressure change: an equivalent increase in osmotic pressure of the sea-water by addition of sucrose causes no appreciable change in impulse frequency. Even a 100 per cent rise in osmotic pressure with sucrose, one that corresponds to more than 200 times the change in threshold required for sodium chloride, is without effect (Fig. 1*b*).

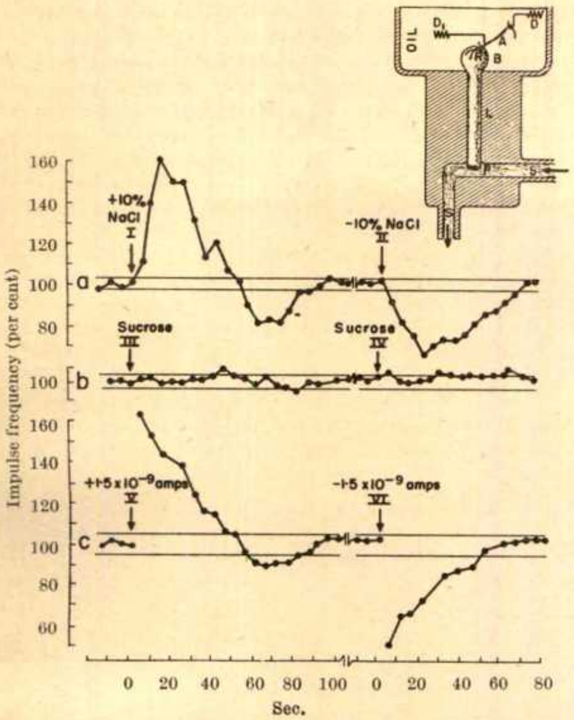

Fig. 1. *a*, Effects of concentration of sodium chloride on impulse discharge of the receptor. The ampulla pore is bathed with normal sea-water; the corresponding mean impulse frequency (100 per cent) and its standard error are delimited by the horizontal lines. The pore is bathed with sea-water the sodium chloride content of which is at I, raised 10 per cent by addition of sodium chloride to normal sea-water; and at II, lowered 10 per cent by dilution. *b*, Effects of increases in osmotic pressure equal to and 10 times that of I by addition of sucrose to normal sea-water. *c*, Effects of a current of $1 \cdot 5 \times 10^{-9}$ amp. through the interior of the ampulla in the direction V, bulb-to-pore; VI, pore-to-bulb

The threshold of the effect is remarkably low. Most ampullæ show a change in impulse frequency with a 5 per cent change in concentration of sodium chloride in the sea-water bathing the pore. The most sensitive ampullæ have thresholds around 0·5 per cent (2 m*M* differential). The sensitivity of the receptor to sodium chloride is several orders of magnitude greater than that of its afferent axon. It is similar to that of some taste[5–9] and hypothalamic 'osmoreceptors'[10–13].

Another striking feature of the effect is the shortness of its latency. The exact value of the latency could not be assessed, because it is shorter than the time resolution of our perfusion system (0·8 sec.). From the considerations which follow, the latency is likely to be of the order of msec. In any event, it is certain to be shorter than 0·8 sec.

This raises the question of how the receptor element located in the bulb can sense with so short a latency an ionic change occurring at the distant pore. In general, one thinks of chemoreceptor excitation as the result of a direct reaction between the chemical agent and the receptor element. Such a mechanism cannot apply here. The receptor element proper is located within the bulb (no nervous structures are found in the rest of the ampulla; in fact, the entire tubule and the peripheral parts of the bulb and jelly can be cut away without the receptor losing its ability to produce rhythmic nerve impulses[4] or to sense ionic changes). The stimulus, namely, the change in concentration of sodium chloride, occurs at the pore of the ampulla, 1–2 cm. away from the receptor element. The entire ampulla up to its pore is filled with a viscous jelly. We have measured the diffusion constant of sodium-24 in the jelly and found it to be $0 \cdot 85 \times 10^{-5}$ cm.²/sec. This is close to the constant of a sodium chloride solution of about equal concentration to that in sea-water (0·5 *M*)[14]. Under the conditions of the experiment of Fig. 1*a*, it would thus take more than 2 hr. to produce a 1 per cent change in sodium concentration at the level of the receptor element by diffusion. Even a change in concentration at the receptor by a factor of 10^{-12}, which would call for a remarkable receptor sensitivity indeed, would require 17 min.—far longer than the observed latent period. A mechanism involving a chemical action of a diffusing agent on the receptor is thus clearly excluded.

An electrochemical mechanism seems more plausible. Murray[15] has already shown that ampullæ *in situ* are sensitive to small voltage gradients in the water around the fish. We have passed electrical currents through the isolated receptor between an electrode fitted to the pore and a micro-electrode placed on the bulb near the terminal portion of an axon. Current through the interior of the ampulla in the direction bulb-to-pore is found to add, at the site of impulse initiation, with the background generator current and, thereby, to enhance the frequency of background impulses. Inverse effects are produced with current in the opposite direction (Figs. 1*c* and 2). The threshold is of the order of 10^{-9} amp. and the latency of the order of msec.

When the concentration of sodium chloride at the ampulla pore is changed by, say, 10 per cent, as in the example of Fig. 1*a*, a potential change is measured at the sea-water-pore junction. The current through the interior of the ampulla resulting from this junction potential is in the direction bulb-to-pore after an increase in sodium chloride, and in the opposite direction after a decrease. (The circuit is closed in most experiments through non-polarizable ground

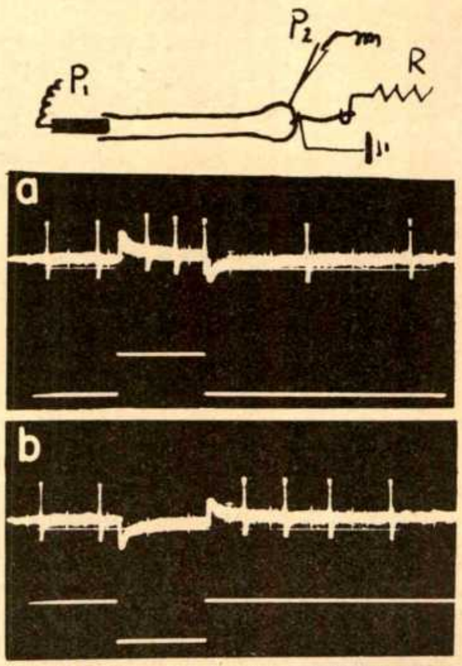

Fig. 2. Enhancement and depression of background receptor discharge by electrical current. Current pulses of 110 msec. duration are passed between electrode P_1 and microelectrode P_2: *a*, in the direction bulb-to-pore; *b*, pore-to-bulb. Time marker, 50 msec.

electrodes in contact with the sea-water and axon.) The current is of the right order of magnitude and in the right direction to account for the observed changes in impulse frequency.

The present results give evidence for a 'chemo'-receptor process of high sensitivity mediated through an electrical mechanism. They do not resolve, however, the question of the physiological role of the ampulla; whether the animal utilizes the ampulla as a sodium chloride receptor, or whether purely electrical[15], pressure[3,4] or other changes are the physiological stimuli, remains to be shown through behaviour studies.

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Injury-induced Synthesis of Deoxyribonucleic Acid in the Lens of the Sea Bass

PREVIOUS work has shown that small mechanical injuries to the epithelium of the rabbit lens can initiate a wave of stimulation to synthesis of DNA and mitosis. The number of cells stimulated to divide is relatively large compared with the size of the injury itself. This reaction is being examined in order to gain a better understanding of the relationship between injury and the induction of cell division.

Examination in cold-blooded animals would offer the advantage of a considerably wider physiological range of temperatures within which the reaction could be studied. Also, the possibility of obtaining this reaction in an *in vitro* system may be greater with the lens of a cold-blooded animal.

The present work demonstrates that small mechanical injuries to the lens epithelium of the bass (*Centropristes striatus*) induces synthesis of DNA in a large number of the surrounding cells. The lenses were injured by insertion of a fine steel needle through the cornea, and into the lens in a manner similar to that previously described[1]. The fish were maintained in tanks of circulating sea water, at approximately 22° C. for 48 hr. At the end of this time the lenses were removed and incubated for 5 hr. in 16 ml. of the following solution at 12° C.: Eagle's basal medium[2] to which was added 0·35 gm. sodium chloride/100 ml., plus tritium-labelled thymidine (sp. act., 3·0 c./mM), at a concentration of 5 μc./ml. The lenses were then fixed in Carnoy's fluid and afterwards prepared for autoradiography[3]. The slides were covered with

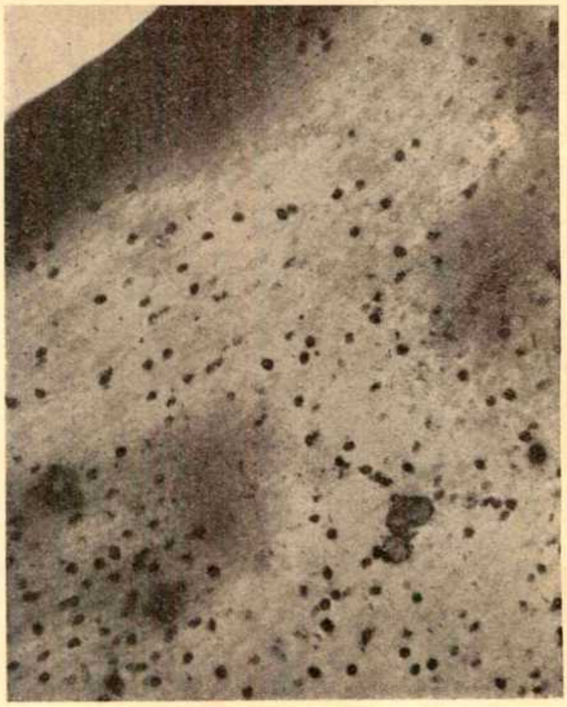

Fig. 1. Autoradiogram of whole-mount of sea bass lens epithelium, fixed 48 hr. after injury. The lens was removed at 46 hr., and immersed in a solution of modified elasmobranch Ringer, containing tritium-labelled thymidine, at 5 μc./ml. Injury site is toward upper left

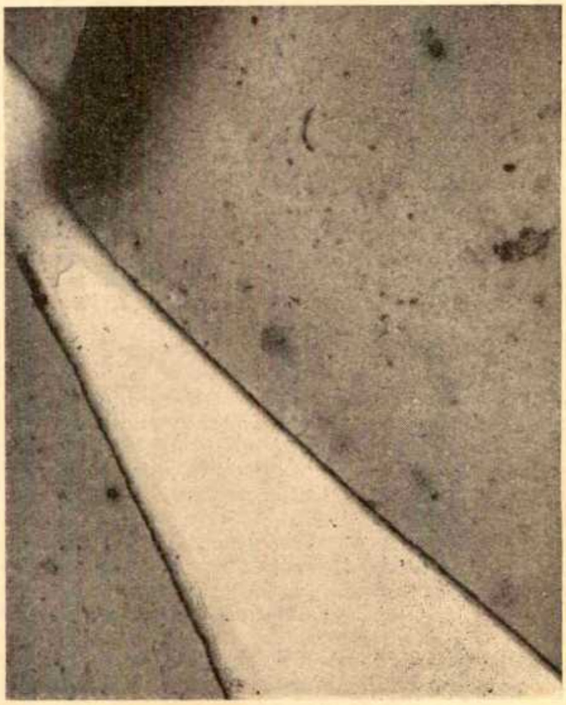

Fig. 2. Autoradiogram of whole-mount of uninjured sea bass lens epithelium. With the exception of the injury, the lens was treated identically to that shown in Fig. 1

Kodak *AR* 10 stripping film, and exposed for 38 days before photographic development. Of the seven experimental preparations obtained, all showed a well-defined injured area. Surrounding this was a large number of cells with radioactive nuclei. Fig. 1 is a photograph of a portion of such a preparation. This large number of reacting cells is comparable with the number seen in the rabbit lens epithelium following injury. Preparations from lenses which had not been injured did not show this number of radioactive nuclei, although a small number of scattered radioactive nuclei were evident in these controls (Fig. 2).

These results indicate that, as in the case of the rabbit lens, a comparatively small mechanical injury can result in the stimulation of synthesis of DNA in a large proportion of the surrounding cells. It is not apparent at the present time whether this stimulation of synthesis of DNA is a propagated response, as has been demonstrated in the rabbit[4]. The number of cells responding, however, is very large compared with the size of the injury itself.

The nature of the stimulus remains obscure. The possibility that the initiation of the cell cycle in these cells is due to the withdrawal of a naturally occurring inhibitor must also be considered. The lens of a cold-blooded animal, such as the bass, may be useful in studying the nature of this reaction under different conditions of temperature, and perhaps in an *in vitro* system.

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Influence of Salicylate on Cortisol Secretion in the Dog

SIMILAR antiphlogistic and biochemical effects of salicylate, adrenocorticotropic hormone (ACTH) and cortisol[1] have stimulated interest in establishing a possible influence of salicylate on the secretion of adrenocortical hormones in man and experimental animals. Studies in dogs, guinea pigs and rats have shown a stimulatory effect of salicylate on cortisol secretion[2]. It has been emphasized, however, that these experiments used a dose per unit of body-weight greater than the comparable therapeutic dose in man[3,4]. Furthermore, an anti-rheumatic effect of salicylate has been demonstrated in a patient whose adrenocortical function had been suppressed by 9-α-fluorohydrocortisone[5]. In view of this conflicting evidence, the present work was designed to determine whether or not salicylate has a direct stimulatory effect on the adrenal cortex.

Adult mongrel dogs, anæsthetized by intravenous administration of sodium pentobarbital, were hypophysectomized by a transbuccal approach[6] 1–3 days before examination. All experiments used an isolated perfused dog adrenal preparation[7]. After several control collections of adrenal venous effluent, the agents under examination were administered directly into the arterial circuit of the perfused glands. The plasma concentrations of sodium salicylate (intravenous preparation, Eli Lilly and Co.) and sodium acetyl salicylate (freshly prepared by dissolving equal parts of sodium bicarbonate and acetyl salicylic acid in water) ranged from 7 to 50 mgm./100 ml. and the duration of administration ranged from 15 to 35 min. At the end of each experiment, an injection of ACTH (intravenous preparation, Upjohn Co.) was given at the rate of 1 unit/min. to verify adrenal viability and to compare the cortisol secretion rates under supramaximal stimulation with the previous salicylate periods. Cortisol concentrations in the adrenal venous effluent were determined by the method of Peterson *et al.*[8]. Sodium salicylate added *in vitro* to plasma containing known concentrations of cortisol did not interfere with the spectrophotometric analysis. The arterial and venous plasma-concentrations of salicylate were determined by the method of Brody *et al.*[9].

Table 1. LACK OF EFFECT OF SALICYLATE ON CORTISOL SECRETION FROM PERFUSED DOG ADRENAL GLANDS

Exp.	Control cortisol (μgm./min.)	Salicylate perfusion Salicylate (perfused	Dose mgm./100 ml.)	Cortisol (μgm./min.)	After ACTH Cortisol (μgm./min.)
1	1·6	Sodium	25	0·9	4·2
			50	0·7	
2	5·3	Sodium	25	3·8	9·1
			50	1·9	
3	0·6	Sodium acetyl	7	0·5	2·5
4	3·4	Sodium acetyl	15	1·5	10·0
			25	1·1	
5	10·7	Sodium	25	8·6	16·3

Table 1 summarizes five experiments in which salicylate compounds perfused directly into adrenal glands resulted in no influence upon cortisol secretion either in the presence or absence of ACTH. In Exp. 4 salicylate concentrations in adrenal venous blood were identical to simultaneously measured arterial concentrations, indicating that the adrenals did not accumulate salicylate. In Exps. 1–4 hypophysectomized dogs were used so that little endogenous ACTH was present. Under these conditions control cortisol secretion-rates from the adrenals were variably low, and there was no increase in cortisol secretion following administration of the salicylate compounds. ACTH given at the end of these experiments showed the expected stimulation of cortisol secretion. In Exp. 5 a non-hypophysectomized preparation was used, and the secretion-rate of cortisol was high during the control period. The addition of sodium salicylate to these adrenals stimulated by endogenous ACTH again failed to alter cortisol secretion significantly.

Adrenocortical response to salicylate administration in man is suggested by the appearance of Cushing's syndrome in a patient treated for acute rheumatic fever[10] and by the observed increase in urinary steroids in patients treated for rheumatic diseases[11]. In animal studies salicylate raised dog and rat plasma cortisol concentrations; similar effects in the guinea pig were abolished by hypophysectomy[2].

On the other hand, normal or low plasma and urine 17-hydroxycorticosteroid values were found in normal humans and in patients with rheumatic diseases receiving salicylate[12-15]. While short-term salicylate administration in man resulted in no increase in cortisol synthesis, prolonged administration was associated with a fall in cortisol turnover[5].

The present acute experiments demonstrate no direct stimulatory influence of salicylate on adrenal cortisol secretion in the dog; in fact, the data may be interpreted as showing a modest suppression of cortisol secretion.

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Fate of Fructose in the Newly Delivered Fœtal Lamb

IT is well known that the blood of the fœtal lamb contains a high concentration of fructose and that this disappears within a few hours of birth[1-4], yet two recent publications have shown that the perfused liver of the fœtal and new-born lamb is unable to metabolize fructose before the fifth day after birth[5,6], and it is known that fructokinase is absent from the liver of the fœtal lamb[7]. In view of these observations, the results of some preliminary experiments on the urinary excretion of fructose in newly delivered fœtal lambs would appear to be of interest.

Six lambs weighing 3·3–5·4 kgm. were delivered by Cæsarean section under spinal anæsthesia from four cross-bred ewes at 138–141 days gestation age (term is about 147 days). Catheters were inserted under local anæsthesia into a femoral artery and into the bladder (via a small abdominal incision) of the fœtus while it was still attached to the mother by the umbilical cord. Blood samples were taken and the bladder was emptied as completely as possible immediately before dividing the umbilical cord and at intervals during the next 6 hr. Blood and urine fructose was measured as described by Bacon and Bell[1].

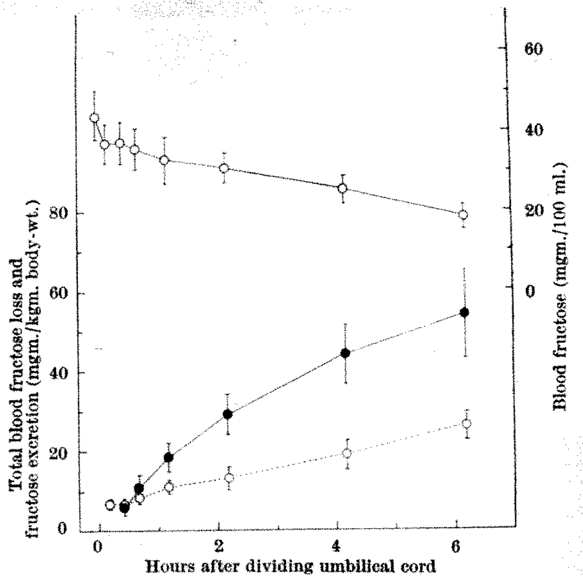

Fig. 1. Blood fructose concentration (○—○), total urinary excretion of fructose (●—●) and apparent total loss of fructose from the blood ○…○) in newly delivered fœtal lambs of 138–141 days gestation age. Means ± standard error, 6 lambs

The results (means ± standard error) are summarized in Fig. 1, which shows the blood fructose concentration, the total amount of fructose excreted in the urine, and the total amount of fructose disappearing from the blood at different time intervals after dividing the cord; this last was calculated on the generous assumption that the blood volume in ml. was equivalent to 10 per cent of the body-weight in gm. There were considerable quantitative differences between individual lambs. Urine was excreted at a mean rate of 5·0 ± 1·56 ml./kgm./hr. over the first 6 hr. and the urinary fructose concentration varied from 42–1,536 mgm./100 ml. Nevertheless the results were wholly consistent. In each experiment, the amount of fructose excreted in the urine was more than sufficient to account for the apparent loss of fructose from the blood; indeed, as shown in Fig. 1, the mean rate of urinary fructose excretion was rather more than twice the apparent rate of fructose loss from the blood. Since the urinary fructose must have been derived mainly from the blood, this difference was presumably due to fructose passing from the tissues and/or extracellular fluid into the blood, so leading to a low estimate of blood fructose loss.

Until some estimate has been made of the total body fructose loss (and of the rate of fructose production), it is not possible to calculate the amount metabolized by the lambs. Nevertheless, as shown in the work on perfused livers[5,6] these experiments suggest that the amount metabolized may be extremely small, and that in the prematurely delivered fœtal lamb the urinary excretion of fructose probably accounts for the greater part, if not all, of the total fructose loss. While it has not yet been shown that this also applies to the fœtal lamb *in utero* and to the new-born lamb delivered naturally, the available information on fœtal urinary fructose excretion and on the rate of fall of blood fructose in normal new-born lambs suggests that they are similar to those observed in the present work. This would seem to suggest that fructose is of little importance as a source of energy to the fœtal and new-born lamb. However, Dickens and Greville[8] have shown that, unlike other fœtal tissues, the fœtal membranes of several species can utilize fructose almost as readily as glucose. Therefore, the possibility remains that the fructose of fœtal ungulates, which have unusually low blood glucose concentrations[4], may provide a source of energy for the amnion and allantois.

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PHARMACOLOGY

Effect of Drugs on the Uptake and Release of ^{3}H-Norepinephrine in the Rat Heart

FOLLOWING the administration of a wide variety of drugs such as psychoactive agents (reserpine, chlorpromazine, imipramine), sympathomimetic amines (tyramine, amphetamine), cocaine, adrenergic blocking agents (phenoxybenzamine), and hypotensive drugs (guanethidine) a lower concentration of injected ^{3}H-noradrenaline was found in the heart, spleen and adrenal glands[1-3]. Most of these drugs also increase the rate of metabolism of ^{3}H-noradrenaline in the whole mouse presumably by preventing the protective binding of the hormone and exposing it to enzymatic attack[4,5]. Although these drugs have many actions, they could produce a common-end result by different mechanisms. They might lower the tissue levels of ^{3}H-noradrenaline by blocking the entry of the circulating catecholamine into the storage site, preventing the binding or causing the release of the bound ^{3}H-noradrenaline or by a combination of these.

If a drug prevents the uptake, it should lower the tissue levels of ^{3}H-noradrenaline only when given before the ^{3}H-catecholamine. If it reduces the concentration when given after ^{3}H-noradrenaline when the hormone is bound in tissues, then it releases the catecholamine. To distinguish between these actions rats were given drugs before or after the intravenous injection of ^{3}H-noradrenaline and the amount of the ^{3}H-catecholamine in the heart was measured.

Male rats (Sprague–Dawley), weighing from 160–180 gm., received 10 μc./100 gm. *dl*-7-^{3}H-noradrenaline (20 mc./mgm.) in the tail vein. The rats were killed after 2, 4 or 24 hr., and the hearts were homogenized with 12 ml. of ice-cold 0·4 *N* perchloric acid. After centrifugation the supernatant solution was assayed for ^{3}H-noradrenaline[6]. Drugs were given before or after ^{3}H-noradrenaline as shown in Table 1. Rats were divided at random in groups of 6–12 for each drug treatment.

Reserpine, amphetamine, *d*-adrenaline and phenoxybenzamine reduced the amount of ^{3}H-noradrenaline in the heart when given after as well as before the ^{3}H-catecholamine (Table 1). Tyramine was previously shown to have this action[7]. It is concluded that these drugs release the bound hormone from its storage site. The experimental technique does not distinguish whether or not these drugs may also inhibit the uptake of ^{3}H-noradrenaline.

Table 1. EFFECT OF DRUGS ON THE UPTAKE AND RELEASE OF ^{3}H-NORADRENALINE IN RAT HEART

Drug	Dose (mgm./kgm.)	Time drug given before or after ^{3}H-noradrenaline	Time rats killed after ^{3}H-noradrenaline (hr.)	^{3}H-Noradrenaline in heart (mμc./gm.)
None	—	—	2	300 ± 15
Chlorpromazine	10	30 min. before	2	112 ± 6 *
Chlorpromazine	10	30 min. after	2	271 ± 14
None	—	—	24	58 ± 9
Chlorpromazine	10, 10	30 min. and 12 hr. after	24	49 ± 10
None	—	—	2	276 ± 15
Imipramine	10	30 min. before	2	132 ± 8 *
Imipramine	10	30 min. after	2	269 ± 25
None	—	—	24	91 ± 11
Imipramine	10, 10	30 min and 12 hr. after	24	94 ± 12
None	—	—	4	216 ± 17
Reserpine	1	30 min. before	4	3 ± 0·5 *
Reserpine	1	30 min. after	4	17 ± 2 *
None	—	—	2	240 ± 28
d-Adrenaline	2	10 min. before	2	115 ± 5 †
d-Adrenaline	2	0·5 min. before	2	99 ± 5 *
d-Adrenaline	2	10 min. after	2	159 ± 22 ‡
None	—	—	4	217 ± 15
Phenoxybenzamine	20	30 min. before	4	111 ± 17 *
Phenoxybenzamine	20	30 min. after	4	119 ± 21 †
None	—	—	4	217 ± 15
Dichloriso-proterenol	20	30 min. before	4	148 ± 11 †
Dichloriso-proterenol	20	30 min. after	4	173 ± 19
None	—	—	4	268 ± 7
Amphetamine	5	30 min. before	4	44 ± 10 *
Amphetamine	5	30 min. after	4	164 ± 14 *

All drugs were given intramuscularly except *d*-adrenaline, which was given intravenously. Twelve animals were used for phenoxybenzamine and dichlorisoproterenol and six for all other drugs.
* $P < 0·001$. † $P < 0·01$. ‡ $P < 0·05$.

Treatment with chlorpromazine, imipramine and dichlorisoproterenol produced a lower concentration of ^{3}H-noradrenaline when given before, but not after, the ^{3}H-catecholamine (Table 1). Cocaine was previously shown to have this action[7]. From these observations, it appears that these drugs block the entry of ^{3}H-noradrenaline into storage sites but do not cause its release.

Because chlorpromazine and imipramine blocked the uptake of ^{3}H-noradrenaline, the ability of these drugs to block its release was examined. Previous studies have shown that monoamine oxidase inhibitors produce a higher concentration of ^{3}H-noradrenaline in the rat after 24 hr. by preventing the spontaneous release of the hormone[8]. Chlorpromazine and imipramine did not appear to block the release of ^{3}H-noradrenaline in 24 hr. (Table 1).

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Morphological Correlate to a Cholinergic Sympathetic Function

THE sympathetic ganglion cells in the cat are heterogeneous with respect to acetylcholinesterase (AcChE) activity when stained histochemically with the thiocholine method[1,2]. Only a limited number possess high AcChE activity (heavy staining) comparable with that of anterior horn cells and other accepted cholinergic cells. The majority of the sympathetic ganglion cells show very faint or no activity. Some cells are intermediate with respect to staining intensity. There has been recent discussion whether the outstandingly 'AcChE-rich' cells are correlated to a peripheral cholinergic function[1-3]; but definite proof for such a hypothesis has been lacking until now. In an earlier paper[2] it was shown that the stellate ganglion is especially rich in cells with high AcChE-activity compared with the superior cervical ganglion and the prevertebral ganglia. This communication explains that finding.

It is known that sectioning of the post-synaptic fibres of nerve cells results in their becoming tigrolytic, that is, loss of ribonucleic acid (RNA)-staining, and it also results in a reduction of their AcChE-activity[4]. The staining methods for RNA and AcChE[5] were used to associate the AcChE-rich nerve cells in the stellate ganglion with their appropriate post-ganglionic outflow. It was found that sectioning of the cardiac nerves had no influence on the AcChE-rich cells while the opposite type of operation (sectioning of all post-ganglionic fibres except the cardiac nerves) resulted in an almost complete disappearance of AcChE from the majority of these cells. Denervation experiments performed in different combinations showed that practically all AcChE-rich nerve cells in the stellate ganglion give rise to post-ganglionic fibres to the fore leg. The changes in the AcChE-rich ganglion cells could be correlated to different degrees of cholinesterase disappearance from the sweat glands of the fore paw. It was now logical to study histochemically the ganglia which innervate the hind leg. When the ganglia of the sympathetic chain were examined AcChE-rich cells were found in significant amounts in the region of *L*6–*S*2. The well-defined cell-type described is very unusual in other paravertebral ganglia (Fig. 1) and seems to be almost lacking in the prevertebral ganglia. A typical frequency diagram for lumbosacral ganglia is shown in Fig. 2.

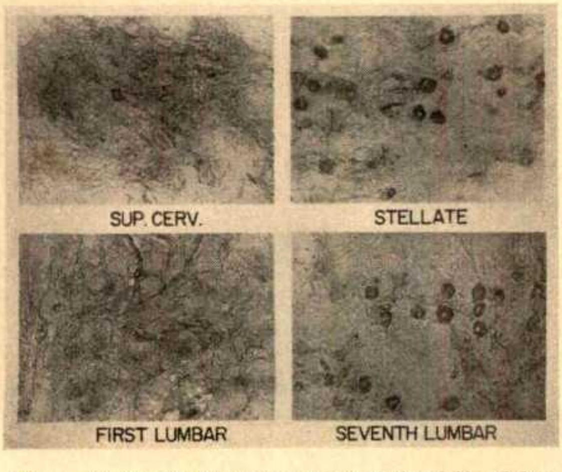

Fig. 1. Photomicrographs from representative fields of four sympathetic ganglia stained for AcChE. Thiocholine method. Incubation time, 30 min. Light microscope. (× *c*. 77)

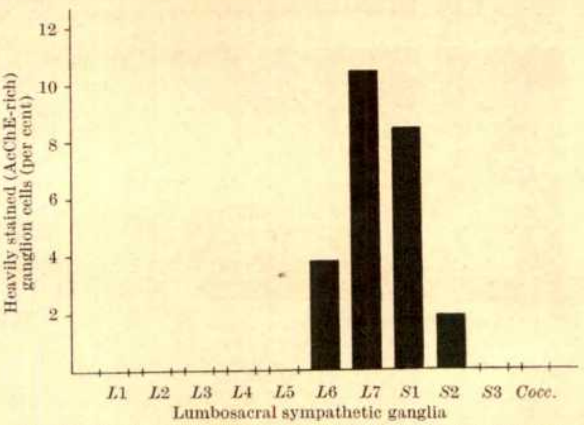

Fig. 2. Frequency diagram showing the number of AcChE-rich ganglion cells in different lumbosacral ganglia after 30 min. incubation

The above-mentioned experiments will be described and discussed in detail in a series of later papers.

It should be pointed out here that the AcChE-rich sympathetic ganglion cells are predominantly localized in those particular ganglia which give rise to post-ganglionic fibres to the cholinergic sweat glands[6], the classical exception in the sympathetic nervous system with respect to neurohumoral transmission[7]. There is a striking similarity between Langley's classical experiments and the findings presented here.

It is evident that the AcChE-rich cells described constitute the morphological correlate to a pharmacologically well-known cholinergic function within the sympathetic nervous system of the cat.

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Physiological Disposition and Fate of Nicotine labelled with Carbon-14 in Mice

COMPARATIVELY few investigations have been reported concerning the tissue distribution of nicotine. These previous studies, which were performed using chemical[1-4] and radiometric[5] methods, showed wide discrepancies. In order to try to elucidate the reason for these diverging results and to obtain further information regarding the pharmacological actions of nicotine we have performed whole-body autoradiography using labelled nicotine. Carbon-14 was introduced in the methyl group by methylating nornicotine with formaldehyde-^{14}C (ref. 6).

White mice weighing about 20 gm. were injected intramuscularly with nicotine labelled with carbon-14. The dosage used was 10 μgm./gm. body-weight corresponding to a radioactive dose of approximately 0·1 μc./gm. body-weight. In a few experiments pregnant mice were used to allow a study of the placental passage of nicotine. The mice were killed 1, 2, 5, 7, 10,

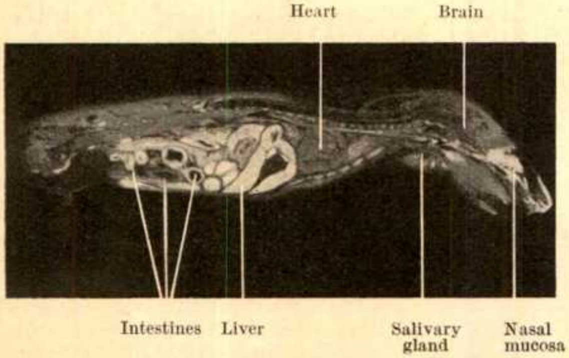

Fig. 1. Autoradiogram showing the distribution of radioactivity in a mouse 15 min. after intramuscular injection of nicotine labelled with carbon-14. White areas correspond to high concentration of radioactivity

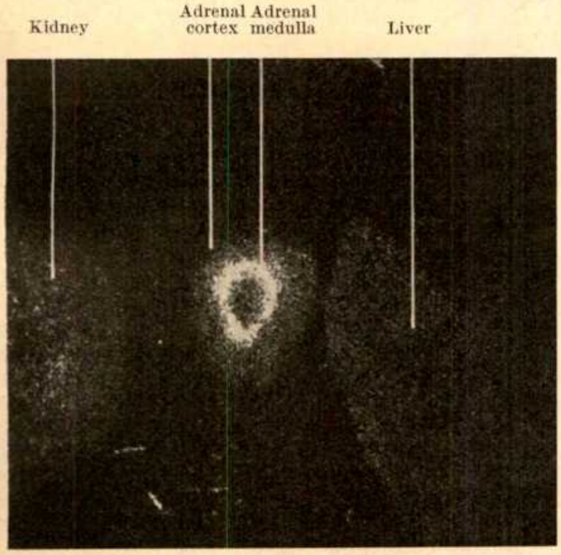

Fig. 2. Enlarged detail of autoradiogram showing the distribution of radioactivity in the adrenal gland 1 hr. after intramuscular injection of labelled nicotine. High activity is observed in the adrenal medulla with the highest concentration in a zone along the border between the medulla and the cortex

30 min. and 1, 2 and 4 hr. after the injection by immersing them into a mixture of acetone and carbon dioxide ice, after which autoradiography was performed[7].

Judging from the whole-body autoradiograms it is obvious that a short time (1–2 min.) after intramuscular injection a considerable part of the radioactive nicotine has been absorbed from the site of injection. The most remarkable finding in these mice is the high brain concentration (Fig. 1). The radioactivity in the brain and spinal cord is considerably higher than in other tissues, and throughout the entire period of examination (up to 4 hr. after injection) the brain shows a high content of radioactivity. 15–30 min. after injection a concentration of radioactivity is noted in the gastric mucosa and radioactivity can also be observed in the lumen of the stomach. The mucous membranes of the nasal cavity show a very high amount of radioactivity. In pregnant mice radioactivity is found in the fœtuses, indicating that nicotine passes the placental barrier.

The main excretory route for nicotine is by the urine. A few minutes after the injection radioactivity can be observed in the renal hilus and in the urinary bladder. Another excretory route for nicotine and its metabolites seems to be the fæces through the bile and the stomach.

Another interesting finding is the high concentration of nicotine in the adrenal medulla. This can be observed a short time after injection, and is persistent during the whole time of observation. In some of the mice a rather sharp zone of radioactivity concentration is observed along the border between medulla and cortex (Fig. 2). Experiments are in progress to localize further nicotine in the adrenal medulla and in ganglion cells by microautoradiographic methods.

Metabolism investigations show that the nicotine is rapidly broken down in the organs. In the liver, the brain and the fœtuses, however, only one metabolite was observed (presumably cotinine). The autoradiographic picture demonstrates thus both nicotine and its metabolites. Experiments are in progress in order to follow the metabolic rate of nicotine in different organs and also to identify the metabolic compounds.

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HISTOCHEMISTRY

Histochemical Adenosine Triphosphatase Activity in Dark and Light Cells of Collecting Tubules in the Mammalian Kidney

IN the kidneys of various species, the collecting tubules are composed of two types of cells. These were apparently first seen by Heidenhain[1], and described in subsequent years as light and dark (intercalated) cells[2]. The name given to these cells alludes to the fact that the cytoplasm of the dark cells stains with various commonly used dyes more intensely than that of the light cells. The dark (or intercalated) cells reveal also strong metachromasia[3]. Electron micrographs show that the dense cytoplasm of the dark cells contains more RNA granules than the cytoplasm of the light cells. Mitochondria are more numerous in the dark cells and are localized not only in the basal but also in the apical portion of the cytoplasm[4]. In contrast, electron-dense granules are more numerous in the light cells than in the dark cells. These granules are composed of phospholipids and proteins and contain one or several crystals with a hexagonal or square lattice[5].

Histochemical reactions which localize enzymatic activity in these cells show a marked difference in staining pattern. This difference has been described previously for succinic dehydrogenase and tri- and diphosphopyridine nucleotide diaphorase in the rat kidney[6]. In this communication, a similar difference in the staining reaction for mitochondrial adenosine triphosphatase is recorded.

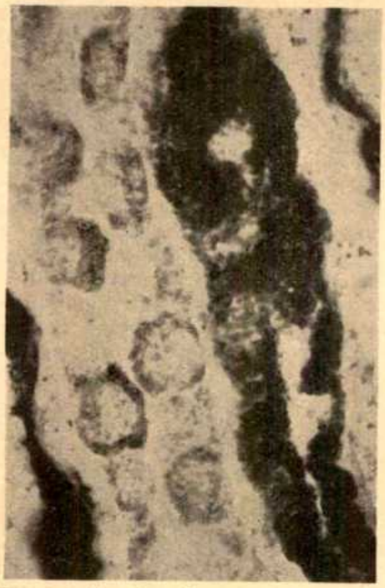

Fig. 1. Frozen section from the outer medulla of the rabbit kidney stained for adenosine triphosphatase activity. To the left a collecting duct is shown. The difference in the granular staining reaction of dark and light cells is striking. To the right an ascending limb of Henle's loop shows intense mitochondrial activity. (× 1,000)

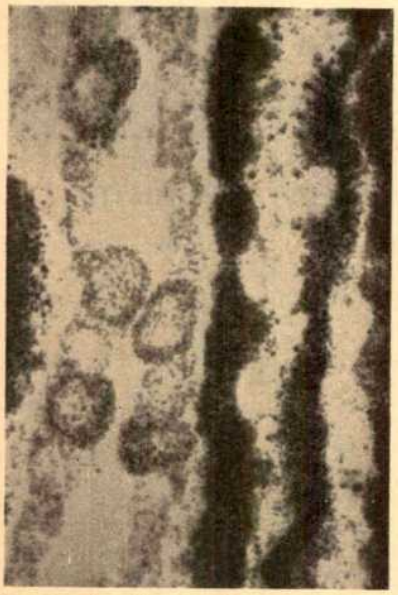

Fig. 2. An adjacent section from the same kidney stained for succinic dehydrogenase (MTT technique of Pearse). A similar enzymatic distribution pattern as that in Fig. 1 is noted. (× 1,000)

Surgically removed human kidneys as well as kidneys of dogs, rabbits, rats and mice were examined. The technique for the demonstration of mitochondrial adenosine triphosphatase has been recently described[7]. Small blocks of tissue are frozen immediately on removal in petroleum-ether or isopentane, cooled to −70° C. Cryostat sections are cut at 5μ and fixed in a neutralized solution of 6 per cent formalin containing 1 per cent calcium chloride, for 5 min. at the freezing point of the mixture (−2 to −3° C.). The slides are then immersed for 15 min. in 0·2 *M tris* maleate buffer *p*H 7·2 at room temperature, and thereafter incubated in the lead-ATP medium of Wachstein and Meisel[8] for 15–30 min. After the usual treatment with ammonium sulphide the sections are mounted in Kaiser's glycerogel. The calcium method for adenosine triphosphatase of Padykula and Herman[9] was also used on cryostat sections that had been fixed for 15 min. in neutral formalin. Results were similar although the method proved to be more capricious.

In all species, the initial portion of the collecting ducts show most of the cells to contain spherical granules corresponding to enzyme-positive mitochondria, while only occasional cells reveal none or only a few of these adenosine triphosphatase positive organelles. The enzyme-rich cells which apparently correspond to the intercalated dark cells decrease in number as the collecting ducts approach the renal pelvis. None is seen in the vicinity of the papilla. At the border of the cortex and medulla the contrast between enzyme-rich and enzyme-poor cells is very striking. This is clearly shown in Fig. 1, which depicts a field from the outer medulla of a rabbit kidney. The neighbouring ascending limbs of Henle's loops are studded with filamentous mitochondria giving a positive reaction for adenosine triphosphatase. Longer fixation of the sections in neutral formalin at 4° C. without the wash in buffer, or fixation of small tissue blocks in cold neutral formalin completely abolishes the mitochondrial staining reaction. This treatment, however, accentuates the staining reaction of vascular capillaries and of some cellular membranes. Fig. 2 shows a fresh-frozen section stained for the demonstration of succinic dehydrogenase for comparison. The similarity between the staining patterns of both methods is obvious. The physiological significance of the marked contrast between the two cell types in the collecting ducts awaits clarification. A marked increase in the number of dark cells in the medullary portions of these tubules occurs in experimentally induced potassium deficiency of the rat, and coincides with the inability to concentrate urine in this experimentally induced renal lesion[10].

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Zinc Enzymes and the Hippocampal Mossy Fibre System

IN 1960[1], I pointed out that some simple modifications in the intravital dithizone (diphenyl-thiocarbazone) method used by histochemists[2,3] for the display of zinc show clearly that the site of the specific (pink) staining in the neighbourhood of the hippocampal

'*CA*3' and '*CA*2' pyramidal cells is topologically identical with the zone occupied by the 'mossy fibre system' (the axons of the granule cells of thefascia dentata); while the conspicuous arc of pink staining within the facia dentata is coincident with the '*CA*4' cell-and-fibre zone. I interpreted these concentrations of zinc as histochemical peculiarities of the histoanatomically peculiar 'syncytial periaxoplasm' which the mossy fibre system exhibits, for most of its course, in ammoniacal-silver preparations[1].

A further slight modification of the dithizone method, namely, leaving slabs of tissue 5-mm. thick in the zinc-acetate-enriched formalin fixative for some 8 hr. before counterstaining with methyl green and cutting 80-μ frozen sections for mounting in glycerine jelly, produces semi-permanent-coloured preparations. The precise location of the pink dye, however, for example, as between the mossy fibres and the shafts of apical dendrites of pyramidal cells, still cannot be microscopically distinguished. To surmount this difficulty, I have more recently slightly modified Timm's[4] silver-sulphide method for the histological display of heavy metals, by varying the intervals between death and fixation as well as the development time, diluting the gum-arabic to 5 per cent, and counterstaining with methylene blue or neutral red. This gives permanent, 10μ, contrasting-coloured sections (Fig. 1) in which different elements of the mossy fibre system can be made to stand out conspicuously from all other structures in the forebrain, and which can be studied informatively at various magnifications.

Low powers of the microscope reveal syncytially skeining long homogeneous brown stripes bestrewn with black particles along their margins wherever they cross the pallisade of apical dendrites of pyramidal cells of '*CA*3' or '*CA*2'. Higher magnifications, with certain variations of the method, and notably in the 'end-bulb' region in the guinea pig after prior dithizone treatment, furnish a clear picture of these black particles outlining the shafts of the apical dendrites in '*CA*3' and '*CA*2' (Fig. 2); and with other variations in the time parameters, give visual confirmation, in the form of pools of homogeneous brown precipitate, of the 'confluence of periaxoplasm at the end-bulb', previously deduced from the ammoniacal-silver studies[1].

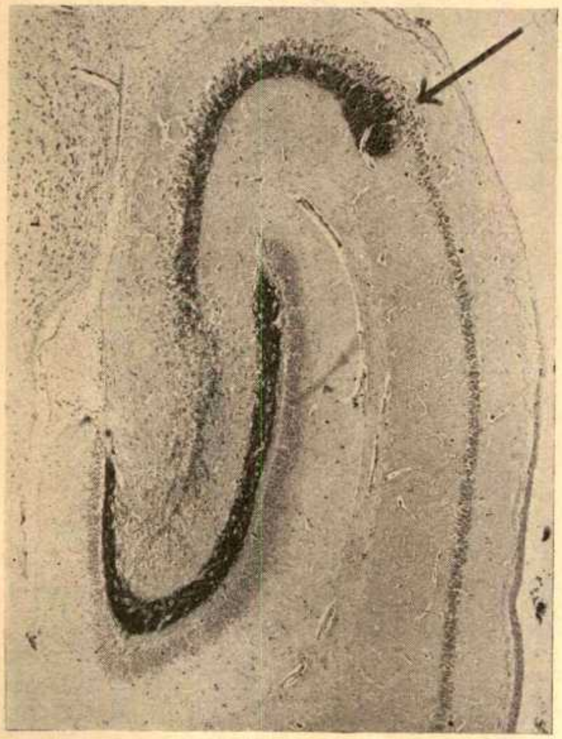

Fig. 1. Transverse section of dorsal hippocampus of guinea pig, showing the mossy fibre system and the '*CA*4 arc' impregnated by silver-sulphide, and the cell-layers counter-stained by methylene blue; 'end-bulb' indicated by arrow. (× 30)

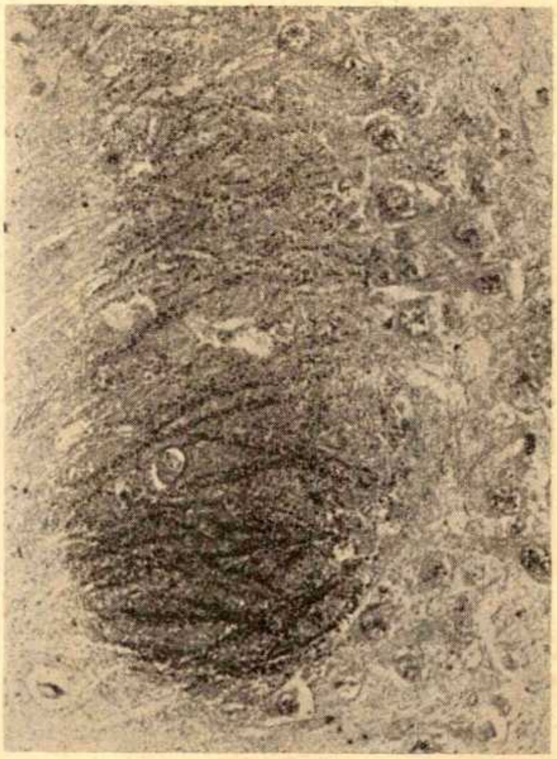

Fig. 2. Enlarged view of the end-bulb region in the guinea pig, prepared as in Fig. 1 after intravital dithizone, showing the 'outlining by black-particles' of the apical dendrites where traversed by mossy fibres. (× 210)

There would seem no reasonable doubt but that the metal responsible for the brown and the black silver-sulphide precipitate is zinc; that this zinc is linked with some diffuse enzymatic protein in the periaxoplasm; and that it is either specially concentrated, or linked with some other enzymatic protein, at the 'mossy' *synapses-de-passage*. My own histo-enzymatic preparations show apparently significant concentrations of acid phosphatase in especially the 'end-bulb': the one part of the mossy fibre system where non-impregnation by ammoniacal-silver had previously given intimation of disappearance of axis-cylinders and a change in the 'chemical specificity'[1]; while succinic dehydrogenase selectively outlines the pallisaded '*CA*3' and '*CA*2' apical dendrites where traversed by the mossy fibres. It is difficult to avoid the working hypothesis (without prejudice to the presence of other enzymes such as acetyl-cholinesterase) that the generality of the periaxoplasm probably contains a zinc-acid-phosphatase, manufactured at '*CA*4' where periaxoplasm first appears[1]; and that each mossy synapse contains an additional zinc-dehydrogenase, presumably elaborated by the mitochondria within each 'presynaptic bag'[5]. Perhaps a third zinc-enzyme must be sought to explain the intensity of the dithizone staining and the diffuse impregnation, by ammoniacal-silver and by silver-sulphide, within the '*CA*4 arc'.

I have elsewhere[6] expounded the thesis that a fibre system possessed of such peculiar morphological and chemical characteristics ought to be investigated intensively for comparably peculiar neurophysiological attributes, having in mind its exploration by pre-modelled sets of pick-up microelectrodes during inputs of species-meaningful, for example, auditory, patterns of stimuli, in non-anæsthetized animals, by people with the facilities for such research.

The silver-sulphide method in the guinea pig also reveals a fine-grain dispersion of zinc-substance between the '*CA*2' somata, as if diffused from the end-bulb. This has interesting possible causal relations with the hippocampal pathology associated with experimental carbon monoxide seizures in the guinea pig, as observed in this laboratory, to be reported on fully elsewhere.

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HISTOLOGY

Use of Fluorescein-labelled Rheumatoid Factor for locating Sites of Antibody Fixation in Tissues

RHEUMATOID factor is a macroglobulin component of the serum of patients with rheumatoid arthritis and can be demonstrated in a variety of serological tests by its reactivity with aggregated γ-globulin or with antibody combined with antigen. Exploiting its peculiar affinity for specifically bound antibody, I have used rheumatoid factor labelled with fluorescein in preliminary experiments as an immunohistological reagent for locating sites of antibody fixation in sections of quick frozen tissue by Coons's technique.

The rheumatoid factor reagent was prepared from a euglobulin fraction of pooled, inactivated rheumatoid serum[1]. The euglobulin was conjugated with fluorescein following the method described by Marshall[2]. Unconjugated fluorescein was removed by dialysis against 0·1 *M* disodium phosphate solution (*p*H 8·6), which minimized re-precipitation of euglobulin. Following conjugation, the sensitized sheep cell agglutination titre of the euglobulin solution had fallen from 1/8,000 to 1/1,000, but there was no further loss of hæmagglutinating activity after dialysis or absorption with guinea pig and human tissue powders.

The reactivity of the rheumatoid factor reagent with tissue-bound antibody was demonstrated by the following procedure. Unfixed sections of quick-frozen normal human kidney (test sections) were exposed overnight to the globulin fraction of an antiserum prepared in the rabbit against isolated human glomeruli. This immune serum, absorbed with human serum, was known to contain antibodies reactive with certain tissue elements in human kidney. Adjacent kidney sections were exposed overnight to normal rabbit globulin (control sections). After washing thoroughly in 0·1 *M* disodium phosphate (*p*H 8·6), the test and control sections were exposed to the rheumatoid factor reagent for 1 hr. at room temperature: in parallel experiments, fluorescein-labelled sheep anti-rabbit globulin antiserum was substituted for the rheumatoid factor reagent.

In the test sections the rheumatoid factor reagent reproduced the distribution of 'staining' observed with the conjugated sheep anti-rabbit globulin antiserum, indicating that the rheumatoid factor reagent had located the sites of specific fixation of rabbit antibody. Antibody fixation could still be demonstrated by the rheumatoid factor reagent when the rabbit antiserum was diluted 1 in 32. It was evident, however, from the degrees of fluorescence produced by the two conjugates that the rheumatoid factor reagent reacts less well with antibody bound to the media of arterioles and the basement membrane of the glomerular capillaries than with antibody at other sites—the basement membrane of the tubules, the adventitia of blood-vessels and the interstitium.

The reaction of the rheumatoid factor reagent with bound antibody in the test sections could be inhibited by unlabelled rheumatoid euglobulin (sheep cell agglutinating titre 1/16,000), but not by the euglobulin fraction of a rheumatoid serum with a negative sheep cell agglutination test and positive *F* II-latex

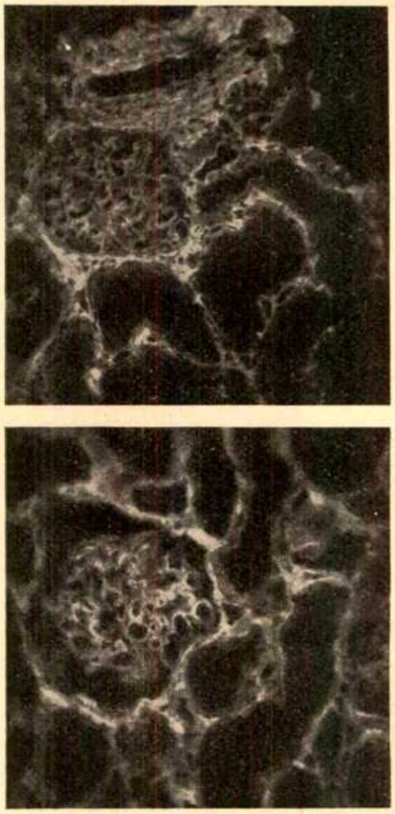

Fig. 1. Fluorescence photomicrographs of human kidney sections. Above, Specifically bound rabbit antibody globulin has been located by fluorescein-labelled sheep anti-rabbit globulin antiserum. The basement membranes of the tubules and glomerular capillaries and the adventitia and media of the artery showed specific green fluorescence. (The internal elastic lamina is visible because of its brilliant white autofluorescence.) Below, The bound rabbit antibody globulin has been located by fluorescein-labelled rheumatoid factor. The basement membranes of the tubules and glomerular capillaries showed specific green fluorescence

fixation test nor by normal human euglobulin or fresh guinea pig serum. After absorption of the rheumatoid factor reagent with insoluble, aggregated human γ-globulin, which reduced the sheep cell agglutinating titre from 1/1,000 to 1/4, there was still a discernible although considerably reduced reaction with bound antibody.

The control sections, treated with normal rabbit globulin instead of the rabbit antiserum, showed no 'staining' with the rheumatoid factor reagent although native human globulin, presumably denatured by freezing, thawing and drying, could be demonstrated by a conjugated specific antiserum. Similarly, there was no reaction with native globulin in sections of normal rabbit tissue.

In this experimental model, rheumatoid serum factor conjugated with fluorescein has proved to be an effective reagent for locating fixed antibody in tissue sections. The possible application of this technique to the problem of detecting specifically bound antibody in other experimental conditions and in spontaneous disease is being explored.

While this work was in progress the Rheumatic Diseases Research Centre was receiving financial support from the Nuffield Foundation and the Empire Rheumatism Council.

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HÆMATOLOGY

Differences in Hæmostatic Behaviour between Arterial and Venous Blood

Previous papers from our laboratory have shown marked differences in the behaviour of arterial and venous blood in hæmostasis. Only citrated arterial blood shows hæmostatic properties when used as a perfusion fluid in the dog's hind leg preparation[1], citrated venous blood being devoid of these properties and, moreover, having a strong inhibitory activity when added to arterial blood[2].

It has been observed that heparin, besides its effects on coagulation, has, at least in some dogs, a marked tendency to prolong determinations of bleeding time. When a standard dose of heparin (300 I.U./kgm.) was injected intravenously into a series of 800 dogs the bleeding time remained normal in 63 per cent (heparin-resistant dogs); 18 per cent showed a moderately prolonged bleeding time; whereas in 19 per cent (heparin-labile dogs) the bleeding time was higher than 30 min.[3]. On the other hand, it has been shown in the dog's hind leg preparation that heparinized arterial blood taken from heparin-resistant or heparin-labile dogs, when in contact with foreign surfaces, changes its hæmostatic behaviour according to the kind of surface[4]. This extreme lability of heparinized blood in respect of its hæmostatic properties makes a sharp contrast with citrated blood, the hæmostatic properties of which remain active for a long time after withdrawal from the animal. Moreover, even in the intact animal it has been observed that rapid contact of heparinized arterial blood flowing through short tubes (placed between the ends of a severed artery) was enough to change its hæmostatic characteristics[5].

The present investigation shows further differences in the hæmostatic behaviour of arterial and venous blood in the intact heparinized animal, these differences being detected when blood is withdrawn and put into contact for some time with a plastic surface and is afterwards re-injected into the circulation of the animal.

The experiments were carried out in two types of dogs previously chosen for their resistance or lability towards a standard dose of heparin. After 10 min. of the intravenous injection of heparin (300 I.U./kgm.) followed by a continuous injection at a rate of 2 I.U./kgm./min., samples of blood were taken from either jugular vein or carotid artery and placed in plastic (cellulose nitrate) tubes, under a layer of mineral oil, at room temperature. The amount of blood taken varied according to the weight of the dog; taking the blood volume as 10 per cent of the body-weight, 2 per cent of the total blood volume was withdrawn for the foreign surface test. After a predetermined time of contact, the blood was re-injected (by the route from which it was withdrawn) and after 3 min. bleeding time determinations were performed by means of skin cuts in the inner aspect of both legs.

When arterial blood from a heparin-resistant dog is placed in contact with a plastic surface and re-injected into the animal, the bleeding time remains normal (5 determinations), but if venous blood is allowed to come into contact with the same surface over the same period of time and then re-injected, hæmostasis is suppressed, as shown by bleeding time values higher than 30 min. (14 determinations).

When arterial blood from heparin-labile dogs is placed into contact with a plastic surface and is again injected into the animal, the bleeding time becomes normal (8 determinations), as would be expected from previous findings on this subject[5]. Venous blood is devoid of these properties, that is, bleeding time remains higher than 30 min. when, after contact with plastic surface, the blood is re-injected into the animal (2 determinations).

In these two sets of experiments the effect on hæmostasis is observed with an amount of venous or arterial blood as small as 2 per cent of the assumed total blood volume and in a period of time higher than 10 min. (10–60 min.). These are critical values, since if smaller quantities are used or the time of contact with the plastic surface is shortened, the phenomenon does not occur (8 determinations). Furthermore, the effect is only observed with heparin administered *in vivo*. The use of heparin *in vitro*, or the application of other anti-coagulants such as sodium citrate (3·8 per cent) and oxalate mixture (1·2 per cent ammonium-oxalate and 0·8 per cent potassium oxalate), are unable to reproduce the phenomenon (11 determinations).

That 2 per cent of the total blood volume, by mere contact with a foreign surface, should acquire the property of changing hæmostasis in the whole animal is rather surprising. Heparin has a broad spectrum of biological activity[6] and seems to sensitize plasma proteins when in contact with foreign surfaces[4,5]. The results given here show that the effect of this sensitization on hæmostasis depends on the source from which the blood is taken, venous blood being active in heparin-resistant dogs, arterial blood in heparin-labile dogs.

As shown previously, the number, agglutinability or clot-retraction activity of platelets do not change after heparin injection[5], their role being, therefore, very questionable in the phenomenon described here. The ability of heparin to make complexes with a variety of plasma proteins is a point that should be stressed, since many observations in our laboratory suggest that the role of plasma proteins is more significant than the participation of blood elements in the control of hæmorrhage in small vessels and capillaries[6]. With the use of heparin, efforts are now being made to isolate and identify the plasma components involved in hæmostasis.

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The First Human Blood, ---/---, which Lacks the 'D-like' Antigen

THE 'D-like' antigen has been demonstrated in all Rh positive and Rh negative human bloods including the rare D--/D-- and r^Gr^G (ref. 1), as well as in rhesus and other lower monkey red blood cells, but it was not found in common laboratory animals[2,3]. One of the characteristics of this antigen is its capacity to induce formation of 'anti-D-like' antibodies in guinea pigs. These red cells which possess this antigen after exposure to its antibody yield eluates exhibiting the 'D-like' specificity.

The unique blood of a normal, female Australian aborigine, Mrs. E. N., which is deprived of all known antigens of the Rh–Hr system, was described recently by Vos, Vos, Kirk and Sanger[4]. As this rare blood was neither Rh positive nor Rh negative, an excellent opportunity was provided to determine whether it carried the 'D-like' antigen. If the ---/--- red cells lacked the 'D-like' property, the question arose of the probable theoretical implications of the relationship of the 'D-like' antigen to basic Rh substance.

Joint parallel studies of the ---/--- blood and the 'D-like' antigen were undertaken based on three criteria: (*a*) absorption with ---/--- red blood cells; (*b*) elution of the antibody; and (*c*) antigenicity in guinea pigs. These investigations demonstrate that this exceptional ---/--- blood devoid of Rh–Hr antigens also does not possess the 'D-like' property.

'Anti-D-like' reagents prepared by absorptions with Rh negative or Rh positive red blood cells did not produce potent reagents since all random bloods possess the 'D-like' antigen; however, using ---/--- red blood cells for absorptions, more potent reagents were obtained which would suggest that these red cells did not carry the 'D-like' property (Table 1).

Eluates were prepared according to the technique described previously[3]. In contrast to Rh negative bloods, the red blood cells of E. N. failed to yield eluates with 'D-like' specificity as indicated in Table 2. While we used a guinea pig anti-rhesus reagent, Vos and his co-workers used a reagent prepared from guinea pigs injected with *rr* red blood cells and confirmed the results shown in Table 2.

Frozen glycerinated ---/--- blood on arrival from Australia was maintained at −70° C. As a control a freshly drawn specimen of Rh negative blood was also preserved in glycerine, frozen and maintained at −70° C. To avoid the loss of red cells incidental to deglycerinization, thawed, unwashed samples were used for injections. (Guinea pigs injected with glycerine revealed no toxic effects.) At three-day intervals each of two series of guinea pigs received two injections of 2·0 ml. of the bloods which contained approximately 1·0 ml. of sediment. When tested ten days later by two absorptions at 1 : 5 with *rr* red blood cells, of the 20 guinea pigs inoculated with the control *rr* blood, 12 produced 'anti-D-like' antibodies; however, none of the 20 guinea pigs inoculated with ---/--- blood showed 'anti-D-like' specificity. Four of the 20 guinea pigs inoculated with ---/--- blood produced an antibody exhibiting anti-N specificity which was not observed in the pre-inoculation specimens. Since the ---/--- red blood cells are of genotype *NN*, the source of stimulation is obvious.

The immunization experiments carried out in Australia were made with heat extracts of group O, *rr* red blood cells and ---/--- red blood cells. While the five guinea pigs injected with heat extracts of ---/--- red cells failed to produce 'anti-D-like', two of these five produced an antibody also identified as anti-N. Of the four guinea pigs injected with heat extracts of *rr* red blood cells, three showed very weak 'anti-D-like' specificity when absorbed with *rr* red cells. After absorbing these sera with the ---/--- red blood cells, all four gave 'anti-D-like' specificity

Table 1

Guinea pig serum (351) diluted 1: 10 and absorbed twice with washed red blood cells	Tested with									
	R_2R_2	R_1R_1	R_1r	R_0r	r′r	r′r	r″r	rr	rr	---/---
---/---	+ + +	+ + +	+ + +	+ + +	0	0	0	0	0	0
R_1R_1	+ +	+ +	+	+	0	0	0	0	0	0
rr	$+^s$	$+^s$	$+^s$	+	0	0	0	0	0	0

Guinea pig No. 351 was injected twice with Rh negative red cells. Readings were taken after incubation for 45 min. at room temperature, light centrifugation and resuspension of the sedimented red cells.

Table 2

Pooled guinea pig anti-rhesus serum 1 : 5	Tested with							
	R_2R_2	R_1R_1	R_1r	R_0r	rr	rr	rr	---/---
Absorbed with rr	+ ±	+ ±	+ +	+ +	±	±	0	0
Eluate from red blood cells :								
---/---	0	0	0	0	0	0	0	0
rr	+ + + +	+ + + +	+ + + +	+ + + +	0	0	0	0

Readings were taken after incubation at room temperature for 1 hr., light centrifugation and resuspension of the sedimented red cells.

of stronger activity than that obtained on absorptions with *rr* red blood cells.

The view that the E. N. genotype, ---/---, is the result of suppression or deletion of that part of the chromosome bearing the Rh locus has been considered by Vos *et al.*[4]. Another possibility may be that ---/--- represents basic material remaining after loss of products of the Rh–Hr genes by one or another genetic mechanism, but, on the other hand, it could represent basic material on which the Rh–Hr genes deposit their products.

Based on our previous observations and the results of this joint study, it would appear that when a gene produces an Rh–Hr antigenic determinant, it simultaneously produces a 'D-like' antigen. We would like to offer the hypothesis that the 'D-like' material constitutes unaltered basic Rh substance which, by a series of mutations in man, has evolved into the complex Rh–Hr situation as it exists to-day. Such a theoretical possibility was mentioned as early as 1952 by Murray and Clark[5]. In any event at this stage of investigation one can conclude that the 'D-like' antigen is associated with all random human bloods bearing any one or more of the antigenic determinant groups of the Rh–Hr system.

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PATHOLOGY

Rat Tail Tendon Denaturation and Rheumatic Diseases

DR. B. J. RIGBY and co-workers[1,2] in their studies on mechanical properties of rat tail tendon have extrapolated a possible explanation for the pathological alterations that occur in rheumatic diseases of humans. They consider the damaging effects of rheumatic diseases to be caused in part by a denaturation of collagen which was a function of temperature. It is our contention that the extrapolation cannot be made for several reasons.

There are a number of observations on rat tail collagen that make it distinct from other mammalian collagens in spite of the similarity of rat collagen's chemical composition and shrinkage temperature to that of other mammalian collagens. Rat tail tendon collagen can be partially solubilized with cold distilled water[3] and approximately 90 per cent of the tendon collagen is dissolved in dilute acetic acid[4]. Most mammalian collagens do not exhibit these properties. Neuman[5] showed that rat tail tendon collagen was completely solubilized by heat treatment in water at 60° C. for 20 min.; whereas the other mammalian tail or Achilles tendons were not solubilized or only solubilized to the extent of 20–30 per cent. However, the solubility and thermal characteristics of rat tail tendon are very similar to those seen in fish collagens.

In our investigations on infectious arthritis, we have been interested in comparing the thermal properties of articular cartilage with that of other collagens. Meyer[6] had originally questioned the similarity of the collagens of tendon and cartilage. Recently we have come across an observation[7] that human costal cartilage has a shrinkage temperature of 83°–87° C.

Table 1. COMPARISON OF BOVINE TENDON AND CARTILAGE COLLAGEN

Temperature	Solubilization* (per cent) Tendon†	Articular cartilage†
37° C.	7·6	1·3
45° C.	13·4	3·5
50° C.	28·1	5·3

* Based on the amount of hydroxyproline that appeared in the filtrate.

† Suspensions of bovine Achilles tendon and bovine articular cartilage were incubated for four days in 0·1 *M tris* buffer, *p*H 8·0, containing 1 : 5,000 dilution of merthiolate.

Our approach was to study the effect of temperature on the solubilization of collagen. Solubilization of collagen was used as a measure of the degree of collagen denaturation. Table 1 shows that bovine tendon collagen is four to six times more solubilized by heat than bovine articular cartilage collagen irrespective of the temperature. These data indicate greater thermal stability of bovine articular cartilage collagen as compared to bovine tendon collagen. We interpret these data to be contrary to the denaturation hypothesis put forth by Rigby *et al.*[2].

Denaturation of collagen as a function of temperature is too broad a phenomenon to explain the specific lesions observed in rheumatic diseases and the variations in the clinical course of the disease process. Denaturation of articular cartilage collagen may still occur in rheumatoid arthritis, but this could well originate in as yet unknown mechanisms involved in the pathological process.

In regard to the importance of the time factor[2] in rheumatic disease, it has been observed frequently that the destructive effects seen in these diseases depend more on the intensity of the rheumatic attack than the duration of a milder form.

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Tissue-Specificity in the Soluble Antigens in Kidney Microsomes

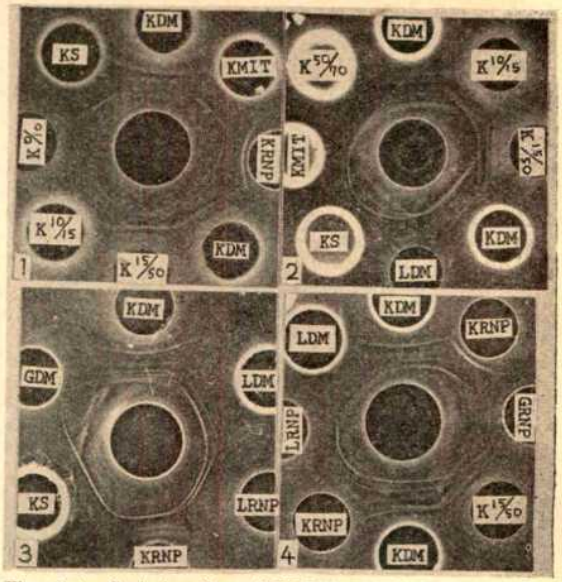
It has been suggested that the membrane of the endoplasmic reticulum contained in the microsomal fraction is the site of the localization of tissue-specific antigens[1]. This structure may, however, be of immunologically complex nature, consisting of a number of antigenically different molecules, only several of which may be tissue-specific. This brief communication is concerned with attempts to identify and partially purify the tissue-specific antigens in the microsomal fraction of chicken kidney. In the immunological test the double diffusion agar technique was adopted, since this method is considered to be most adequate for the analysis of the complex antigen–antibody system and, in fact, it has been successfully used for the immunological characterization of the constituents of the different cell fractions of rat liver[2].

The microsomal fraction was isolated from adult chicken kidneys by a routine method[3], and this was injected into rabbits to prepare the antisera. Sometimes the microsomal pellet was dispersed in a medium composed of 0·14 *M* sodium chloride, 0·1 *M* phosphate buffer and 2 per cent ethylenediamine tetra-acetic acid (EDTA) (*p*H 8·0). The residue obtained by centrifugation after such treatment was also used as antigen for preparation of the antisera. Since preliminary tests revealed that the EDTA-treated microsome, in spite of removal of some soluble proteins together with RNA, produced more potent antisera than untreated microsome without causing any essential difference in the results, the following account concerns results obtained with the anti-EDTA-treated microsomal sera as reagents.

For testing, the homogenates of kidneys, livers and lungs of adult chickens were separately fractionated according to methods described by D'Amelio and Perlmann[2] to prepare the following samples; 0·5 per cent deoxycholate extract of mitochondria, 0·5 per cent deoxycholate extract of microsome (DM-fraction in the following description), EDTA extract of ribosome and cell sap. Double diffusion test in agar was run at 37° C. for 2 days, and then the dishes were kept under 4° C. for 2 further weeks. Concentration of the antigens to be tested had been previously adjusted to contain approximately 1·0–2·0 mgm. protein/ml.

When the antigen–antibody precipitates became stationary, the antisera gave at least 5 major lines and several minor ones in reaction with the DM-fraction of the homologous organ. These lines can be separated into two distinctly different groups. One is a rapidly migrating group containing 2 major lines, and it is common to all the fractions of kidney. In contrast, the other group, which is slowly migrating, is highly specific for DM-fraction (Figs. 1 and 2). Moreover, a heavy precipitin-line appeared closest to the well containing the EDTA extract of ribosome, which may be caused by an antigen specifically predominant in this fraction.

The antigenic components of the slowly migrating group identified in DM-fraction of kidney are not only highly fraction-specific, but some of them display a high degree of tissue-specificity. DM-fraction of lung scarcely gave any visible lines to fuse with the lines belonging to this group, while in the reaction with the same fraction prepared from liver 2–3 faint identical lines became perceptible. But at least 1 major and 1–2 minor antigenic components of the slowly migrating group of kidneys were always left as specific for this tissue (Figs. 3 and 4). The antigens of the rapidly migrating group were shown to be non-specific, being common to DM-fractions prepared from kidney, liver and lung, though the concentration of the last one was very low. Only in the reaction with DM-fraction of lung another rapidly migrating band appeared. This may be caused by antigens of blood cells, which might occur due to the extreme difficulty of complete removal of these cells from the lung.



Figs. 1–4. Central wells, anti-EDTA-treated microsomal sera. Peripheral wells, antigen solutions to be tested: *KDM*, DM-fraction of kidney; *KMIT*, deoxycholate extract of kidney mitochondria; *KRNP*, EDTA extract of kidney ribosome; *K*0/10, precipitate obtained from DM-fraction of kidney at 10 per cent saturation of ammonium sulphate; *K*10/15, *SF*10/15 of DM-fraction of kidney; *K*15/50, *SF*15/50 of DM-fraction of kidney; *K*50/70, *SF*50/70 of DM-fraction of kidney; *KS*, cell sap of kidney; *LDM*, DM-fraction of liver; *LRNP*, EDTA extract of liver ribosome; *GDM*, DM-fraction of lung; *GRNP*, EDTA extract of lung ribosome

Next, efforts were made to separate the tissue-specific components from DM-fraction of kidney by means of the fractional precipitation of ammonium sulphate. Throughout the process *p*H was kept at 7·5 with *tris*-buffer. The precipitate obtained at 10 per cent saturation was found to be insoluble with deoxycholate as well as with saline or EDTA solution at any *p*H between 7·0 and 8·5. The proteins precipitated between 10 per cent and 70 per cent saturation were easily dissolved with water without adding any detergent and were able to be used for immunological test as aqueous solution. The reaction of these water-soluble proteins with the antisera represented a full spectrum of the precipitin-lines recognized between the antisera and DM-fraction of kidney. Further fractional precipitation revealed the following points (Figs. 1 and 2): (1) The proteins precipitated between 10 per cent and 15 per cent saturation (*SF* 10/15) usually gave one or two lines of the slowly migrating group; these were common to DM-fraction of liver. (2) Viscous and yellowish solution obtained from the precipitates between 15 per cent and 50 per cent saturation (*SF* 15/50) contained all the antigenic components which were identified to be kidney-specific; contamination with the components of *SF* 10/15 occurred sometimes, varying from preparation to preparation. (3) The precipitate between 50 per cent and 70 per cent saturation gave a colourless solution and contained all the components of the rapidly migrating group.

Confirmatory results were obtained with the use of the proteins similarly sub-fractionated from DM-fraction of liver or lung. No perceptible lines appeared in the reaction between the antisera and *SF* 15/50 of lung. As expected, *SF* 15/50 of liver was devoid of the kidney-specific components which were contained in the same sub-fraction of kidney.

Preliminary chemical analysis of the separated sub-fractions has indicated that *SF* 15/50 can be characterized by its high lipid content. In fact, the precipitin lines which appeared when this sub-fraction was allowed to react with the antisera were stainable with sudan black and oil red *O*.

Naturally, a considerable number of microsomal antigens which cannot be rendered soluble may be left undetectable in the present test. The results reported briefly here, however, permit us to assume that the water-soluble *SF* 15/50, which is probably extracted from the membrane of the endoplasmic reticulum[4], may contain antigenically different molecules in each tissue.

I thank Mr. A. G. Sato for his collaboration.

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IMMUNOLOGY

'Microspot' Test applied to Cellulose Acetate Membranes

WIDE interest has been demonstrated in the antigen–antibody 'microspot' test on agar gel-coated cover-glasses[1]. The technique has now been extended to permit use of cellulose acetate membranes, the advantages of which over agar gel for immuno-precipitation purposes has been pointed out by Kohn[2].

Strips of cellulose acetate membrane (Oxo, Ltd.), 5 cm. × 2 cm., are ruled with a longitudinal line down the middle and five transverse lines 1 cm. apart, the latter starting 0·5 cm. in from one end of the strip. This produces eight ruled squares, 1 cm. × 1 cm., which may be numbered consecutively in one corner. The lines and numbers may be made with pencil; or more vivid marking can be obtained with 'Polynk' (George T. Gurr, Ltd.), applied with a fine pen.

The strips are immersed in a suitable dilution of antiserum in buffered saline (for example, pH 7·6) and lightly blotted to remove excess moisture. A 10 per cent concentration is sufficient for strong antisera, but higher concentrations can be used, if an antiserum is weak. Control strips are prepared by impregnation with a similar concentration of normal serum.

A microdrop of test antigen is spotted on the centre of each of the squares on the serum-impregnated strip. Thus, eight dilutions of a single antigen, or antigenic samples from eight different sources, may be tested on a single strip. Capillary melting point tubes drawn to fine apertures can be used for applying the microdrops; but more uniform results are obtained if they are applied with 1 µl. Drummond microcap disposable micropipettes (Shandon Scientific Co., Ltd.).

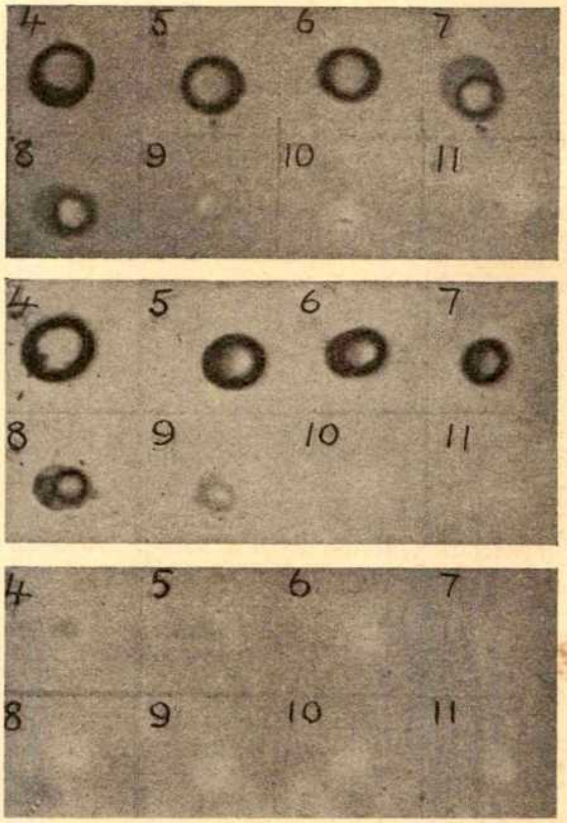

Fig. 1. 'Microspot' test for human γ-globulin on antiserum-impregnated cellulose acetate strips with dilutions 4–11 of five-fold serial dilutions of human serum. Top strip incubated 1 hr.; centre strip, 24 hr.; bottom (control) strip, impregnated with normal rabbit serum, incubated 24 hr. Positive reaction can be seen, even after 1-hr. incubation, with dilution 9 (1 : 390,615). Note halos at unreacted spotting sites

The spotted strips are incubated in a moist chamber, or under a light mineral oil as recommended by Kohn[2]. As little as 1 hr. may be sufficient to allow the antigen–antibody precipitation to go to completion.

The strips are then washed in buffered saline for 1–2 hr. (preceded by a quick rinse in a mild detergent solution, if mineral oil immersion has been used) and stained. I have found Kohn's suggestion of 0·002 per cent nigrosine in 2 per cent acetic acid an excellent dye for 'microspot' strips. Results are readily read on the dye-wetted strips within 1 hr.; but the staining is allowed to continue overnight for possible appearance of exceedingly weak precipitation reactions. Depending on the relative concentrations and purity of the antigen–antibody system, positive reactions appear as stained spots or concentric bands of precipitate against an uncoloured, or lightly coloured, background.

Offsetting the advantage of the direct visibility of the precipitate in the agar-gel 'microspot' test is the much greater sensitivity of the cellulose acetate membrane 'microspot' test. Whereas on the gel[1] I was able to detect the γ-globulin in pooled human serum diluted 1 : 100, using the same reactants on the membrane I have obtained a positive 'microspot' test for γ-globulin with a 1 : 400,000 dilution of the pooled human serum (Fig. 1). This represents a γ-globulin concentration of about 2×10^{-2} μgm./ml., or an absolute equivalent of 2×10^{-5} μgm. of γ-globulin detected on the test strip. This should make the test most suitable for the forensic laboratory for determining the nature and origin of minute quantities of antigenic substances.

Though the use of serum-impregnated membranes is alone described in this communication, I have used a reversed form of the test by impregnating the strip with antigen and using it for the detection of antibody. In either case, speed of reaction and extreme economy of reactants are outstanding properties of the test.

I thank A. W. Wheeler for technical assistance.

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Enhancement of the Complement Fixation Reaction with a Soluble Antigen from *Mycoplasma mycoides*

THE soluble polysaccharide (galactan)[1] isolated from *Mycoplasma mycoides* var. *mycoides* (*M. mycoides*) can be used in precipitin or indirect hæmagglutination reactions to detect antibody to *M. mycoides*. When the galactan was used in place of the standard particulate antigen in the complement-fixation test for contagious bovine pleuropneumonia[2] with known positive sera, fixation of complement was usually weak or absent. As unheated normal bovine serum has been shown to potentiate complement-fixation with heated bovine[3,4] and swine[5] antisera, its effect on complement-fixation by the galactan–bovine antibody system was tested.

Normal bovine sera free from demonstrable bactericidal and complement-fixation antibody to *M. mycoides* were obtained from 4–6-month-old calves, and were used when fresh or within 3 weeks when stored at −12° C. Bovine antisera of known titre to the standard complement-fixation test[2] and the hæmolytic system of the standard test were used. Dilutions were made in veronal buffer[6].

Constant antigen and antiserum concentrations were tested with and without added normal bovine serum in the presence of dilutions of complement. To 0·4 ml. of a 1/40 dilution of the test serum was added 0·5 ml. of each complement dilution and 0·2 ml. of a 1/40 dilution of normal bovine serum, then 0·2 ml. galactan solution (6 μgm./ml.) was added and the mixture was allowed to stand at 3–4° C. overnight. In control tubes reagents omitted were replaced by buffer. In the morning the tubes were warmed to 37° C., 0·2 ml. of hæmolytic system was added, and the test was read after incubation at 37° C. for 30 min. Fixation of complement did not occur when galactan and antibody were mixed unless unheated normal bovine serum was present (Table 1).

Table 1. EFFECT OF NORMAL BOVINE SERUM ON COMPLEMENT-FIXATION BY GALACTAN ANTISERUM TO *M. mycoides*

Reagents present (+)			Dilutions of complement (reciprocals) with degree of fixation					
Galactan 6 μgm./ml.	Antiserum No. 24 (1/40)	Normal bovine serum *D*75 (1/40)	12·6	20	32	50	80	100
+		+	–	–	–	–	–	3
+	+		–	–	–	–	–	3
+	+	+	–	3	4	4	4	4
	+	+	–	–	–	–	–	3

4, Complete fixation; –, no fixation.

When either the antigen or antiserum concentration was varied independently in the presence of constant concentrations of complement and normal bovine serum, the optimal concentration of antigen was in the range of 6–12 μgm./ml. Antisera tested with galactan at this concentration gave lower titres than when tested with the standard particulate antigen.

Four of five sera obtained from calves aged between 4 and 6 months potentiated complement-fixation with galactan and antiserum to *M. mycoides*.

The complement-fixation potentiating effect of normal bovine serum was found to be destroyed by heating to 56° C. for 30 min.[3]. Further work on the nature of the serum factor(s) is proceeding.

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BIOLOGY

Factors affecting the Human Sex Ratio

WE present here evidence suggesting the existence of at least two kinds of factors affecting the human sex ratio. As the sex ratio we use the number of male births per 100 female births. The evidence is based on family data collected by Geissler[1] in 1889 and by ourselves[2,3].

In Table 1 the sex ratio among the *N*th children in the two series of families is given as a function of the number of boys (*N*–1, *N*–2, . . . or 0) preceding this child. From these data two rules emerge: (1) Children of a certain parity tend to be boys if all the preceding children of the family have been boys, and they tend to be girls if all the preceding children have been girls. (2) If the 'unisexual' families exhibiting the above tendency (the highest and lowest figure in each column of Table 1) are excluded, it will be found that the sex ratio is the lower, the greater is the number of preceding male pregnancies. For this comparison, families with four or more children can be used (the encircled areas in Table 1). The only column which does not follow this rule is that of the fifth children of our series, and in this column the sex ratios are based on small numbers of families tested. From Table 2 it can be seen that in Geissler's series of families the downward trend of the sex ratio with the growing number of preceding boys is statistically significant. In our own series there is an identical 5 per cent decrease in the four-child families, but owing to the smaller number of families tested it is not statistically significant.

Table 3 shows that after one boy and two girls the sex ratio of the fourth child is high and after two boys and one girl it is low regardless of the order of birth of the preceding children. Thus the excess of boys or girls is not due to a switch of some families from a gynecophilic to an androphilic period or vice versa, an event postulated by Schützenberg[4].

The data can be explained by postulating that, among other factors, there are the following two

Table 1. SEX RATIO OF THE SECOND, THIRD, FOURTH AND FIFTH CHILD OF A FAMILY COMPARED WITH THE NO. OF BOYS PRECEDING THIS CHILD

No. of boys preceding the child whose sex is under test	Sex ratio and its standard deviation Second child	Third child	Fourth child	Fifth child	
0	100·4 ± 1·2	98·5 ± 2·2	91·3 ± 3·8	94·2 ± 6·3	
1	107·6 ± 1·2	103·1 ± 1·6	104·7 ± 2·2	106·1 ± 3·3	Present author's data
2	—	102·9 ± 2·1	99·9 ± 2·2	105·2 ± 2·7	
3	—	—	105·3 ± 3·7	105·8 ± 3·2	
4	—	—	—	106·4 ± 6·0	
0	103·1 ± 0·6	104·8 ± 1·0	101·4 ± 1·5	104·3 ± 2·4	
1	108·0 ± 0·6	106·8 ± 0·7	107·3 ± 0·9	111·7 ± 1·2	Geissler's data (ref. 1)
2	—	108·9 ± 0·9	102·9 ± 0·8	105·3 ± 0·9	
3	—	—	107·4 ± 1·4	101·4 ± 1·1	
4	—	—	—	107·0 ± 2·2	
5	—	—	—	—	

Table 2. SEX RATIO OF THE FOURTH AND FIFTH CHILD OF A FAMILY COMPARED WITH THE NO. OF BOYS PRECEDING THIS CHILD

No. of boys preceding the child whose sex is under test	Fourth child Sex ratio	Difference	*S.D.* of difference	Fifth child Sex ratio	Difference	*S.D.* of difference
0	101·4			104·3		
1	107·3	5·9	1·9	111·7	7·4	2·6
2	102·9	4·4	1·4	105·3	6·4	1·6
3	107·4	4·5	1·7	101·4	3·9	1·5
4	—			107·0	5·6	2·5

Data from Geissler's report (ref. 1).

Table 3. DEPENDENCE OF SEX RATIO ON THE ORDER OF BIRTH OF THE PRECEDING BOYS AND GIRLS

Preceding children	Total No. of fourth children	Sex ratio of the fourth children
mmf	2,892	101·8
mfm	2,668	96·6
fmm	2,689	101·1
ffm	2,666	105·1
fmf	2,568	107·1
mff	2,749	102·1

affecting p (the probability of a coming child being a boy): (1) p is inherently different in different families. This factor manifests itself in the tendency of certain families to have predominantly boys, and others to get predominantly girls. This was postulated by Edwards[5] in 1958. (2) Apart from the tendency mentioned here, male pregnancies render couples less likely to obtain further boys. This could come about if male pregnancies immunize a small portion of mothers against male (Y) antigens, and this immunization is then harmful to subsequent male fœtuses. The third male fœtus of a mother would be often affected, the fourth more so still, etc.

Our analysis does not give information as to whether the second or even the first boy of a couple could be affected, but data given by Novitski and Sandler[6] may be relevant in this respect. They show that the sex ratio decreases with the increasing age of the family. Supposing that this decrease is mainly caused by the immunization of mothers against male antigens—not necessarily a correct assumption—the data show that generally the first boy is not affected, because the curve is not a 'one hit' curve. On the other hand, the data do show that the second boy can be affected, because the sex ratio of the second child is much lower than that of the first.

The above hypothesis needs an extra assumption, that the undisturbed (not disturbed, for example, by immunization deaths) sex ratio of, say, the fourth child is higher than the sex ratio of the first child. This does not seem inconceivable although there is no direct evidence favouring this assumption.

The hypothesis would lead to certain predictions which lend themselves to investigation. One of them is that among still-births and abortions there should be an excess of boys, and this excess should be greater if boys have previously been born to the family. Some studies on this line have been made, but the results are contradictory[7,8]. Another prediction is that the sex ratio should decrease with increasing parity. This has been demonstrated[6], and there are data indicating that the decrease is dependent on the paternal age and/or birth order rather than on maternal age[9].

A detailed report of this study will be given elsewhere[3]. The investigations have been supported by the Sigrid Jusélius Foundation.

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Kidney Transplantation in the Rat

THIS report describes a new technique for transplantation of the rat kidney from one animal to another by vascular anastomosis[1]. The rats were all members of an inbred line of the Lewis strain, originally obtained from a colony at the Wistar Institute. Skin grafts from one member of this colony to another are accepted uniformly. In the present experiment, the kidney donor weighed approximately 100 gm. It was first anæsthetized with 'Cyclopal' (Upjohn) and then the left kidney, including its vessels and ureter, was exposed through a midline incision. All the vascular branches of the abdominal aorta and vena cava, except the left renal vessels, were tied and divided from the level of the superior mesenteric artery to the origin of the iliac artery and vein.

The donor was then given 1,000 units of heparin intravenously to prevent clotting during the surgical procedure. Small slits were made in the aorta and vena cava above the origin of the iliac vessels; through these openings, plastic tubes were inserted. The tubes were pushed forward until they extended a few centimetres above the origin of the renal vessels. The aorta and vena cava were then divided at the level of the mesenteric artery. The vessels tend to shorten when cut so that the plastic tubes protrude. To prevent the tubes from slipping, ties were placed on the plastic supports where they enter the slits in the abdominal vessels. The remaining vascular connexions, located below the distal ligatures, were cut; and then the kidney was lifted from the abdomen of the donor and placed in the neck of the host (Fig. 1).

The recipient was a 450-gm. Lewis strain rat. It was anæsthetized, placed on its back, and the carotid artery and jugular vein were isolated, clamped and divided. The diameter of these vessels in the neck of the recipient approximates the calibre of the abdominal aorta and vena cava of the 100-gm. donor. The arterial anastomosis was begun by placing

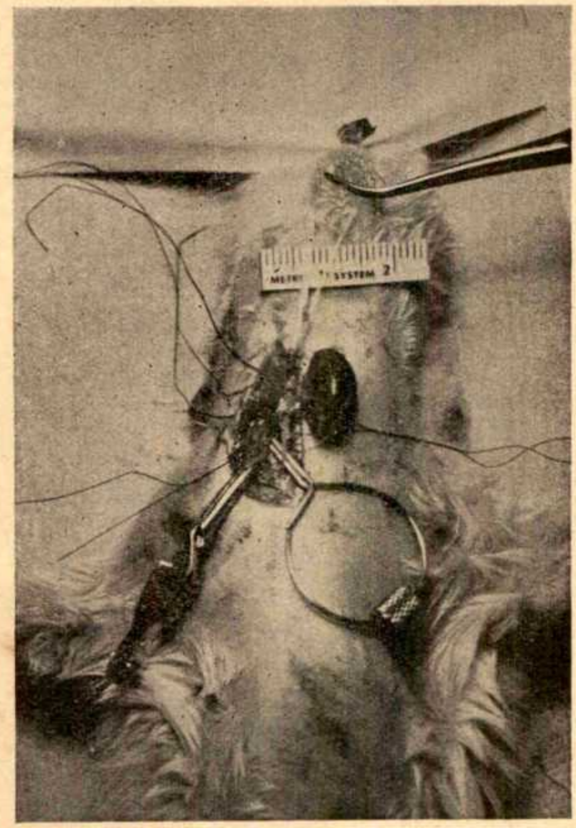

Fig. 1. The donor kidney is shown in the neck of the recipient. The plastic tubes are inserted into the recipient vessels and the arterial holding sutures have been secured. After completion of the anastomoses the plastic tubes will be removed

two silk sutures to connect the aorta and the proximal stump of the carotid artery (Fig. 1). The plastic tube protruding from the anterior end of the aorta was inserted into the carotid artery. The arterial vessels were anastomosed by means of a continuous suture technique. The plastic tube aids in the suturing by preventing collapse of both the donor and the host arteries; it also helps insure against accidental closure of the vascular lumen. On completion of the anastomosis, the plastic tube was removed through the open end of the aorta. The aorta was then tied off just above the renal vessels. When the arterial anastomosis was completed, the vena cava was connected to the jugular vein by a procedure similar to that used for the arteries. On removal of the vascular clamps, blood flowed from the host's carotid artery into the donor's aorta and renal artery. The return flow was through the renal vein and vena cava of the donor to the jugular vein of the recipient. The kidney was secured under the skin and its ureter brought to the exterior through a separate stab wound.

The important features of the procedure are the following: (1) the use of a T-section of vessels, so that the aorta and vena cava are available for anastomoses; (2) the temporary insertion of plastic tubes to maintain the patency of the vessels during surgery; (3) the transfer of a kidney from a 100-gm. rat to a 400-gm. rat, so that the large abdominal vessels of the donor will match the carotid artery and jugular vein of the recipient.

When these precautions are followed it is possible to obtain long-term transplants within the Lewis strain. In some of our early experiments, difficulties were encountered when scar tissue closed the ureteral opening on the skin. In some instances, repeated reconstruction of the ureteral opening has been necessary to avoid hydronephrosis. Infection in the transplant was controlled by administering sulphamethazine, substituting a saturated solution of this compound for the drinking water.

In a series of isologous transplants, six out of seven consecutive operations were successfully completed. Four of these kidneys were biopsied 35 days after the operation. There was no evidence of the rejection process in histological sections. One rat survived 71 days after its own kidneys had been removed. This animal died of an unknown cause; but the transplant had not been rejected.

It is believed that this type of vascular anastomosis on very small vessels may find application in fields of surgery other than transplantation.

This work was carried out during the tenure of an advanced research fellowship of the American Heart Association to Dr. Nathan. Grants from the U.S. Public Health Service ((*A*-2938) and the American Heart Association (61-*G*-131) helped support this work. The authors thank Mrs. Barbara Bouldin and Miss Kathleen Robb for their valuable assistance.

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Nerve Endings on Chromaffin Cells in the Rat Adrenal Medulla

SINCE Fusari[1] and Dogiel[2] published accounts of the adrenal medullary nerve plexuses many different opinions have been expressed concerning the question of nerve-endings in relation to chromaffin cells. The majority of workers claim to have seen extracellular endings in the form of platelets or *boutons* and Willard[3] described end-*boutons* and *boutons de passage*. Nerve fibres ending in a terminal anastomosing net of neurofibrils—the so-called 'terminal reticulum'—were described by Stöhr[4] and Sato[5]. Hillarp[6], however, using a methylene blue technique found no evidence of nerve-endings and stated that the nerve fibres run in fine bundles in a terminal Schwann plasmodium, the composite mass forming the 'nervous ground plexus'.

Using a cholinesterase technique for the demonstration of nerve fibres and supporting elements, Coupland and Holmes[7] demonstrated a coarse three-dimensional network composed of Schwann cell cytoplasm and fine nerve fibres in the adrenal medulla. This net was found to correspond in general form with Hillarp's 'nervous ground plexus'. However, the same workers also noted that the histochemical technique for the demonstration of true cholinesterase revealed not only the coarse network, but also fine branches which left the main net and exhibited swellings, corresponding in size and shape to Willard's end-*boutons* and *boutons de passage*, in relation to chromaffin cells.

The problem has been pursued further using electron microscopic techniques. Adrenal glands of Sprague–Dawley albino rats were sectioned and fixed in Caulfield's buffered osmium tetroxide (*p*H 7·3)–sucrose mixture, embedded in araldite and sectioned on a Cambridge–Huxley ultramicrotome. Sections were stained on the grid with an aqueous 1 per cent solution of potassium permanganate and examined with a Metropolitan–Vickers *EM* 6 electron microscope.

Nerve fibres and endings have been observed in large numbers in all specimens. In the rat adrenal medulla the majority of fibres are unmyelinated and less than 1 per cent of all those seen in sections exhibited evidence of myelination. The unmyelinated axons are always associated with Schwann cell cytoplasm (Fig. 1) and run in bundles between groups of chromaffin cells. From these bundles of axons fine axis cylinders arise and may be traced for a variable distance between adjacent chromaffin cells before ending. As each fine axis cylinder runs between chromaffin cells it is accompanied by an extension of Schwann cytoplasm; this initially surrounds the whole fibre, but as the nerve-ending is approached it covers only one aspect of it with a tongue-like process.

Nerve fibres exhibit synaptic-type endings on or 'in' chromaffin cells (Fig. 2). At the nerve-ending the axis cylinder contains numerous pre-synaptic vesicles and mitochondria. The pre- and post-synaptic membranes are thickened (Fig. 3): especially the latter. Some multiple synaptic-type endings have been seen on single chromaffin cells and appear to involve the same nerve fibre.

A minority of nerve fibres invaginate the plasma membrane of chromaffin cells before terminating at a variable distance from the cell centre in a typical synaptic-type of ending. During its 'intra-cellular course' the nerve fibre is surrounded by a tubular invagination of the chromaffin cell's plasma membrane: protoplasmic continuity never occurs. This unusual arrangement may well account for the so-called 'intra-cellular nerve-endings' described by some earlier workers, including Fusari[1] and Alpert[3].

The findings indicate that chromaffin cells, which are developmentally and functionally closely related to the autonomic nervous system, also resemble neurones in so far as they are associated with synaptic-type nerve-endings.

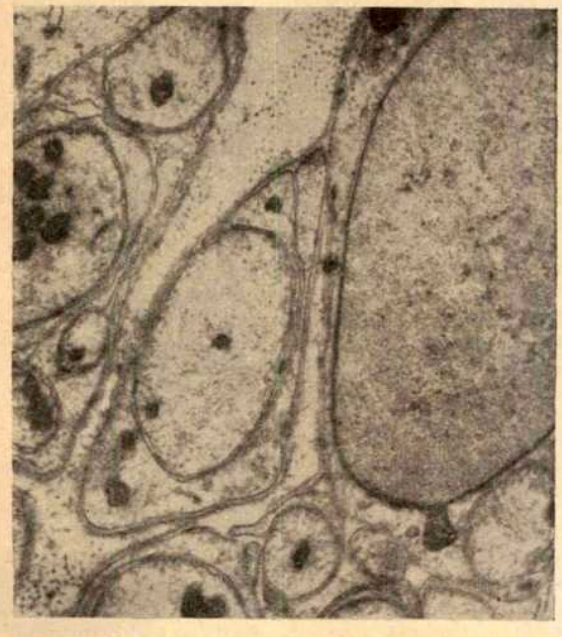

Fig. 1. Groups of non-myelinated axons and associated Schwann cell cytoplasm and nucleus in rat adrenal medulla. (EM. mag. × 13,000, photo mag. × 1·2)

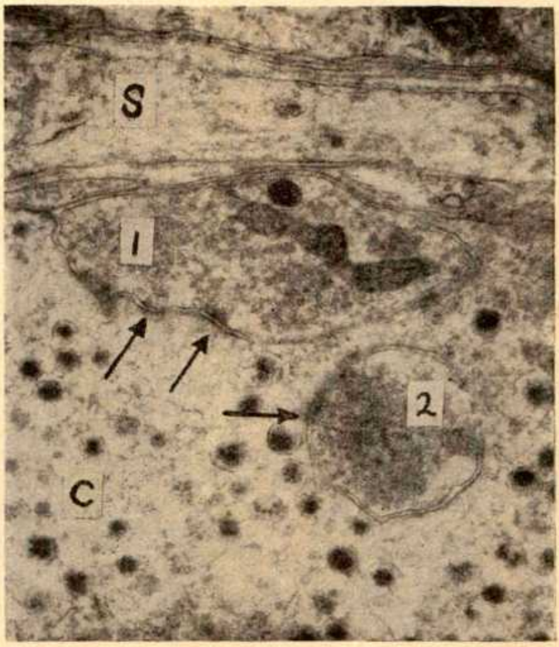

Fig. 2. Nerve ending on surface of (1) and in interior of (2) a chromaffin cell (*C*). Synaptic thickenings indicated by arrows. *S*, Schwann cell cytoplasm. (EM. mag. × 16,000, photo mag. × 1·2)

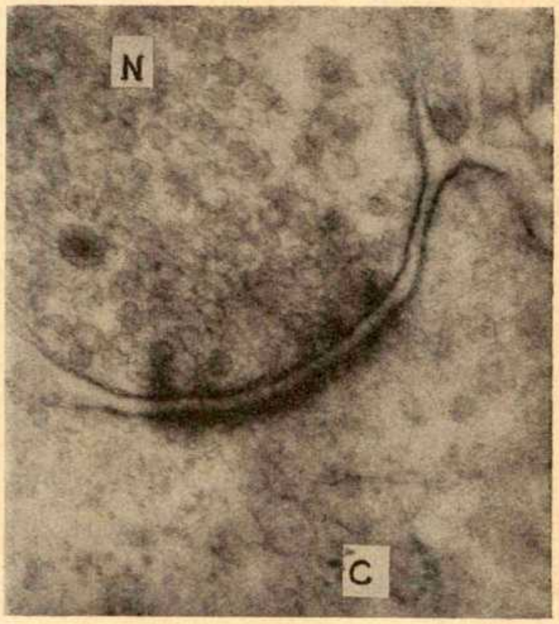

Fig. 3. Synaptic-type nerve ending on a chromaffin cell. Nerve fibre (*N*) containing pre-synaptic vesicles. *C*, chromaffin cell cytoplasm. (EM. mag. × 60,000, photo mag. × 1·3)

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Furunculosis in Salmon Kelts

INVESTIGATIONS into the occurrence of furunculosis among spawning or spawned salmon were undertaken in Scotland during the spawning seasons of 1958–59 and 1959–60[1]. In the former season 96 salmon kelts were examined and 85 of these had furunculosis; in the latter season, up to the time of going to press, 9 out of 24 kelts examined had furunculosis. In the same season 14 kelts were examined in England and Wales and 11 of these gave a positive reaction.

In order to determine to what extent furunculosis occurred among kelts in Ireland it was decided to collect dead and dying kelts from four Irish rivers during the 1960–61 spawning season. In all, between December 15, 1960, and May 17, 1961, a total of 159 kelts were examined from the following rivers:

River	No. of kelts examined	Hatchery or naturally spawned
River Owenea (Co. Donegal)	121	Majority hatchery stripped
River Erne (Co. Donegal)	17	Majority naturally spawned
River Shannon (Co. Limerick)	12	All hatchery stripped
River Boyne (Co. Meath)	9	All naturally spawned

With the exception of one fish from the Owenea River all were negative for furunculosis. It is hoped to continue these investigations during the next spawning season 1961–62.

I thank Mr. E. Hirsch and his staff of the Veterinary College of Ireland, Ballsbridge, Dublin, who undertook bacteriological examination of these fish.

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Differences in the *p*H Optimum of the Photosynthetic Fixation of Carbon Dioxide in Isolated Whole and Broken Chloroplasts

RECENTLY, Gibbs *et al.*[1–4] emphasized the differences between whole and broken chloroplasts regarding their sensitivity against inhibitors during fixation of carbon dioxide and the distribution of carbon-14 in the sugars so formed. In our experiments an additional difference, the markedly lower *p*H optimum of fixation of carbon dioxide in whole chloroplasts, as compared with broken chloroplasts, was observed.

Chloroplasts were isolated from spinach leaves by the method of Arnon *et al.*[5] and Gibbs *et al.*[1,2], using 0·35 *M* sodium chloride solution with 0·015 *M* *tris*-hydrochloride *p*H 7·5 and 0·01 *M* sodium ascorbate. In order to obtain broken chloroplasts, washed whole chloroplasts were resuspended in 0·01 *M* sodium ascorbate solution. All experiments were carried out at light saturation (25,000 lux).

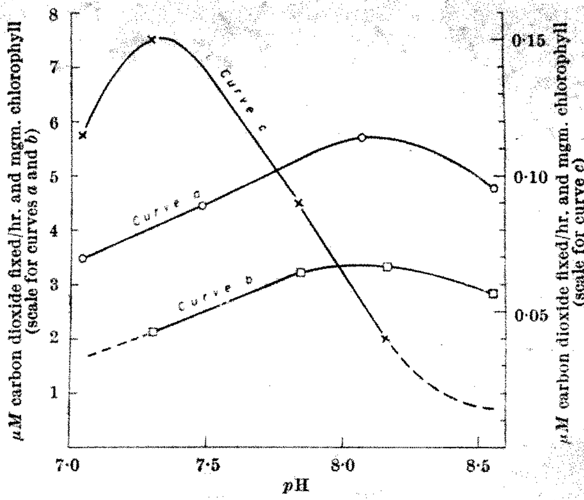

Fig. 1. *p*H optimum of the photosynthetic fixation of carbon dioxide by whole chloroplasts without (curve *c*), and with co-factors (curve *b*), and by broken chloroplasts (curve *a*). $t = 20°$ C.; gas phase nitrogen. 1 ml. of the reaction mixture contained: curve *c*, 200 μgm. chlorophyll; in μ*M*, sodium chloride 350; *tris* 35; manganese chloride 0·8; magnesium chloride 2·0; sodium ascorbate 10; $Na^{14}HCO_3$ 2, specific activity 24·47 μc./μ*M*. Curve *b*: like curve *c* plus ADP 2·0; TPN 0·12; FMN 4×10^{-4}; R-5-P 1·0; GSH 3·0; $Na^{14}HCO_3$ 10, specific activity 0·28 μc./μ*M*. Curve *a*: broken chloroplasts, conditions like curve *b*, plus chloroplast extract equivalent to 400 μgm. chlorophyll minus sodium chloride

The results of a typical experiment are shown in Fig. 1. The curve for broken chloroplasts (*a*) shows a flat maximum at 8·1, whereas the curve for whole chloroplasts (*c*) has a marked optimum around 7·3. When the co-factors are given to whole chloroplasts, the total fixation is increased by more than 10-fold, thus reaching the level of broken chloroplasts. In this case the *p*H optimum is identical with that of the broken chloroplasts (curve *b*).

This behaviour probably results from the presence of only a small portion of physiologically intact chloroplasts compared with a big portion of physiologically defect and broken chloroplasts in the preparations of the so-called 'whole' chloroplasts. Jacobi and Perner[6], as well as Erikson *et al.*[7], came to the same conclusion in their morphological work. Then, after the addition of co-factors, the fixation by broken chloroplasts exceeds that of the whole chloroplasts to such an extent that practically only the *p*H optimum of the first is measured. Also the strong variation of the rate of fixation of carbon dioxide of the whole chloroplasts expressed in Table 1 may best be explained by the assumption that the percentage of really intact chloroplasts varies greatly, depending on the condition of the leaf material.

Furthermore, it can be seen from Table 1 that the *p*H optimum of the fixation of carbon dioxide in whole and broken chloroplasts is not shifted by a variation of the absolute level of fixation. For whole chloroplasts it is between 7·0 and 7·5, whereas for broken chloroplasts between 8·0 and 8·5. So far as whole chloroplasts are concerned, our results are in agreement with Gibbs and Calo[1]. In the case of broken chloroplasts, however, their results[2] differ from ours, since they obtained the same *p*H optimum as with whole chloroplasts. While measuring light phosphorylation, Allen *et al.*[8] found a *p*H optimum around 8·3 for broken chloroplasts.

The difference between whole and broken chloroplasts, in the *p*H optimum of their photosynthetic fixation of carbon dioxide, may be explained in three ways: (1) the *p*H dependent permeability for the

Table 1. pH-Dependence of the Photosynthetic Fixation of Carbon Dioxide of Whole Chloroplasts without Co-factors and of Broken Chloroplasts with Co-factors in Different Preparations with a Varying Activity of Fixation of Carbon Dioxide. (For Reaction Conditions, see Fig. 1)

	Date of experiment	μM carbon dioxide fixed per hr. and mgm. chlorophyll at the corresponding pH					
		7·15	7·30	7·70	8·00	8·30	8·50
Whole chloroplasts	12.8.1960	1·35	1·27	0·78	0·46	—	—
	30.8.1960	2·38	2·13	1·55	1·30	—	—
	7.8.1961	0·04	0·05	0·02	0·01	—	—
	9.8.1961	0·05	0·07	0·02	0·01	—	—
	29.8.1961	0·13	0·15	0·11	0·06	—	—
Broken chloroplasts	5.5.1961	—	2·65	6·37	8·80	9·90	—
	10.5.1961	—	1·35	3·05	6·95	7·90	—
	30.6.1961	—	—	3·00	4·47	4·90	4·33
	3.7.1961	—	—	2·83	3·73	3·85	3·60
	23.8.1961	—	—	3·00	4·45	4·90	4·68

bicarbonate ion or the carbon dioxide molecule, respectively, is the limiting factor for the assimilation of carbon dioxide in whole chloroplasts; there is no such restriction in broken chloroplasts since the permeability barrier is absent.

(2) By 'breaking' the chloroplasts, reactions of the mechanism of photosynthesis with a more alkaline pH optimum become limiting for the fixation of carbon dioxide compared with the pH-optimum of those which are limiting in whole chloroplasts.

(3) The reaction mechanism of the photosynthetic fixation of carbon dioxide and reduction in whole chloroplasts is different from that in broken chloroplasts[9,10].

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Changes in Endogenous Gibberellins in Relation to Chilling of Dormant Seeds

EMERGENCE from the rest period in potato tubers has been correlated with an increase in the level of endogenous gibberellin[1]. There have been no reports of similar observations with respect to breaking of seed dormancy although a 'germination stimulator' has been demonstrated in after-ripened ash embryos[2]. No difference in gibberellin-level was found between light-activated and dark-imbibed lettuce seeds[3]. Increases in gibberellins, however, have been demonstrated in connexion with the induction of flowering both by low-temperature treatment[4] and by long photoperiods[4-6].

An examination was made of gibberellins in hazel (*Corylus avellana*) and beech (*Fagus sylvatica*) seeds, both of which are dormant at the time of harvest and require several weeks' moist storage at 0–5° C. for after-ripening. A chilling period of about 12 weeks was necessary for intact nuts to show a high germination percentage. Wheat coleoptile and oat mesocotyl section bio-assays had been carried out on 80 per cent methanol extracts of chilled and unchilled seeds, no significant differences being found in the levels of growth promoters and inhibitors. In both beech and hazel seed extracts an auxin-like growth promoter was found at R_F 0·4–0·6 following paper chromatographic separation with the solvent isopropanol/1/100 × 0·88 s.g. ammonium hydroxide (4 : 1).

For gibberellin studies seeds were crushed and extracted at room temperature with 80 per cent methanol. Following removal of the methanol by distillation under reduced pressure the aqueous residue was purified by a procedure based essentially on that described by Radley[7]. The purified material was dissolved in a small volume of 'dry' methanol and stored at −15° C. Attempts to bio-assay such 'purified' extracts directly with dwarf (d–1) maize and dwarf (variety, Meteor) pea seedlings were unsuccessful due to the presence of inhibitory or toxic substances having a damaging effect on the leaf to which the extract was applied. Further purification was therefore carried out using paper chromatography. Several solvents were tried, butanol saturated with 1/100 × 0·88 s.g. ammonium hydroxide being found the most satisfactory for separating gibberellin and inhibitor zones in the case of hazel extracts.

Two bio-assay techniques, which were more convenient for use following paper chromatographic separation, were used. These were: (1) a lettuce hypocotyl test[8]; (2) a maize leaf section test. The latter was based on a technique described by Boyarkin and Dmitrieva[9] with the difference that 'dwarf-1' mutant maize seedlings were used in place of normal maize seedlings. This test was used in preference to the lettuce hypocotyl test, being more sensitive to gibberellins and apparently less sensitive to the presence of inhibitory materials in the extract. The maize-leaf section test was carried out as follows: Maize grains were sown in sand in the dark at 26–28° C. and dwarf seedlings were selected after 7–8 days, when the first leaf had unfolded. Seedlings were divided into several arbitrary groups on the basis of degree of unfolding of first and second leaves, as a means of randomizing seedling sections among the various treatments. The mesocotyl of each seedling was cut 5 mm. below the coleoptilar node and the coleoptile plus enclosed leaves cut 20 mm. above the node. 12 such leaf-plus-mesocotyl sections were used per treatment and were placed in a vertical position in 3 ml. of the solution under test with the mesocotyl end of the section dipping in the solution. Prior to the bio-assay chromatogram segments (R_F 0–0·1, 0·1–0·2, etc.) were cut into small pieces, placed in 1·7 × 5 cm. specimen tubes and eluted overnight at 5° C. with 3 ml. distilled water. Specimen tubes with leaf sections were kept in the dark at 25° C. and the length of the first leaf was measured after 2 days. The two extreme measurements from each treatment were discarded, the mean increase in length of the first leaf being based on 10 replicates. The response of 'dwarf-1' maize leaf sections to various concentrations of gibberellic acid is shown in Fig. 1.

In a preliminary experiment an extract of wheat grains, known to contain gibberellins[7], was assayed using the above techniques. Gibberellin activity was demonstrated at R_F 0·2–0·3 in butanol/dilute ammonia. (Gibberellic acid has an R_F of about 0·26 in this solvent.) The gibberellin contents of two independent extracts of unchilled hazel seeds (imbibed

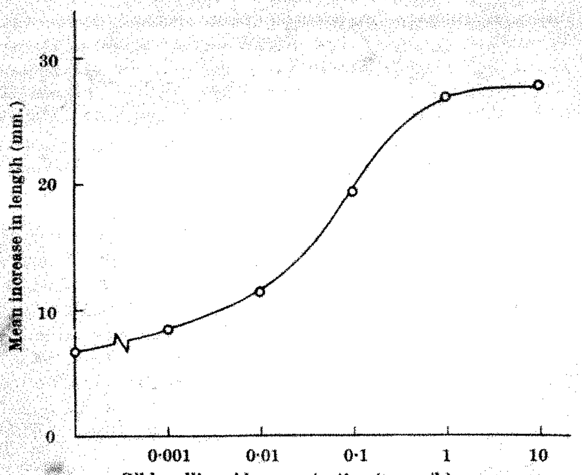

Fig. 1. Effect of gibberellic acid on extension of first leaf in leaf-plus-mesocotyl sections of 'dwarf-1' maize seedlings. *L.S.D.* (for $P = 0·05$) is 3·5

48 hr. at room temperature) were compared with those of extracts of 6-week chilled and 12-week chilled seeds. The equivalent of 100 gm. dry weight of seed was used for each bio-assay. No significant gibberellin activity was detected in extracts of unchilled seeds but two zones of activity, at R_F 0–0·1 and R_F 0·2–0·4, were found in chilled seeds (Fig. 2). The gibberellin growth response obtained was very small, being roughly equivalent to 0·02–0·1 µgm., gibberellic acid per kgm. dry weight of seed. The failure of unchilled seed extracts to give a gibberellin response was not due to the presence of inhibitory material. A chromatogram eluate (R_F 0·1–0·4) of unchilled seed extract only slightly decreased the response of maize leaf sections to 0·01 mgm./l. gibberellic acid.

In the case of beech a comparison of gibberellin contents was made between unchilled (imbibed 48 hr. at room temperature) and 12-week chilled nuts. The chilled nuts were germinating with radicles 0·5–3 cm. in length (beech nuts, unlike hazel, germinate readily at the chilling temperature). Gibberellin activity was found in both unchilled and chilled nuts although there appeared to be qualitative differences in the chromatographic pattern of activity. In unchilled extracts there was a significant response at R_F 0·2–0·3 and evidence of activity at R_F 0·5–0·6, whereas in chilled extracts there was a response at R_F 0·4–0·5 only.

In both hazel and beech nuts there were changes in gibberellin content during after-ripening, although only in hazel was there a simple increase in the level of gibberellin. These observations, coupled with the fact that gibberellic acid will stimulate the germination of dormant hazel and beech seeds[10], provide some evidence that endogenous gibberellins are involved in the after-ripening process in these seeds.

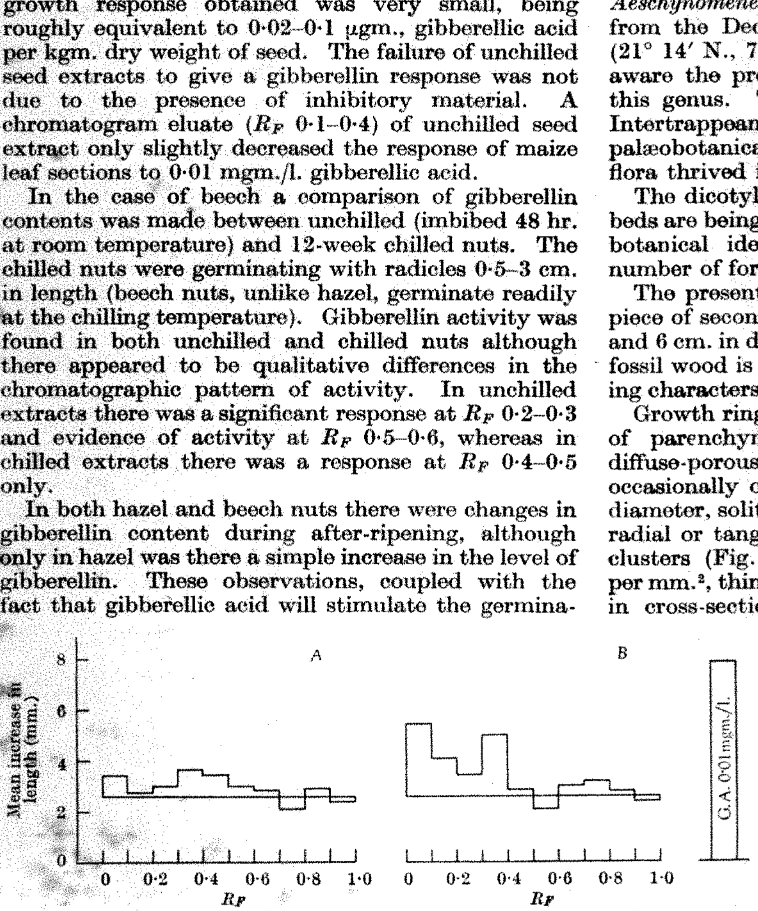

Fig. 2. Maize leaf section bioassay of purified extracts of hazel seeds. *A*, Unchilled; *B*, chilled 12 weeks. Following paper chromatography with butanol/dilute ammonia. Extension of control sections, 2·6 mm. *L.S.D.* (for $P = 0·05$) is 1·2

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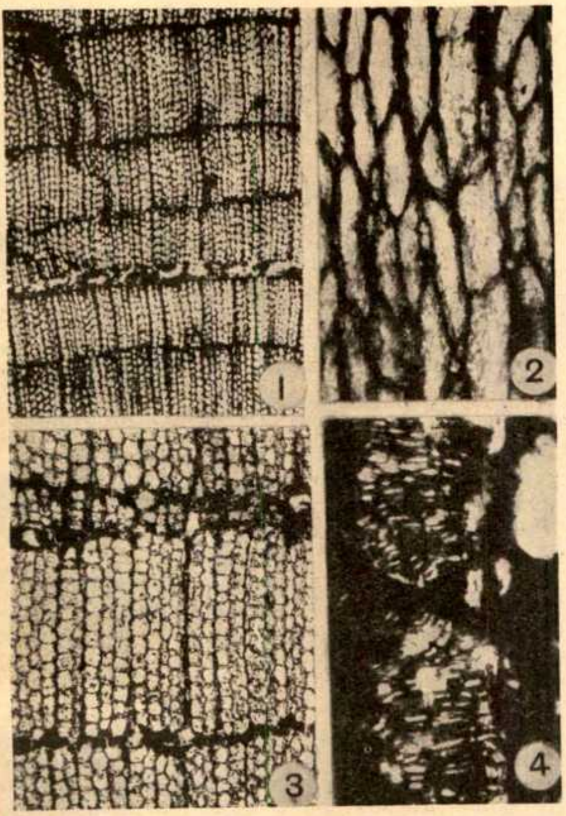
Aeschynomene tertiara, a Fossil Wood from the Deccan Intertrappean Beds of Mahurzari, near Nagpur

In the present communication, a new species of *Aeschynomene* (Leguminosae), *A. tertiara*, is described from the Deccan Intertrappean beds of Mahurzari (21° 14′ N., 79° 1′ E.) near Nagpur. So far as I am aware the present description is the first record of this genus. The existence of *Aeschynomene* in the Intertrappean beds furnishes further proof to the palæobotanical evidence that most of the Deccan flora thrived in pools, lakes and swampy habitats.

The dicotyledonous woods from the Intertrappean beds are being intensively studied with regard to their botanical identification and interpretation and a number of form genera have now been determined.

The present material consists of a single petrified piece of secondary xylem measuring 11 cm. in length and 6 cm. in diameter. Structural preservation of the fossil wood is remarkably good. It shows the following characters:

Growth rings inconspicuous, with tangential bands of parenchyma simulating growth rings; wood diffuse-porous (Fig. 1). Vessels usually small, occasionally of medium size, 45–110µ in tangential diameter, solitary as well as in pairs and sometimes in radial or tangential rows of 3–5 cells, very rarely in clusters (Fig. 3), widely spaced, about 2–3 vessels per mm.², thin-walled, mostly angular, sometimes oval in cross-section. Vessel elements short, 150–210µ long with truncated ends. Perforation plates exclusively simple, horizontal to slightly inclined. Intervascular pit-pairs bordered, alternate to opposite or sub-opposite and often almost of scalariform-type with linear to extended apertures (Fig. 4). Tyloses absent but vessels commonly plugged with gummy deposits. Parenchyma scanty, occurring either as 1–2 (3–4) seriate, narrow, continuous or broken tangential lines (Figs. 1, 3), almost parallel to each other or as 1–2 or few diffuse cells, also sometimes in



Figs. 1–4. *Aeschynomene tertiara.* Fig. 1, Cross-section showing thin tangential parenchyma bands simulating growth rings. (× 13·5). Fig. 2, Tangential longitudinal section to show the distribution and type of xylem rays. (× 75). Fig. 3, Cross-section magnified to show the vessels, fibres and parenchyma cells. (× 35). Fig. 4, Intravascular pit-pairs. (× 260)

association with the vessels; cells usually tangentially elongated in cross-section, 40–50μ × 15–25μ in size, rarely crystalliferous; both fusiform and strand parenchyma appears to be present. Xylem rays homogeneous composed of procumbent cells, mostly uniseriate (Fig. 2), rarely partially biseriate and unstoried; 1–11 (usually 1–6) cells or 40–560μ high; up to 8–12 rays per mm. Ray cells usually oblong or narrow and vertically elongate as seen in tangential section. Imperforate tracheary elements fibres forming the ground-mass of the secondary xylem, quadrangular to polygonal in cross-section (4–6-sided), 45–80μ × 41–100μ in size, walls thin with a large lumen (Fig. 3); cells fusiform (Fig. 2), short, about 188–408μ in length; unstoried; pits simple, more numerous on the radial than on the tangential walls. In cross-section fibres look very much like the vessels except that the vessels are slightly thicker-walled and often plugged with black deposits.

Structural features of the fossil wood indicate that its closest affinities are with the modern genus *Aeschynomene*. A survey of all available woods of this genus shows that the nearest resemblance of the fossil is with *Aeschynomene* sp. from Siam (Yale Wood Collection No. 16552), although there are some obvious differences between the two, namely, in size of the vessels, length of fibres and in relative thickness and spacing of tangential parenchyma bands.

I thank Profs. Elso S. Barghoorn and I. W. Bailey for many helpful suggestions. I also thank Dr. William L. Stern (then of Yale School of Forestry), curator, Division of Woods, Smithsonian Institution, Washington, D.C., for kindly sending the modern woods of the genus *Aeschynomene*.

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MICROBIOLOGY

Relationship between Sugar Utilization and the Action of Cycloheximide on Diverse Fungi

CYCLOHEXIMIDE ('Actidione', Upjohn) is an antibiotic which inhibits growth of protozoa[1], mammalian cells[2] and many fungi[3]. It has been proposed that cells having strong aerobic fermentative capability are especially sensitive to this antibiotic[4]. In the present work, oxygen consumption and aerobic carbon dioxide evolution by 17 fungi grown in glucose medium was measured by standard manometric techniques[5]. Drug sensitivity was determined by the tube-dilution method. No correlation was found between respiratory quotient and susceptibility to cycloheximide (Table 1). Respiration and fermentation of glucose by *Saccharomyces pastorianus* were not inhibited appreciably by 2 μgm. cycloheximide/ml. although 0·5 μgm./ml. halted growth. Cells of *S. pastorianus* fermented maltose when cultivated in maltose medium, but not when cultivated in glucose medium. Fermentation by maltose-adapted cells was not retarded significantly by 2 μgm. antibiotic/ml. but the inductive process was suppressed completely (Table 2). Cells exposed to the drug were still viable at the end of the experiment. Cycloheximide does not inhibit significantly activity of glycolytic enzymes but does prevent new enzyme synthesis. Protein or nucleic acid synthesis, therefore, seems to be one vulnerable site of cycloheximide action[6].

Table 1. RESPIRATORY QUOTIENT (RQ) OF DIVERSE FUNGI AND MINIMAL CONCENTRATION OF CYCLOHEXIMIDE-INHIBITING GROWTH (MIC)

Organism	RQ	MIC (μgm./ml.)
Schizosaccharomyces pombe	> 40	15
Zygosaccharomyces fermentati	3·2	300
Z. marxianus	3·1	> 300
Saccharomyces pastorianus	3·1	0·3
Z. globiformis	2·4	3
Torulaspora rosei	2·4	0·5
Z. lactis	1·9	> 350
Z. casei	1·8	> 300
Saccharomyces fragilis	1·8	> 350
Hansenula saturnus	1·5	0·5
Candida stellatoidea	1·2	275
Cryptococcus sp.	1·2	350
Mycoderma cerevisiae	1	0·5
Pichia membranaefaciens	1	0·4
Endomycopsis lindneri	1	50
Trichosporon sp.	1	75
Debaryomyces klockeri	1	2

Respiratory quotient was determined with 5 mgm. dry weight of washed cells, 100 mgm. glucose, air atmosphere, 0·067 *M* $NaK(H_2PO_4)_2$ buffer *p*H 4·5, with or without 20 per cent potassium hydroxide in the centre well of the reaction vessel.

Table 2. SUPPRESSION OF INDUCTION OF MALTASE BY CYCLOHEXIMIDE

Inducing medium	μl. CO_2 evolved/10 min. from Glucose	μl. CO_2 evolved/10 min. from Maltose
Glucose	177	5
Glucose + 2μgm. cycloheximide/ml.	149	4
Maltose	153	98
Maltose + 2 μgm. cycloheximide/ml.	131	4

Glucose grown *S. pastorianus* cells were incubated aerobically for 2 hr. at 30° C. in maltose or glucose medium with or without antibiotic. Carbon dioxide evolution was measured manometrically with 5 mgm. dry weight of washed cells, 100 mgm. sugar, 0·067 *M* $NaK(H_2PO_4)_2$ buffer *p*H 4·5 and a nitrogen atmosphere.

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Department of Microbiology,
University of Minnesota, Minneapolis.

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Identification of Purported *Agaricus campestris* Strains (*NRRL* 2334, 2335, 2336) as *Beauveria tenella* (Delacroix Siem.)

Comparative morphological and physiological investigations were conducted with different European strains of the cultivated mushroom and with three strains of *Agaricus campestris* (*NRRL* 2334, 2335, 2336) from the Northern Regional Research Laboratory, Peoria, Illinois. These have been described as mutants arising from normal cultivated mushrooms under conditions of submerged culture[1].

These strains differed greatly from the normal strains in substrate demand, speed of growth, mycelium yield, enzymatic qualities, and colony growth as well as in microscopic appearance on solid media and liquid culture. In addition, no fruiting bodies were produced[1-7].

It is, however, improbable that these *NRRL* strains which appeared in 1948 in Humfeld's laboratory during submerged culture experiments, and described by him as spontaneous mutants, could be mutants. The possibility that so many independent qualities were altered during one or few mutation steps is very remote. In my own experiments with an even larger selection of strains it was impossible to detect such mutants.

An exhaustive examination of these *NRRL* strains in the Centraalbureau voor Schimmelcultures, Baarn, Holland, showed that under certain conditions a form of asexual reproduction occurred. The oval, smooth, hyaline conidiospores (1·8–4·5μ × 1·8–3·2μ) and oblong macrospores (6·6–9·4μ × 2·4–3·2μ) arising thereby developed on sporogenous cells which were usually swollen and bottle-shaped.

According to my observations, these strains do not belong to the Basidiomycetes, but to the Fungi Imperfecti, family Moniliaceae, genus *Beauveria*. Comparisons with authentic *Beauveria* strains confirmed the identity of the *NRRL* strains as members of the genus *Beauveria*.

A repetition of these investigations with *NRRL* 2334, 2335, 2336 strains, again received from Peoria, and the agreement of my own findings with those from previous investigators, eliminated the possibility of an infection occurring since the first description of these strains[2].

It was also confirmed that the *Beauveria* strains, like other Moniliaceae, were able to live saprophytically on the fruiting bodies of cultivated mushrooms. It is therefore possible that the *Beauveria* infection was carried with the inoculum (tissue-culture of mushroom fruiting bodies[1,5]) into the submerged cultures or entered the culture through some other agent. Probably the infection overgrew the slow-growing mushroom mycelium. *Beauveria* species are commonly encountered where mushrooms are cultivated[8].

On the basis of these findings, it is not surprising that attempts to produce mushroom mycelium commercially in submerged culture with the strains *NRRL* 2334, 2335, 2336 have been abandoned because of the lack of the typical mushroom flavour in the mycelium harvested[9].

Peter Molitoris

Institut für Mikrobiologie der Universität,
Göttingen.

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MISCELLANEOUS

Publication of Proceedings of Conferences and Symposia

I have read with the greatest interest and indeed gratitude the remarks of Sir Lindor Brown concerning the publication of proceedings of conferences and symposia, and the editorial comments on the same subject[1].

Much of the trouble I believe arises because, as Sir Lindor points out, complete publication of a symposium is sometimes achieved only one or two years after the event; in consequence, the report is out of date when it appears, and few of those who presented papers have been able to resist the temptation to publish their material elsewhere. But this delay, though unfortunately common, is quite unnecessary. Some years ago I attended a symposium on biological problems of grafting in Liège, which I had hoped would have served as a model for the future. Communications had to be submitted a few weeks before the symposium, and when we arrived they were all in galley proof. Each participant received a proof of his own contribution with firm instructions to correct it immediately and return it to the secretariat. The discussion was taken down more or less verbatim, typed, and distributed to participants just after dinner each evening. We were then expected to edit our own discussion and return an acceptable version to the secretariat of the conference by breakfast time the following morning. The result was that when we left Liège all the contributed articles were set up in type, and the discussion was ready to go to the printers. Publication of the report of the symposium was not quite as quick as had been hoped; but the delay was partly due to the fact that two languages were used at the meeting. I should have thought that if this pattern were followed at a meeting where only one language was used it should be possible to have an official report of the symposium on sale 4–6 weeks after the meeting.

I fully agree with the view that there should be many symposia of which no reports are published; clearly, however, publication is sometimes worth while, and in this event it should, and could, be done quickly.

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Edinburgh.

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[1] *Nature*, **193**, 709, 724 (1962).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Wednesday, April 25

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m. Prof. L. F. Lamerton: "Health Aspects of Ionizing Radiation—Facts and Fancies".

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Television Standards Conversion" opened by Mr. A. V. Lord and Mr. B. Marsden.

Society of Chemical Industry, Food Group—Nutrition Panel (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by meeting on "Foods Low in Phenylalanine". Dr. L. I. Woolf. "Clinical Aspects"; Dr. F. A. Robinson: "Manufacture and Technological Difficulties".

Oil and Colour Chemists' Association (at the Criterion Restaurant, London, W.1), at 8 p.m.—Twenty-fourth Annual General Meeting.

Thursday, April 26

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10 a.m.—Symposium on "Some Developments in Techniques for Temperature Measurement".

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. E. O. Lilge (University of Alberta): "Hydrocyclone Fundamentals".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. W. S. Cassels: "Arithmetic on an Elliptic Curve".

Society of Instrument Technology, Control Section (at Manson House, 26 Portland Place, London, W.1), at 5 p.m.—Exposition—"Self-Adaptive Control Systems".

University of London (at the London School of Economics and Political Science. Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. Gilbert Ryle. "A Rational Animal" (Fifth Auguste Comte Memorial Lecture).*

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. B. Bleaney, F.R.S.: "Radiospectroscopy" (Fifty-third Kelvin Lecture).

Society of Chemical Industry, Microbiology Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Sir Cyril Hinshelwood, F.R.S.: "The Chemical Basis of Cell Properties".

Thursday, April 26—Friday, April 27

British Institute of Radiology (at Church House, Westminster, London, S.W.1—Twenty-third Annual Congress and Exhibition.

Friday, April 27

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Symposium on "Flameproofing, Intrinsic Safety and other Safeguards in Electrical Instrument Practice".

Institution of Mechanical Engineers, Graduates' Section (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. A. Salek: "The Air Cylinder—a Versatile Aid to Production".

Monday, April 30

University of London (at the London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. John R. Raeburn: "Economic Development and Growth: Agricultural Aspects".*

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5 30 p.m.—Discussion on "The Applications Engineer—Control Schemes and Automation".

Monday, April 30—Saturday, May 5

Production Exhibition (at Olympia, London, S.W.7).*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer or Lecturer in the Department of Philosophy—The Registrar, University College of Wales, Aberystwyth (April 30).

Keeper (with an honours degree and/or Museums Diploma, and some knowledge of anthropology and primitive art) of Ethnology—The Director, Liverpool Museums, William Brown Street, Liverpool 3 (April 30).

Lecturer or Assistant Lecturer (with special interests and qualifications in palæontology and stratigraphy) in Geology—The Registrar, The University, Manchester 13 (April 30).

Lecturer (Grade I or Grade II) in Physical Chemistry—The Registrar, The University, Leicester (May 1).

Lecturer or Assistant Lecturer (with a special interest in the plastic deformation or fabrication of metals, and preferably with research, teaching or industrial experience) in the Department of Metallurgy—The Registrar, University College, Singleton Park, Swansea (May 1).

Lecturer (preferably with an interest in thermodynamics) in Physical Chemistry—The Registrar, The University, Leicester (May 1).

Assistant Lecturer (preferably with an interest in cryptogamic botany) in Botany—The Registrar, The University, Nottingham (May 4).

Assistant Lecturer in Statistics—The Registrar, The University, Sheffield (May 5).

Temporary Lecturer or Assistant Lecturer (with special interests in inorganic chemistry) in Chemistry—The Registrar, The University, Sheffield (May 5).

Assistant Lecturer in Physics—The Principal, Royal Holloway College, Englefield Green, Surrey (May 7).

Senior Lecturer (with considerable teaching and research experience) in Chemistry; and Lecturers or Assistant Lecturers in Applied Mathematics, Physics, Botany and Zoology at the University College of Cape Coast, Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (May 7).

University Demonstrator in the Department of Geography—Dr. I. D. Muir, Department of Mineralogy and Petrology, The University, Downing Place, Cambridge (May 10).

Lecturer in Metallurgy, and an Assistant Lecturer in Metallurgy—The Registrar, The University, Sheffield (May 12).

Lecturer in Refractories and Ceramics Technology—The Registrar, The University, Sheffield (May 12).

Lecturer (with a good honours degree in physics or mathematics and some experience in research) in Astronomy—The Secretary, The University, Edinburgh (May 12).

Lecturer or Assistant Lecturer in Statistics—The Registrar, The University, Leeds 2 (May 14).

Lecturer (with qualifications in either pure or applied mathematics) in Mathematics—The Registrar, (Room 22, O.R.B.), The University, Reading (May 14).

Assistant Lecturer or Lecturer in the Department of Philosophy—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 15).

Assistants (2) in Mathematics—Secretary of University Court, The University, Glasgow (May 15).

Reader in Meteorology—The Secretary of the Faculties, University Registry, Oxford (May 15).

Reader in Physical Metallurgy at the Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 17).

Dewar Research Fellow, to engage in research in radiation chemistry—The Secretary, The University, Edinburgh (May 18).

Lecturer in the Department of Biochemistry—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (May 20).

Geologists (with at least a first- or upper second-class honours degree in geology, and preferably specialized in petrology) with the British Antarctic Survey, for service in the Antarctic for two years—Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M3C/53810/TA.

Lecturer in Educational Psychology—The Warden, Goldsmiths' College (University of London), New Cross, London, S.E.14.

Lecturer in Numerical Analysis—Head of the Department of Mathematics, Imperial College of Science and Technology, London, S.W.7.

Lecturer (with a Ph.D. degree or equivalent, also teaching and research interests in one of the following fields· genetics of lower plants; morphogenesis, or host-parasite relationships) in Botany—Dr. E. R. Waygood, Head, Department of Botany, University of Manitoba, Winnipeg, Manitoba, Canada.

Master (graduate) to teach Chemistry to Sixth Form-level and Lower School General Science—The Headmaster, Monmouth School, Monmouth.

Principal Lecturer (with experience of supervising research of an academic standard); a Senior Lecturer and a Lecturer in the Physics Department—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

Reader in Civil Engineering, and a Reader in Mechanical Engineering—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C 1.

Research Assistant (preferably postdoctoral, with some experience in neuroanatomy) in Neuroanatomy, for a joint research programme of the Department of Zoology and the Institute of Experimental Psychology, scheduled to last five years—Dr. Juan Delius, Department of Zoology Annexe, The University, 13 Bevington Road, Oxford.

Research Assistant (with a good honours degree and appropriate research experience) in the Department of Zoology and Applied Entomology, Imperial College of Science and Technology, to work with Mr. M. J. Way at the College Field Station at Ascot on the ecology of crop insects—The Director, Silwood Park, Sunninghill, Ascot, Berks.

Senior Lecturer and a Lecturer (with the qualifications and experience to teach mathematics for Diploma in Technology courses and for degree courses in engineering and general science) in Mathematics—The Registrar, Bradford Institute of Technology, Bradford 7.

Temporary Research Assistant or Technician—The Secretary, Wright–Fleming Institute, St. Mary's Hospital Medical School, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Zoology. Vol. 8, No. 4: A Revision of Certain *Barbus* (Pisces, Cyprinidae) from East, Central and South Africa By P. H. Greenwood. Pp. 151–208. (London: British Museum (Natural History), 1962.) 20s. [241

British Leather Manufacturers' Research Association. Annual Report, 1960–61. Pp. 58. (Egham, Surrey: British Leather Manufacturers' Research Association, 1961.) [261

The Facts About Margarine. Revised edition. Pp. 52. (London: Van den Berghs and Jurgens, Ltd., 1961.) [261

Royal Observatory Bulletins. No. 45: The Radial Velocity and Spectrum of HD 134646. By B. E. J. Pagel. Pp. i+E367–376. 2*s.* net. No. 46: Studies in the Equilibrium of Globular Clusters. 5: Rotation. By R. v.d.R. Woolley and R. J. Dickens. Pp. i+E377–386. 2*s.* net. (London: H.M. Stationery Office, 1961.) [261

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Planning, Vol. 28, No. 458 (29 January, 1962). Long-range Weather Prediction. Pp. 1–20. (London: Political and Economic Planning, 1962.) 3*s.* 6*d.* [12

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The Royal Institute of Chemistry. Lecture Series, 1961. No. 4: Ramsay, Chemistry and the Electrical Industry. By R. C. Chirnside. Pp. 13+5 plates. 4*s.* No. 5: Tobacco Smoke and Lung Cancer. By Dr. J. W. Cook. Pp. 18. 4*s.* No. 6: Recent Development in the Chemistry of Free-Radical Addition Reactions. By Dr. J. I. G. Cadogan. Pp. 28. 5*s.* (London: Royal Institute of Chemistry, 1961.) [12

The Colombo Plan for Co-operative Economic Development in South and South-East Asia. Tenth Annual Report of the Consultative Committee, Kuala Lumpur, October-November 1961. Pp. 233. (Cmnd. 1600). (London: H.M. Stationery Office, 1962.) 13*s.* net. [12

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>
> Charles Morgan : *The House of Macmillan* (1843–1943)

NOT even the present Editor's work is excepted; it is not so long since a book of which he was co-author received an adverse review in the columns of *Nature*. But, even *Nature* can make a mistake, not, one hopes, as often as those we see in the weekly and daily Press for there is not in *Nature* the same excuse of the deadline. There are some errors, however, which even the demands of time cannot excuse. The great Lord Northcliffe would never accept an excuse for an error of fact which could have been checked either by reference to the person concerned or elsewhere. He would, therefore, never have excused 'Atticus' for writing in the *Sunday Times* (London) of April 15 that "the Prime Minister's son, Maurice Macmillan, M.P. . . . edits 'Nature', the scientific journal". A small error one might think, which could do little harm. In fact, this wound, though unobtrusive like that of a stiletto, pierces deep and causes hidden injury, since it thrusts into the heart of the policy carried out by *Nature* itself and by Macmillan and Co., Ltd., its publishers, since its foundation. Some botanists claim that the least important characteristic of a plant is its name. Be that as it may, it is certainly true of editors. The first editor of *Nature*, the astrophysicist Sir Norman Lockyer, occupied the editorial chair for exactly half a century, from the foundation in 1869. The *names* of those who followed him and that of the present Editor are of no account. The main characteristic of *Nature* is that it is a scientific journal, known and used throughout the world of science. As such, it has always been, and still is, owned, but only owned, by the House of Macmillan. Scientists should be grateful, for although Macmillan do all they can to help and promote the journal they never attempt to influence, much less to interfere with, its policy. This is an attitude to publishing for which *Nature* can be grateful and the House of Macmillan proud.

It may be true that the least important characteristic of a plant is its name, but the recent statement in the *Sunday Times* (that it is the "Prime Minister's son" who edits *Nature*) shows that it is certainly not true that a rose by any other name smells just as sweet. For there is real and deep concern that it may in some sense be true that there is some outside interference in the editorial policy of *Nature*. After all, the Prime Minister himself is a member of the family which publishes the journal: his son is also a Member of Parliament. As Charles Morgan said, this is "a naughty world", and we have become suspicious of such coincidences. In these days when science is exerting such a profound influence on society it is all the more important that a scientific journal such as *Nature* should remain independent.

Nature must not only be independent, but also must be seen clearly to be so, for it goes to every part of the world where scientific research or teaching is being carried out. If the statement made in the *Sunday Times* were accepted as true in these countries it could be unfortunate and, in some cases, disastrous. 'Atticus' has made a silly error of fact. One day perhaps there will be a scientific Macmillan capable of editing *Nature*. There is no doubt that if there were he, too, would be as independent in his editorial policy from the rest of the family and from the firm that publishes *Nature* as is the present Editor and as have been the editors of the past.

Nearly twenty years ago Charles Morgan wrote the lines quoted at the beginning of this article. If he were alive to-day he could write them again with the same truth.

INTERNATIONAL INVESTMENT IN SCIENCE

THE eleventh annual report of the National Science Foundation[*], covering the year ended June 30, 1961, is similar in pattern to the previous year's report. Following the short statement of the director, Dr. A. T. Waterman, almost half the report is occupied by accounts of the programme activities of the Foundation. The remainder of the report comprises appendixes detailing the membership of the Board, its staff, committees and advisory panels, financial report, lists of research grants, fellowship awards and of publications (see p. 341 of this issue of *Nature*).

Of special interest at the present time is the section of the report dealing with the measurement and appraisal of the national investment in science, including the intensive study of science and technology as stimulant of growth in the national economy. The total research and development effort of the United States is now about 14,000 million dollars, or some 2·5 times the expenditure of 1953. A report issued by the Foundation in July 1961, *Investing in Scientific Progress*, estimated that the expenditure during 1961 for scientific and engineering education and for basic research in colleges and universities of 3,000 million dollars should grow to more than 8,200 million dollars in 1970, of which about 2,700 million dollars would be for basic research at universities. The figures in this report are based on the work of the Foundation's Office of Special Studies, which is engaged in collecting, analysing and publishing facts regarding the scientific resources of the United States. These studies, which have established the predominant role of the Government or public sector in the finance of research and development, are now being broadened to cover all scientific activities in relation to the economy, including the organization and management of science and their relation to the broad structure of social and economic institutions in the United States.

The statistics do not indicate the direction policy should take, but they reflect, quantitatively, some of the inter-relations which have been the subject of much public discussion. When full account is taken of the inquiry into the management of research in Britain, made under the chairmanship of Sir Solly Zuckerman, it must be admitted that Mr. Denzil Freeth's reply in the House of Commons on February 27 to a question regarding the staffing of the Office of the Minister for Science would have been more convincing were there evidence that the Minister had already at his disposal the information required for sound objective decisions on the many matters of scientific and technical significance which call for his attention. Only too often, as in hydrological research or nature conservancy, he has to admit that the information has still to be obtained.

The National Science Foundation's report shows, it is true, that frequently the essential information has still to be obtained in the United States also. There, as in Britain, greater understanding is needed as to what types of economic and technological conditions and managerial motivation lead some industries as a whole or certain firms within an industry to make the risk decisions to invest in research and development while others do not take this step. The President's Science Advisory Committee, reporting on *Scientific Progress, the Universities, and the Federal Government*, discussed some of the questions in this field that are just as critical in Britain as in the United States. However, in Britain more information is desirable before it will be possible to reach wise decisions as to the expansion of the research resources in the universities and colleges of technology, or of the appropriate use of other research institutions, including Government research stations and national or international institutions in particular fields.

Commenting on this study, *Investing in Scientific Progress*, in his statement, Dr. Waterman remarks that the steadiness of the increasing trend in output of scientific manpower, which is expected to double by 1970, suggests real interest and purpose on the part of the younger generation in the values of such careers. This trend must be maintained and possibly accelerated to meet the estimated needs during this decade, and this goal should be achieved without drawing on more than about 4 per cent of the United States highest talent in intelligence quotient. Nevertheless, insufficient preparation is being made for these essential increases in trained manpower, and Dr. Waterman adds that the United States is headed steadily towards an accelerating general activity in science and technology without adequate provision for the magnitude and cost of the effort, and he points out that if provision is to be made it must be based on public support and understanding.

Dr. Waterman's statement is of particular interest in its discussion of the factors involved in the provision of an adequate supply of trained manpower, and the interplay of science and technology with the economy. It is not simply that scientific and technical education needs to be expanded in scale: its quality and content also need attention, and it is imperative also to give more care to the ways in which trained manpower is used and especially its creative ability fostered. Here, in words that reflect much of Lord Hailsham's attitude, Dr. Waterman stresses the dependence of the progress of science on the personal initiative and independence of the individuals and groups engaged in research and the pitfalls that beset any attempt at central planning or direction even in respect of the content of programmes or the determination of priorities. He recognizes that national science policy and its supporting organization present a most important and challenging problem, but he neither attempts to define such policy nor suggests that the structure established in the United States is necessarily adapted for use elsewhere. It may well be that due account will be taken of the work and functions of the National Science Foundation, the President's Science Advisory Committee and the Federal Council for Science and Technology by the Advisory Council on Scientific Policy in its continuing inquiry into the organization of scientific research and also in the review of the existing organization for promoting civil science by Government agencies which the Government is now undertaking. Certainly the devices, including methods for improving the speed and accuracy of survey analysis, techniques for dealing with masses of detailed information and methodology based on statistics and communication theory noted in this report, should be utilized to the full in the comparison of needs and priorities with trends and potentialities with respect to manpower, facilities, funds and findings of research.

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[*] National Science Foundation. Eleventh Annual Report for the Fiscal Year ended June 30, 1961. Pp. xxi+325. (Washington, D.C.: Government Printing Office, 1962.) 1.25 dollars.

It is, in fact, the considerations which have led to the Foundation establishing an Office of Science Resources Planning that have prompted doubts as to whether the Office of the Minister for Science and the Advisory Council on Scientific Policy are adequately staffed and supported for the inquiries they need to conduct. The National Science Foundation system includes adequate provision for individual and local initiative and independence, and those intake elements need not be sacrificed in efforts to acquire the awareness and the information as to activities in progress and the disposition of scientific manpower and other resources which are essential elements in the decisions which must inevitably be taken at the centre. Moreover, leaving on one side the actual support for scientific research afforded through the National Science Foundation or the extensive fellowship and studies designed to extend the opportunities of, and also the qualities of, training in science and technology, the Foundation is making a major contribution in the dissemination of scientific information and in international co-operation in science which at least merits the direct attention of the Minister for Science himself. Its bearing on the effective use of scientific and technological manpower is too important for this to be left entirely to the Department of Scientific and Industrial Research.

The Foundation is seeking to promote development of new and better techniques for handling scientific information and also to improve existing methods for disseminating scientific information, and in all made some 166 grants totalling about 5·38 million dollars during the year for these purposes. While the tendency during the year has been increasingly to support the principle that dissemination of the results of research is an integral element of the total cost of research, generally the report records the continuance of activities along existing lines, with some emphasis on the analysis of existing patterns of scientific communication, and the ways in which scientists and engineers use existing publications and information services. The projects which the Foundation is supporting in the use of notation systems, mechanical translation and the like are, however, of international importance even where they do not call for the immediate co-operation of scientists outside the United States; they illustrate a field in which courtesy alone would suggest that no opportunity of co-operation with the Foundation should be missed by scientists in Britain.

This is clearly contemplated by the Foundation itself, science information and the improvement of the communication of science generally being specifically mentioned among the responsibilities of its Office of Special International Programmes, though in this present report the major attention is directed to educational matters, including the encouragement of contacts between educationists and scholars in the United States and in other countries and the improvement of the content of science courses and the training of teachers of science. Some grants were made to enable outstanding scientists from the United States to visit other countries to prepare research reviews in their special fields, and the Foundation is also exploring experimental approaches to co-operating in international support of scientific centres in developing areas; and one such project, providing partial support for an international team of physiologists in a tour of India, is supported also by the Royal Society.

AN OVERSEAS INDIAN COMMUNITY MADE VISIBLE

Peasants in the Pacific

A Study of Fiji Indian Rural Society. By Adrian C. Mayer. (International Library of Sociology and Social Reconstruction.) Pp. xiii+202+16 plates. (London: Routledge and Kegan Paul, Ltd., 1961.) 35s. net.

ONE of the points of attack on the problems of social and culture change has been comparison of established overseas communities with the society and culture of their homelands. Studies of immigrant groups, especially in the United States, and the number of recent studies on overseas Chinese communities, are cases in point. The author aims this work toward extending the coverage of such studies to overseas Indian communities. His work is done against the background of earlier field work in south India. While he does not attempt any direct comparison with India, he has designed this study to provide material for such comparison. He carried out his field work in Fiji during 1950–51. In his material he compares three rural Fiji Indian communities, chosen to provide clear variations in geographical location and physical setting.

As yet, no single theoretical framework exists for making systematic comparisons of this kind. Such comparative work as has been done is limited to comparing the changes within overseas communities in institutions or cultural complexes specific to the homeland. The author here approaches his communities more holistically. He gives, for example, but passing attention to what has happened to caste among Fiji Indians, on the grounds that he found caste to play only a very minor part in the social relations of his subjects. Contrariwise, he does a superb job of tracing the historical development of settlement patterns among rural Fiji Indian communities, and of connecting this development with ecological factors locally in play. Equally, he shows in excellent detail the way internal differentiation within these communities has been based on felt cultural allegiances to broad regions of origin in India—North India as compared with South India—and to original religious differences, such as Muslim, Sikh and Hindu. There is implicit that these allegiances have acted to replace the caste allegiances which in most Indian rural communities act to produce very similar internal differentiation, though the author does not attempt to draw out any comparative significance.

The main part of the work is concerned with an analysis of local community politics. Politics he defines in terms of the functions it performs, arguing reasonably enough that the relationships he deals with as political do the same thing, on a small scale, that formal political bodies do. He examines such groups as local school committees, cane harvesting gangs, informal councils for mediating disputes, and factional groups the leaders of which are competing for local political power and social influence. He examines in detail the contexts of political action, the modes of leadership, and the accompanying patterns of factionalism. The descriptive treatment is highly convincing. Again, there is obvious comparative significance in the close similarity of factional modes and motivations to those widely reported for India which, true to his approach, the author does not try to underline.

In the final section he does an excellent job of drawing out the diverse strands of opinion and stereotype in the view the Indian community have of the outside—the Fijians, the Europeans, and especially well done, their ambivalent view of India. Throughout, the documentation and illustrative material is of high order, and kept very well under control. If there are any doubts on the work, they lie in the author's intention that his shall be a descriptive contribution to a comparative analysis as yet unframed. His description, to be comprehensive, necessarily has been stretched rather thin over some of his topics, particularly on kinship and economics. The difficulty here is that when some comparative analysis is finally attempted, it is likely that the analyst will find he needs greater depth in the materials he uses than the author provides here. This is no criticism unique to this work, which as a descriptive contribution is outstanding, but is one general to all studies that do not try to tackle the analytical problems of the comparative work toward which they aim to contribute. R. R. Jay

LOCUST RESEARCH IN INDIA

The Desert Locust in India

By Y. Ramchandra Rao. (Monograph, No. 21.) Pp. xix + 721 + 59 plates. (New Delhi: Indian Council of Agricultural Research, 1960.) Rs. 73.50; 111*s*.

SERIOUS depredations by the desert locust (*Schistocerca gregaria* Forsk.) in the pre-partition India in the late 'twenties stimulated the Board of Agriculture not only to initiate measures aiming at saving the crops but also to realize the need for long-term research on this recurrent problem. A scheme for extensive investigations of the biology and ecology of the locust was established and the work, based on Karachi but extending over the locust habitats both in Pakistan and India, has continued for nine years under the leadership of the author of the book with a small staff of both nationalities. The book, however, is not merely a detailed report of the work carried out during the period, but also the results of the latter have been most usefully augmented by the abundant data carefully extracted from a mass of local documents and critically examined in the light of the later findings. The result is a comprehensive monograph of the desert locust problem in the Indo-Pakistan sub-continent on a scale and with the wealth of detail which have no parallel in any other locust-infested part of the world.

An introductory chapter, describing the general physiographical, climatic and vegetational background, is followed by a detailed account of the extensive field investigations. These were of particular value because they concentrated on the study of the local non-swarming locust populations which were suspected as the source of devastating swarms. Methods of routine population counts were elaborated and a network of observation posts established throughout the main locust-breeding areas, where continual observations on the various aspects of locust biology and ecology were organized in the field and in open-air cages.

What is most important, the data assembled were thoughtfully analysed, leading to a series of conclusions, many of which anticipated the results of much later investigations in other countries. These results included original data on the effects of temperature and humidity on the rates of development of the different stages of the locust; its food plants and their effects on the growth and sexual maturation; its natural enemies; the correlation of the annual life-cycle with the seasonal weather; changes in the morphometrics of wild populations in relation to the phase change, etc.

Among the most original and important results was the finding, well supported by field observations, that non-swarming locusts carry out seasonal migrations fully comparable with those of swarms, thus taking advantage of the seasonal shifts in the areas of rainfall which is essential for their breeding. As it was generally believed at the time (and is still maintained now in some text-books and popular works) that the solitary phase of the locust is also non-migratory, the Indian evidence was of outstanding importance for research elsewhere. Moreover, it was also established that migrations of solitary locusts were under the direct influence of seasonal winds, including the regular monsoons and the less-regular cyclonic depressions. A close connexion between the pattern of locust migrations and the dynamics of weather has thus been established for the Indo-Pakistan region many years before the connexion was analysed in more detail by joint efforts of entomologists and meteorologists elsewhere. This finding is amply supported in the later chapters of the book where all available records of movements of locust swarms and breeding are analysed against the background of weather for the locust plague periods 1920–39, 1908–19, 1888–1907 and 1860–88.

This analysis, partly illustrated by annual maps, both of locust distribution and weather, led to another important conclusion that the invasions by the desert locust of India were synchronous with similar events in Iran, Middle East and Africa, and, in most cases, appeared to originate outside India. On the other hand, it was also established that swarms produced in India and Pakistan during the monsoon rains mostly migrated westwards to breed in the winter rain areas in the west, the progeny again invading Pakistan and India in the next year. Thus, the interdependence of geographically very distant breeding areas was confirmed by the Indian work.

The author has also attempted to investigate the periodicity of locust plagues in relation to sun-spot periods and thought that there was some evidence of the outbreaks following the sun-spot minima, that is, relatively dry periods. He was cautious enough to admit, however, that the possibility of forecasting locust plagues must depend on a much better knowledge both of the present distribution and movements of locust populations and of the weather throughout the whole vast region of the pest. A special chapter of the book deals with two other locusts occasionally swarming in India, *Patanga succincta* and *Locusta migratoria*; again, their seasonal cycles and migrations are discussed against the meteorological background.

It was most regrettable that the book, completed in 1945 and actually set in print in 1951, was not published before 1960. Although some of the most important extracts from it were published in England, an earlier appearance of the complete work would not have failed to benefit from the progress of locust research elsewhere and such a long delay in its publication for, apparently, purely administrative reasons, is difficult to justify. The standard of the book's production is very low, the paper being of poor quality; illustrations badly reproduced (though the originals of the coloured plates must have been of high quality); there are innumerable misprints; plates and maps are mostly unnumbered and scattered throughout the book without any relation to the relevant text; and the binding of the unused book is already falling to pieces; all this reflects little credit on the official publishers. Nevertheless, the intrinsic value of the book is so great that it will always remain a classic example of a well-conceived investigation of a major regional problem, carried out with more inspiration and determination than the material resources and resulting in far-reaching conclusions of exceptional general value. B. P. UVAROV

A FOUNDATION-STONE OF PLANT TAXONOMY

Species Plantarum

By Carl Linneaus. With an Introduction by W. T. Stearn and an Appendix by J. L. Heller and W. T. Stearn. Vol. 1: Pp. xiv+1–560. 50*s*. Vol. 2: Pp. xv+561–1200. 60*s*. (London: The Ray Society, 1957 and 1959. Sold by Bernard Quaritch, Ltd., 11 Grafton Street, W.1.)

THE Ray Society and Dr. W. T. Stearn have performed a great service to botanists throughout the world by producing this excellent facsimile of *Species Plantarum* at so modest a price.

Species Plantarum is, of course, familiar, at least by name, to every systematic botanist. It is the starting point of binary nomenclature and established a general system which rendered possible for the first time the organization of information about the rapidly increasing number of plants which were becoming known to botanists in the eighteenth century.

It is, I suspect, seldom studied except by specialists on nomenclature. Indeed, the understanding of its content and of its wider significance has made too great a demand on time and special knowledge for it to be undertaken by most botanists.

Dr. Stearn's introduction to this facsimile, occupying the first 176 pages, is a feast of interesting and valuable information which, in turn, makes Linnæus's great work comprehensible to the modern botanist.

An historical survey summarizes the principal events in the life of Linnæus and traces the development of his work through his travels, studies and publications. In the course of this the reader is given an idea of the range of interests of the 'Prince of Naturalists' as indicated, among other things, by the titles of the 186 dissertations for which he was primarily responsible. A small sample will serve to show the kind and variety of subjects dealt with: *Peloria, Crystallorum Generatione, Surinamensia Grilliana, Taenia, Splachnum, Plantae Hybridae, Noctiluca Marina, Cynographia, Flora Palestina* and *Medicamenta Purgantia.*

Dr. Stearn traces also the history of the major botanical publications such as *Genera Plantarum, Hortus Cliffortianus* and, of course, *Species Plantarum* itself, and makes clear the development of the concepts necessary for classification and of the binary nomenclature. He also explains the methods to be used for, and the pitfalls which beset the unwary in, the typification of Linnean genera and species, matters of practical importance for the taxonomist.

The section on the sources, format, method and language of the *Species Plantarum* is particularly interesting. Linnæus was the creator of botanical Latin with "its avoidance of verbs, its restricted number of nouns . . . and its rich store of adjectives", which has already been a living language for longer than classical Latin was.

Other matters of practical importance discussed in this scholarly introduction are the history and present location of the various surviving Linnean herbaria and of other herbaria to which Linnæus had access, and the meaning of the geographical names used for describing the distribution or localities of plants. These are both of great assistance to those who have to attempt the often difficult task of typifying Linnean species, and, like the rest of the introduction, should be read carefully by systematists as simple-minded as myself.

The appendix to the second volume includes an exhaustive index of authors and books cited by Linnæus which has been compiled with great attention to detail by J. L. Heller of the University of Illinois. This is followed by notes on illustrations and an account of four other Linnean publications by Stearn.

The latter, beside providing some additional nomenclatural information, are of particular interest for the light they throw on Linnæus's methods and concepts; they should be studied in conjunction with the section on his species-concept in the introduction to Volume 1. It becomes evident that the artificial 'sexual system' was no more than an aid to identification and that Linnæus was always searching for a 'natural' classification. But, as he writes in *Ordines Naturales* (1764), "A multitude of *Genera* is a burden on the memory to be lightened by System", and he provided a very useful system.

All three sections of the work are fully indexed, and that to *Species Plantarum* itself is made additionally useful by the indication of the species which have been proposed as generic types.

No systematic botanist should be without this facsimile, even though he be the fortunate possessor of the original work.

T. G. TUTIN

PROGRESS IN BIOCHEMISTRY

Annual Review of Biochemistry

Vol. 30. Edited by J. Murray Luck, in association with Frank W. Allen and Gordon Mackinney. Pp. viii + 758. (Palo Alto, Calif.: Annual Reviews, Inc., 1961.) 7 dollars.

WHEN, thirty years ago, Prof. J. Murray Luck and his three advisory colleagues wrote their preface to the first volume of the *Annual Review of Biochemistry*, they offered an apology for starting a new publication. They need not have apologized, for they were launching what has proved to be one of the most important publications in biochemistry; moreover, they were setting the pattern for the various 'annual reviews' in other biological subjects which were to come from California. The parent *Review* has now reached its thirtieth volume, still under the editorship of Prof. Luck. His thirty-year record of service to biochemical literature certainly deserves special mention and thanks.

The introductory chapter in the present volume was written by Prof. J. H. Northrop, pioneer in crystallization of enzymes. He likens present-day discussions on the nature of viruses to those which were taking place on enzymes fifty years ago. Perhaps his general theme may be summed up by quoting the following provocative sentence: "chemists (and physicists) have great respect for the Reverend Occam's razor and endeavour to limit their assumptions to the minimal number essential for an explanation, in accordance with the principle of the conservation of hypotheses; whereas some biologists have no respect for the Reverend's weapon and fearlessly bolster an ailing (and unnecessary) assumption by another similar one". This chapter is followed by articles on the subjects which are usually covered year by year: biological oxidation, chemistry and metabolism of carbohydrates, fats and proteins, biochemistry of muscle, vitamins and so on. Indeed, many of the chapter headings are the same as those in Volume 1 of this *Review*, and it is interesting and impressive to compare the contents of corresponding chapters thirty years apart. The present volume includes a well-illustrated article on X-ray studies of biological compounds (A. Rich and D. W. Green), and chapters on protein synthesis (P. Berg), nucleotides (A. M. Michelson), nucleic acid biosynthesis (R. Abrams), and on the polyamines spermidine and spermine (H. Tabor, C. W. Tabor and S. M. Rosenthal). The final three chapters deal with the biochemistry of (1) cultured mammalian cells (L. Levintow and H. Eagle), (2) genetic factors (C. Levinthal and P. F. Davison) and (3) the dividing cell (D. Mazia).

The editorial committee has followed the recommendation of the Commission on Enzymes in adopting the coenzyme abbreviations NAD and NADP. This, presumably, will finally seal the fate of DPN and TPN and, incidentally, provide an excuse for a new edition of almost every text-book of biochemistry. Articles on biochemistry in the U.S.S.R. appeared in Volumes 26–28; there have been none since, though there are occasional references to Russian work in the general articles in the present volume. A third decennial subject and author index would normally now be due, but according to the preface of Vol. 29 this will not be published. In view of the usefulness of the separate cumulative indexes covering Volumes 1–20, this decision seems a pity. Biochemists owe a debt of gratitude to the 38 authors from seven countries who have contributed to this volume. To write one of these chapters is no light undertaking.

D. C. HARRISON

SOIL ORGANIC MATTER

Soil Organic Matter

Its Nature, Its Role in Soil Formation and in Soil Fertility. By M. M. Kononova. Translated from the Russian by Dr. T. Z. Nowakowski and G. A. Greenwood. Pp. 450. (London and New York: Pergamon Press, 1961.) 80*s*. net.

THIS book was first published in Russian by the Academy of Sciences of the U.S.S.R. in 1951, but this recent English translation has been revised and brought up to date by the author. The last comprehensive review in English of this subject was the familiar work of Waksman, published twenty-four years ago, and so Mme. Kononova's book is a welcome addition on two accounts: it brings together many of the recent advances which have been made towards the elucidation of the chemical nature and role of soil organic matter, but, perhaps more important still, it provides a full account of the work of the Russian scientists in this field, most of which has not been available in English before.

The first chapter is devoted to a historical account of the development of the subject from the beginning of the eighteenth century and provides a valuable and interesting sidelight on the divergence of ideas relating to the chemical nature of humus. Earlier British and American workers suggested that because of the relative resistance of plant lignin to microbial decomposition, it must be a major component, in a modified form, of humus. On the other hand, Russian and German workers believe that humus is a product of microbial synthesis, plant materials merely acting as a substrate for the micro-organisms. The author then elaborates on the theory that humus is a mixture of heterogeneous polymers produced by random condensation of polyphenols and amino-acids, both products of microbial decomposition, in the presence of oxidizing enzymes. The heterogeneous nature of the polymers is claimed to explain their resistance to microbial decomposition. A great deal of evidence is provided to support this theory, much of it having been obtained by Mme. Kononova and her colleagues at the Dokuchaev Institute.

A discussion follows on the role of soil organic matter in soil fertility and soil formation in the light of this theory. Unfortunately, the section on fertility only touches on nitrification with scarcely a mention of organic phosphorus and sulphur compounds. Its role in soil formation and the changes brought about by different types of cultivation are reviewed at length. Methods of maintaining the levels of organic matter in soils are dealt with, including the rotation theory of V. R. Williams, which is receiving a good deal of attention in the U.S.S.R. to-day. The section on soil formation is placed in perspective by a final short chapter contributed by N. N. Rozov on the major soil types of the U.S.S.R.

Analytical methods are confined to an appendix, thus making for easier reading, and here mention should be made of the excellent work of the translators. It is a pity that the quality of the book has been lowered by the use of inferior quality ink, but on the credit side the value of the book has been enhanced by an offer from Pergamon Press to supply readers with a translation of any article referred to in the text, at a reasonable price.

J. W. Parsons

YOUTH SCIENCE ENCYCLOPÆDIA

Junior Science Encyclopaedia

Consultant Editorial Board: Dr. Maurice Burton, Prof. J. M. Cassels, Sir James Chadwick, Sir John Cockcroft, Prof. L. Dudley Stamp, Prof. F. G. Young, and Prof. J. Z. Young. General Editor: Leslie Basford. Vol. 1: Pp. 1–88. Vol. 2: Pp. 89–176. Vol. 3: Pp. 177–264. Vol. 4: Pp. 265–352. Vol. 5: Pp. 353–440. Vol. 6: Pp. 441–528. Vol. 7: Pp. 529–616. Vol. 8: Pp. 617–703. (London: Sampson Low, Marston and Co., Ltd., 1961.) 104*s.* the set, or 13*s.* each volume.

We are unquestionably living in the golden age of science, at a time when the sciences, the scientists, and their promoters have 'never had it so good'. Since the turn of the century, science has risen to a position of such great political, social, and technological significance that it now seems unnecessary even to apologize for rockets and capsules to the moon, or laboratories and equipment in the schools, or reactors and refineries on the sea-shore. We are also growing accustomed to the ever-increasing number of scientific books which purport to satisfy a long-felt need, or offer no apology for their appearance at all.

Junior Science Encyclopaedia is one such set of books which has appeared on the market without a preface. However, the general editor, Mr. Leslie Basford, does supply on the cover and the title-page of every volume the names of the eminent scientists who form the consultant editorial board. Sir Lawrence Bragg and six of the consultant board have contributed short introductions to the study of physics, zoology, science, electronics, geology, biochemistry and anatomy. These introductions are arbitrarily allotted one to each of Volumes 2–8. I found myself wondering why Prof. Cassels did not contribute an article, and why Sir John Cockcroft did not autograph his contribution. Volume 1 contains, of course, a short introduction to the consultant editorial board.

The reader, therefore, is left to judge for himself the aim and scope of this set of books. This is by no means an easy task. To begin with, the title is somewhat misleading. The word "Junior" usually relates to children of not much more than twelve years of age. However, these volumes have little to offer such children apart from the glossy, attractive covers, and the exaggerated use of vivid colours: the only things in black and white are a few photographs (including those of the above-mentioned scientists) and most of the print. Furthermore, the term "Science Encyclopædia" rather implies a wealth of scientific information for both the young and the old. However, the cover illustrations and the presentation of the material indicate clearly that these volumes are not primarily designed for the adult. It would appear, therefore, that they are intended for children in their early teens.

The topics range widely from absolute temperature to calorimeters (Volume 1), Cambrian period to Doppler effect (Volume 2), double stars to graphs (Volume 3), gravitation to loglines (Volume 4), longitude to petrified forests (Volume 5), petroleum to satellites (Volume 6), saturation to triple-expansion steam engines (Volume 7), and tropical medicine to zoology (Volume 8). The last volume also contains an index with ample cross-references to related topics, short articles on subjects of recent scientific research and on scientific method, a supplementary glossary of scientific terms, and forty-eight charts of the night sky. There is a superabundance of illustrations which are invariably highly coloured. Some of these, such as the domestic electricity meter on p. 194, the camera lens on p. 336, and the guns on pp. 269–270, are quite impressive. However, many, such as the amphibians on p. 19, the distillation apparatus on p. 172, and the minerals on p. 381, are very poor: this is the result of rather inferior colour printing and inaccurate draughtsmanship.

Since Sir James Chadwick ends his introduction to the study of science with the comment that "there is some relief to be found in the fact that it is now easier than it was to have access to reliable sources of information", there seems to be the suggestion that these books are reliable sources of scientific information. The editor must, of course, omit much of interest and importance. He must also omit unnecessary complications and admit convenient simplifications. He must then run the risk of creating a false impression and of publishing a less-reliable source of information. A most surprising omission from the list of 116 famous scientists (pp. 532–540) is the name of John Dalton, 'founder' of the atomic theory.

On pp. 45–46, the relative sizes of the atoms are indicated by spheres in an assortment of colours from yellow and green to mauve and chocolate-brown. These colours are of little significance since a different set of coloured spheres is used on p. 111 to represent 'molecules' of sodium chloride and other well-known ionic compounds. These illustrations are fortunately small. The calcium carbonate 'molecule' also appears on p. 388. This time, for no apparent reason, it is inverted and occupies half the page. In both these illustrations the relative sizes of the atoms are preserved and the links between them are in accord with their valencies. Unfortunately, it is well established that all three oxygen atoms are attached to the carbon atom. The linking between oxygen atoms represented by these illustrations probably occurs only in ozone and ozonides.

Certain small errors were also noticeable to me. For example, chemists rarely ever do acid-base titrations using litmus indicator (p. 21), a colloidal solution is called a 'sol' and not a 'soll' (p. 131), the illustration of the structure of graphite is slightly inaccurate (p. 155), and 'allotrope' rather than 'allotropy' is intended in the glossary of terms (p. 696).

The overall impression of this set of books is likely to be that they have something of interest to offer the budding young scientists, but that they are too expensive at 104*s*.

Michael C. Cox

Introduction à l'Astrophysique : les Étoiles

Par Prof. Jean Dufay. (Collection Armand Colin: Section de Mathématique, No. 352.) Pp. 218. (Paris: Librairie Armand Colin, 1961.) 4.50 N.F.

PROF. DUFAY gives us an account of present-day techniques in stellar spectroscopy and photometry, but deliberately omits any discussion of variable stars or extra-galactic objects.

A preliminary chapter introduces the definitions of absolute and apparent magnitude, and discusses the effects of atmospheric absorption and agitation. The account of selective absorption by the constituents of the Earth's atmosphere is very condensed. The final chapter is taken up by a discussion of Saha's equation and its consequences, together with a brief account of the theory of stellar evolution.

The main portion of the book describes the instruments and methods of stellar spectroscopy and photometry in the optical and near ultra-violet regions in considerable detail, attention being directed to the corrections required for each method. The spectra of the main classes are described, and the characteristic features of spectra of Wolf-Raynet and other peculiar non-variable stars are listed. The continuous spectra are classed in terms of colour gradient, and a brief account of three-colour photometry given. The three-parameter photometric classification of Barbier, Chalonge and Diran is described.

The book should be of interest to those reading honours courses in astronomy. Its usefulness for other purposes is marred by the author's failure to give any references when citing a paper, an inadequacy not removed by the short selection of other works listed in the bibliography.

M. R. C. McDowell

Introduction to the Dynamics of Automatic Regulating of Electrical Machines.

By M. V. Meerov. Translated by J. S. Shapiro. Pp. xxii+411. (London: Butterworth and Co. (Publishers), Ltd., 1961. Published in association with the Department of Scientific and Industrial Research.) 100*s*.

PART of the interest in reading this translation of a text first published in the U.S.S.R. in 1956 lies in meeting an approach to familiar material which differs in flavour if not in substance from British conventional fare. The core of the book is a study of the stability, quality and synthesis of regulating systems. Stability is assessed by conventional techniques—the *D*-partition theorem of Neimark turns out to be a slightly generalized form of the Nyquist diagram. A criterion of aperiodic stability is also presented. Quality is assessed by a modified form of the Routh–Hurwitz criterion, various integral criteria and the use of the frequency response characteristic to estimate the transient behaviour of the system. Some of the integral criteria are interesting as they are not usually met in our texts: one of these, due to Krasovskii, develops a Lyapunov function which is then minimized with respect to certain parameters of the system; another minimizes the 'closeness' of the regulating system to a standard linear system. Synthesis is achieved by adding stabilizing links and various configurations utilizing such links are analysed.

The book commences with a fairly detailed analysis of electrical machines, amplifying and measuring devices and includes sections on sampled-data systems, non-linear systems and systems with time delays. The author also devotes a chapter to the examination of the effect of neglecting small parameters in the analysis of a system. The book is a competent study of a limited field—the deterministic behaviour of regulating systems incorporating electrical machines.

D. Q. Mayne

Carbon-14 Compounds

By John R. Catch. Pp. vii+128+5 plates. (London : Butterworth and Co. (Publishers), Ltd., 1961.) 30*s*.

IT is probably true to say that carbon-14 is used more extensively than any other radioactive isotope as a radioactive tracer, and a very large body of literature has built up during the past decade, largely in the form of research papers. The aim of this book is to provide a guide to the literature of carbon-14 preparations which will be particularly useful to the newcomer in the field of tracer work. It takes the form of a series of reviews covering the production of carbon-14, chemical synthesis of labelled compounds, biological methods of labelling, peculiar features of carbon-14 compounds, the analysis of labelled compounds, measurement of carbon-14, and precautions in the use of carbon-14 compounds. A short appendix summarizes recent work which appeared during the preparation of the book.

The literature of the subject is now so extensive that a book of this type must inevitably be selective in its treatment and thus tend to invite criticism on the grounds of omissions. However, the newcomer should indeed find it a very valuable guide, and the expert may well profit from it, too, outside his own narrow field of specialization. The chapters dealing with the peculiar features of carbon-14 compounds and the significance of analytical purity are most useful in directing attention to those factors which must be clearly understood if errors are to be avoided in the interpretation of experimental results.

H. Barker

The Year Book of the Royal Society of London, 1962

Pp. 320. (No. 66.) (London: The Royal Society, 1962.) 21*s*.

THE *Year Book of the Royal Society* continues in its tradition of providing a compendium of useful information concerning the Society. In its 320 pages, details of Fellows and Foreign Members are shown, together with membership of the many committees and boards, standing orders, Council procedure, medal awards, special memberships and appointments, and a report of Council for the year ended September 30, 1961. Reports of holders of Royal Society research fellowships and professorships are appended, and short histories of bequests and endowments made for the purposes of research are included elsewhere. The remaining appendixes list the representation of the Society at international scientific meetings, Parliamentary grants-in-aid for scientific investigations and publications, and additions to the Society's library. It is a pity that the *Year Book* is published immediately before the annual election of new Fellows. This renders the list of Fellows and Foreign Members given at the front of the book out of date within a few weeks of publication. A delay of only a month or so before publication would surely make it possible to include the new Fellows.

PINCHED LIGHTNING

By B. T. MATTHIAS and S. J. BUCHSBAUM

Bell Telephone Laboratories, Inc., Murray Hill, New Jersey

WE have obtained what we believe to be a unique photograph of a pinched lightning discharge.

The photograph was taken in late August 1961 at Los Alamos, New Mexico, during a severe thunderstorm. The camera (Elmar 3·5 : 1 lens, focal length 50 mm., *f* number 22) was pointed from behind a window-pane in a darkened room at an approaching thundercloud which was estimated to be about a kilometre away. The camera was inclined to the horizontal at an angle of about 15° and was, of course, focused at infinity. Six frames of Kodachrome colour film *ASA* speed 10 were exposed for 15 min. each. Four were found blank, one showed a standard lightning stroke, and the sixth is reproduced in Fig. 1. Over the years we have taken hundreds of photographs of lightning discharges, and this is our first photographic evidence of a pinched lightning.

We estimate the distance of the lightning to the camera at between 200 to 1,000 metres, and thus, the transverse dimension of the lightning between 1 and 5 m. Somewhat puzzling is the apparent much larger intensity of the integrated luminosity of the pinched lightning when compared with the luminosity of the aforementioned standard lightning stroke.

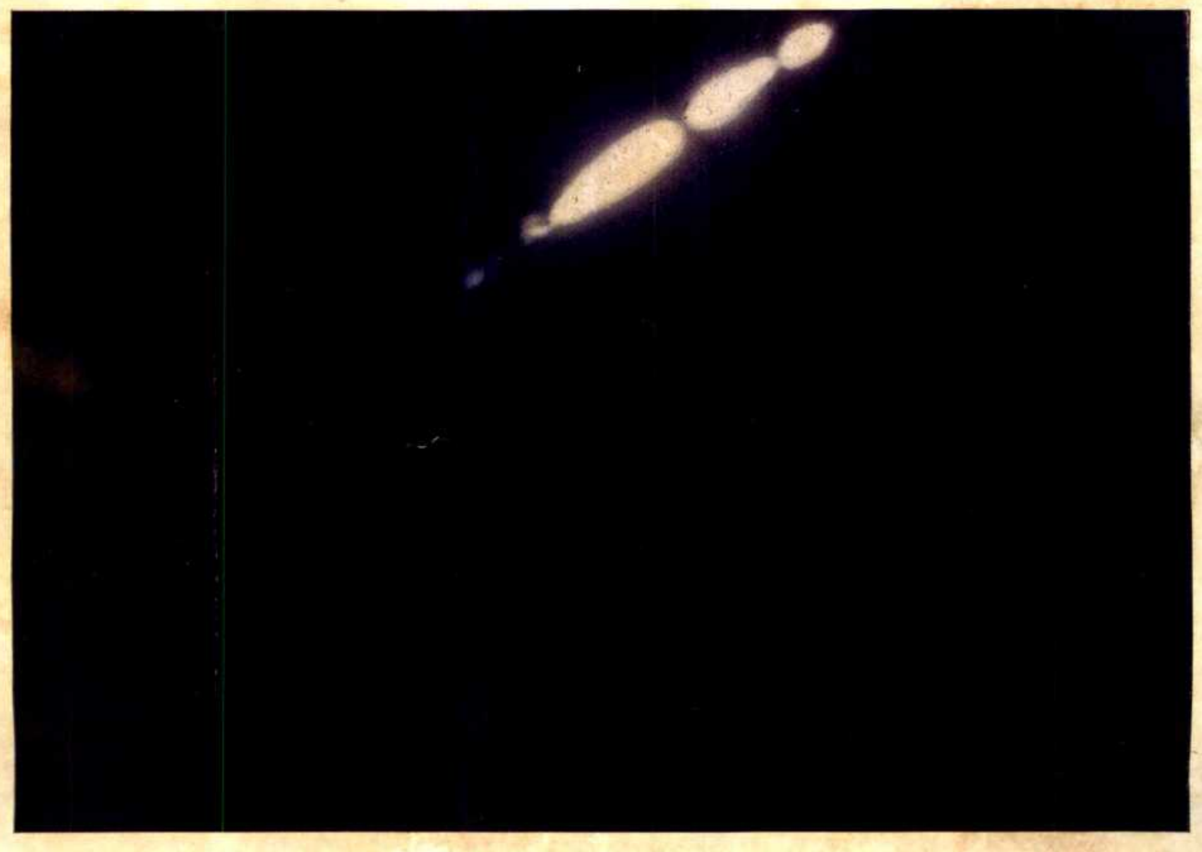

Fig. 1. Photograph of pinched lightning

DATING THE GALAXY BY URANIUM DECAY

By Prof. R. H. DICKE
Palmer Physical Laboratory, Princeton University

ARP[1] has recently discussed the metal content of stars in clusters of various ages. One striking thing about Arp's data is the indication that the heavy elements were generated primarily by early stars in the halo population. He finds that the very old galactic cluster *NGC* 188 (16 × 10^9 years old) has approximately the same heavy element content in its envelope as does the Sun. While there is an indication of slightly more heavy element in very young clusters than in *NGC* 188, the increase is small (~ 30 per cent) and it may not be statistically significant. While the data are not so firm as to make this conclusion certain, I propose to assume here that the heavy elements were formed primarily by the halo population.

There existed in the early halo population a type of star no longer present in the Galaxy, the massive Population II star. This star may have been supernova prone; it may have been the chief source of the heavy elements.

There appears to be no systematic variation, from one cluster to another, in the ratios of the heavy element abundances. This suggests that uranium was produced at the same time as the other elements, and that Arp's data can be assumed to apply also to the production of uranium.

With this assumption it is easy to obtain an age for the Galaxy based on the radioactive decay of uranium-235. The technique is similar to that first used by Fowler and Hoyle[2]. However, the end result differs by a factor of 2. The production ratio (uranium-235/uranium-238 = 1·64) obtained by Burbidge, Burbidge, Fowler, and Hoyle[3] is used. To show how uncritical this assumed production ratio is, ages are calculated also with assumed ratios of 2·0 and 1·0.

Designating by N the fractional abundance of uranium-235 (or uranium-238), that is, the number of atoms of uranium in the interstellar medium per atom of the interstellar medium, N satisfies the differential equation:

$$\frac{dN}{dt} + \gamma N = S(t) \qquad (1)$$

Here γ is the decay constant ($\gamma_{235} = 0{\cdot}97 \times 10^{-9}$ years^{-1}; $\gamma_{238} = 1{\cdot}54 \times 10^{-10}$ years^{-1}), and S is the fractional production-rate of the isotope ($S_{235}/S_{238} = 1{\cdot}64$)[3].

Possible production distribution curves are plotted in Fig. 1 against time expressed in units of the present age of the Galaxy (T). Curve (1) is determined by the requirement that the heavy elements be generated prior to the time of formation of *NGC* 188 ($t = 0{\cdot}4T$).

This relative time of formation of *NGC* 188 and the other clusters plotted in Fig. 1 is based on the stellar evolution time-scale which is here being questioned. There is considerable uncertainty about the linearity of the stellar evolutionary time-scale, first because of uncertainties in the evolutionary theory, particularly of Population II stars, secondly because of a possible secular decrease of the gravitational constant, a variation which, if it occurred, would serve to stretch substantially the time-scale for older stars, making an old star appear much older than it actually is[4–7].

If Salpeter's model[8] of stellar evolution is used, stars being produced at a rate proportional to the amount of gas present in the interstellar medium, it is found that the duration of the halo-forming period is about $0{\cdot}2T$. There is an indication from the very large velocity dispersion in the halo population that even this short period is too long. It is generally agreed that these stellar velocities are fossil remains of the initial high turbulence velocities in the primordial gas of the Galaxy, the stars continuing to move with the original velocities of the gas out of which they were formed. The relaxation-time for this early turbulence to die out was probably less than 2 × 10^8 years (ref. 9). If the turbulence was maintained for a longer period by radiation from massive stars in the initial population, the duration would be limited by the life-time of these stars to less than 10^9 years. These possibilities are mirrored in curve No. 2 of Fig. 1.

If, as is suggested by Arp's data, the heavy elements were not produced completely by the early Population II stars, but are still being produced to some small extent, it would be reasonable to assume, for the fractional production-rate, the function plotted as curve No. 3 in Fig. 1. This curve represents 60 per cent production associated with the function S_2 (curve No. 2), and 40 per cent distributed uniformly from 0 to T. Consistent with Arp's data, this production distribution curve would give an excess of heavy elements in young clusters of roughly 30 per cent. The constant value of the fractional production-rate, after the halo formation period, is consistent with Salpeter's model of galactic evolution. Fowler and Hoyle[2] assumed that Salpeter's model implied that an exponential function should be used for the production-rate. While this is true of the total production-rate, the fractional production-rate is approximately constant with Salpeter's model.

The solution to equation (1) giving $N(T–4{\cdot}7)$, the fractional abundance at the time of formation of the solar system, assumed to be 4·7 × 10^9 years ago, is:

$$N(T-4{\cdot}7) = \int_0^{T-4\cdot7} S(t)\,\exp(t + 4{\cdot}7 - T)\,dt \qquad (2)$$

For curves 1 and 2, it is convenient to take $S(t)$ to be in the form of a Gauss error curve:

$$S(t) \sim \exp\left[-\delta\left(\frac{t}{T}\right)^2\right] \qquad (3)$$

and for curve No. 3 it is:

$$S(t) \sim 0{\cdot}4\,\frac{1}{T} + 0{\cdot}6\,\frac{2}{\sqrt{\pi}}\,\frac{\delta^{1/2}}{T}\,\exp\left[-\delta\left(\frac{t}{T}\right)^2\right] \qquad (4)$$

For the three curves of Fig. 1, Nos. 1, 2 and 3, δ assumes the values 24, 72 and 72 respectively. The ratio of the two values of N for N_{235} and N_{238} at the time of formation of the solar system, computed from the present observed ratio of 0·0072, is:

$$\frac{N_{235}(T-4{\cdot}7)}{N_{238}(T-4{\cdot}7)} = 0{\cdot}34 \qquad (5)$$

Table 1. Age of Galaxy from Uranium Decay for the Three Assumed Fractional Production-Rates plotted in Fig. 1

Curve from Fig. 1	Assumed production ratio $^{235}U/^{238}U$ 1·0	1·65	2·0
No. 1	7·1 × 10^9 yr.	7·7 × 10^9 yr.	8 0 × 10^9 yr.
No. 2	6·6 × 10^9 yr.	7·2 × 10^9 yr.	7·50 × 10^9 yr.
No. 3	—	7·4 × 10^9 yr.	—

Equations (2) and (3) or (4) are substituted in equation (5) which is solved for T to determine the present age of the Galaxy. The various results for curves No. 1, 2 and 3, and for the 3 assumed values of the production ratio, are given in Table 1.

Fowler and Hoyle's[2] determination of 15 × 10^9 years for the age of the Galaxy is based on the decay of thorium-232 as well as uranium-235 and uranium-238. Because of the long mean life of thorium-232 (20·1 × 10^9 years), an age determination based on this element is quite sensitive to the assumed production ratio thorium-232/uranium-238 = 1·65 (ref. 3) and to the present abundance ratio, which, because of the difference in chemistry, cannot be determined with precision. Fowler and Hoyle take this ratio to be 3·8 ± 0·3.

Instead of using the thorium-232/uranium-238 ratio to help determine the age, the procedure can be reversed and the above determined ages can be used to calculate a production ratio. With an assumed age of the Galaxy of 7·2 × 10^9 years and a production-rate given by curve No. 2, the assumed present ratio thorium-232/uranium-238 of 3·8 ± 0·3 implies a production ratio thorium-232/uranium-238 of 1·9 ± 0·2. This to be compared with a value calculated from the theory of nucleosynthesis[3] of 1·65 ± 0·15. The agreement is good.

It should be noted that an age of the Galaxy of 7·2 × 10^9 years is perfectly consistent with an evolutionary universe with a Hubble age of 12 × 10^9 years. If, as has been suggested, the universe is closed, but with a large radius, its evolutionary age would be but slightly less than $\frac{2}{3} \times 12 \times 10^9 = 8 \times 10^9$ years (assuming zero for the cosmological constant). This is an encouraging result and indicates that finally the Hubble age of the universe may be settling down to something like its final value.

The difference between the age of 7·4 ± 0·5 × 10^9 years determined here, and the age of approximately 15 × 10^9 years determined earlier by Fowler and Hoyle may be traced to two factors, the different use made of the thorium data and a different assumption about the form of the production curve. These ages also disagree with the present stellar evolution age of 26 × 10^9 years (ref. 10). A possible reason for this was discussed[7] previously.

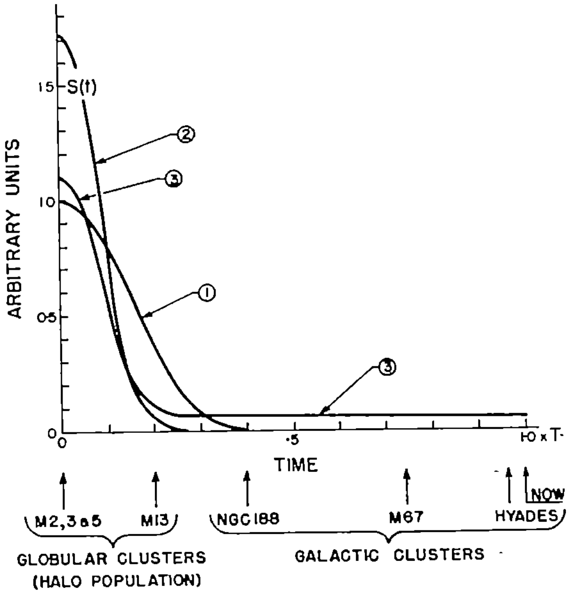

Fig. 1. Uranium production-rate (arbitrary units). T represents the present age of the Galaxy

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THE DESERT LOCUST AND TROPICAL CONVERGENCE

By H. J. SAYER

Desert Locust Survey, East Africa High Commission, Nairobi

THE extended use of aircraft against swarms of the desert locust (*Schistocerca gregaria* Forsk.) in eastern Africa during the past decade has provided further evidence that flying swarms of locusts are displaced downwind[1–3], and the net effect of such downwind movement will, in general, bring locusts into zones of wind convergence, which are areas where there is an excess inward flow of air on the horizontal plane, the excess being accounted for, by vertical air currents. In the tropical zone infested by the desert locust there are several regularly identifiable semi-permanent convergence zones often characterized by asymptotic streamlines having easily recognized annual cycles which probably dominate and certainly largely affect the annual movement and breeding of the locust. Locust movement on the inter-continental scale has been extensively studied for many years and is relatively well known[4]; but recently it has been possible to make detailed observations, hour by hour and day by day, on the complex system of swarms and storms which often occurs at a zone of convergence. Of the several regularly occurring tropical convergence zones, the most significant as regards the desert locust is that which is called the 'intertropical convergence zone' or the 'intertropical front', the meeting

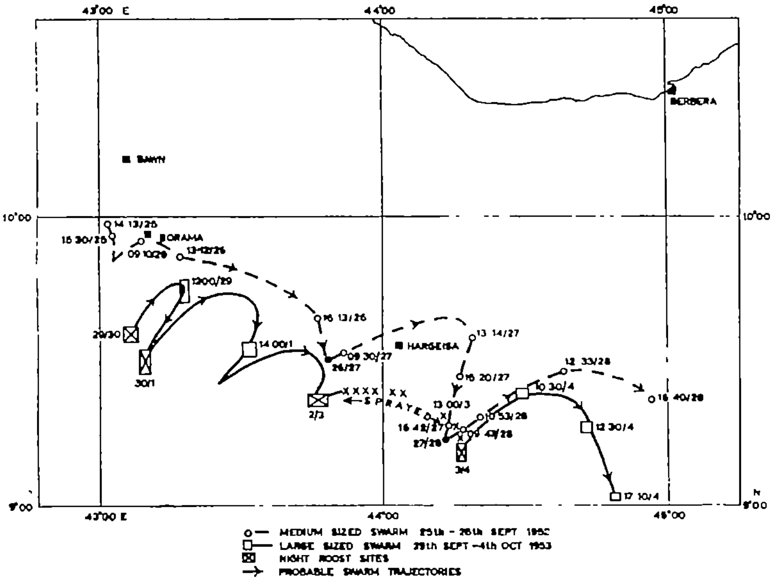
43°00 E
44°00
45°00
10°00
9°00
N
BERBERA
BAWN
BORAMA
HARGEISA
MEDIUM SIZED SWARM 25th – 26th SEPT 1953
LARGE SIZED SWARM 29th SEPT – 4th OCT 1953
NIGHT ROOST SITES
PROBABLE SWARM TRAJECTORIES

Fig. 1. Plots of trajectories of two large swarms across Somaliland during September 25–October 4, 1953. The front at this time and place is beginning to move southwards and therefore the swarms spend longer in the frontal system, while night-roost sites are located successively farther south

place around the tropical belt between the trade winds or monsoons of the northern and southern hemispheres[5]. On most synoptic meteorological charts prepared in tropical Africa, the intertropical convergence zone is identified as lying between stations having large differences in wind direction, and sometimes, though not always, differences in other physical properties of the air-streams, such as temperature and dew-point. Because of the low density of meteorological stations over most of this tropical belt, the exact position of the intertropical convergence zone is often doubtful.

There are three distinct and superimposed movements of the intertropical convergence zone differing in both amplitude and duration. The largest movement is an annual north–south movement following the Sun with the usual month or so of lag common to all Sun-generated weather cycles. This thermally controlled movement is greatest over the continent, especially the deserts, and least over the sea, and can have a range over land of 40° of latitude[6]. The smallest oscillation is a daily movement involving southward movement during the earlier part of the day with a reversal in the late afternoon, and has an amplitude of the order of a few tens of miles. Lastly, there is an intermediate scale of movement which can have an amplitude of the order of one hundred miles or more around a mean position, and which lasts several days.

Detailed observations on the effect of the two latter small-scale movements were first made in Somaliland from September until October 1953 (ref. 5) during the first air control operations in that area, and the map (Fig. 1) shows the trajectories of two swarms followed during that period. It can be seen that swarms moved daily on a zig-zag course, north-east–south-west, making a small net displacement eastwards each day. Wind observations made at that time show that over Hargeisa, a south-westerly was established in the morning followed by a period of doldrums at 13.00–14.00 hr., with a reversal to north–north-easterly during the remainder of the afternoon. The swarms moved away from their night-roost site at about 09.00 hr., progressing north-easterly, reversing their tracks in early afternoon to south-south-westerly and maintaining this course most of the afternoon, followed sometimes by a reversal near sunset, to the night-roost site.

A large-scale infestation of locusts in the same area during the period June–August, 1960, presented an opportunity to make further detailed observations on these complex locust and weather situations characteristic of this area and time of year, and previously described as a chaotic jumble of swarms and storms. Two or three of the many observations made at that time serve to illustrate the most important phenomena which were repeatedly seen in this area during the period.

The east–west elongation of swarms in the late afternoon was demonstrated by a swarm stretching along the Somali coast (on June 14) for 40 miles from Berbera westwards, found in the middle of the afternoon, the coast here being roughly east–west, and on June 28 by another swarm measuring 30 miles east–west and 2–3 miles north–south found to the north-west of the operational base at Borama in the late afternoon. The latter swarm appeared, when viewed along the east–west axis, as a dense black streak sloping upwards for several thousand feet from the ground, with its top towards the south. During traverses made by the reconnaissance aircraft along the 2–3 mile north–south axis of the swarm (the extent of which was recognized by locusts impacting on the aircraft and not by flying over them), a rate of ascent of several hundred feet per minute with a maximum of more than one thousand feet per minute for a short distance near the centre, was recorded on the aircraft instruments. From these and other observations made during the period it became obvious that the towering, sloping masses of flying locusts were situated in a wind discontinuity. Smoke generators dropped from an aircraft showed opposing winds at less than 10 miles apart, and incidental observations made while travelling on a north–south road, suggested a transition even more abrupt than this.

At the intertropical convergence zone in the Sudan, E. Kruger (unpublished results) has similarly recorded opposing winds at stations less than 13 miles apart. From several such air traverses, together with pilot balloon data, made at the base at Borama and at Bawn (about 20 miles north of Borama) and orientated photographs of the locusts it was possible to construct the schematic north–south cross-section shown in Fig. 2, which is believed to be representative of the discontinuity as most frequently found near Borama during June–August. The wind from the south side was almost consistently from 220° to 230°, the south-east monsoon having crossed the equator by some 10° of latitude into the northern hemisphere, and therefore having been deflected by the Coriolis force to a south-wester. The wind from the north side appeared to be more variable between north and north-east, possibly due to the influence of the nearby Ethiopian highlands. Near the ground, a strong upward velocity appeared to be reached at a relatively narrow 'neck'; but at higher levels a more extensive but generally weaker ascent was recorded, probably due to turbulent mixing and entrainment of air from the north and south surfaces. At ground-level there was an area of doldrums, with occasional light puffs of wind from no constant direction. The slope of the discontinuity in this area, indicated by swarm photographs, was about 35° with the top towards the equator, at least to the highest levels recorded (about 5,000 ft. a.g.l.). This slope would seem to be due to the northern air mass, which is of mainly continental origin, being slightly higher in temperature (3° C.) and probably less dense than the air mass from the south, which, on its south-easterly course south of the equator, has come over the Indian Ocean during the latter part of this track.

In its diurnal movement the discontinuity, starting from its most northerly position in the early hours, progresses southward during the day to its southern limit and intensifies until late afternoon, after which there is a weakening and often a reversal of movement at sundown. This daily cycle, it will be shown, together with local topography and the associated change of temperature with altitude, determines the daily changing distribution of the associated adult locust populations. As was first shown in the 1953 observations, any swarms on their night-roost site which are in the south-westerly wind in the morning will depart in a north-easterly direction. The south-westerly wind is strong, 15 or more knots, and the drifting of the locusts away from the roost site for a period of an hour or more results in a low-flying, low-density swarm elongated north-east–south-west. At some time during the early afternoon the northward-moving locusts meet the southward-moving intertropical convergence zone and a striking difference is immediately seen in the swarm appearance. A towering dense mass of flying locusts rapidly rises upwards until a more or less stable appearance is established.

When viewed from either the north or south it appears as a sheet of flying locusts with very dense striations, rising sometimes to several thousand feet above the ground, topped by an undulating, dense mass. Viewed along the east–west axis, the flying locusts appear as a dense black streak sloping upwards with the top nearest to the equator and often with a broad, dense, umbrella-like top (Fig. 3). The density of flying locusts in the front (as determined from photographic records[8]) can be more than a hundred times that of the locusts on the approach to the front. It is suggested that this very obvious change in the appearance of the swarm in the intertropical convergence zone, compared with the pattern outside it, can be interpreted in terms of the structure of the front as indicated in the cross-section shown in Fig. 2. The inflowing air from both north and south has velocities often comparable with or greater than the flying speed of the locust (3·8–4·5 m./sec. (ref. 9)) and even if all the flying locusts were orientated either north or south they would be unable to make any progress away from the front. By analogy with observations on orientation in swarms elsewhere, it is more likely that flying locusts are effectively randomly orientated[10], the front acting as a concentrator on the north–south axis but not restricting movement along the front, that is, along the east–west axis.

The dense undulating appearance of the top of the locusts can most likely be explained as an effect of a combination of the decrease in temperature with height (found to be at the dry adiabatic lapse rate) and the widening and slowing down of the vertical air stream. Since the body temperature of the locusts is very close to that of the air in which they are flying, they will, correspondingly, cool with height. An altitude will be reached at which the locusts' body temperature will be less than the temperature at

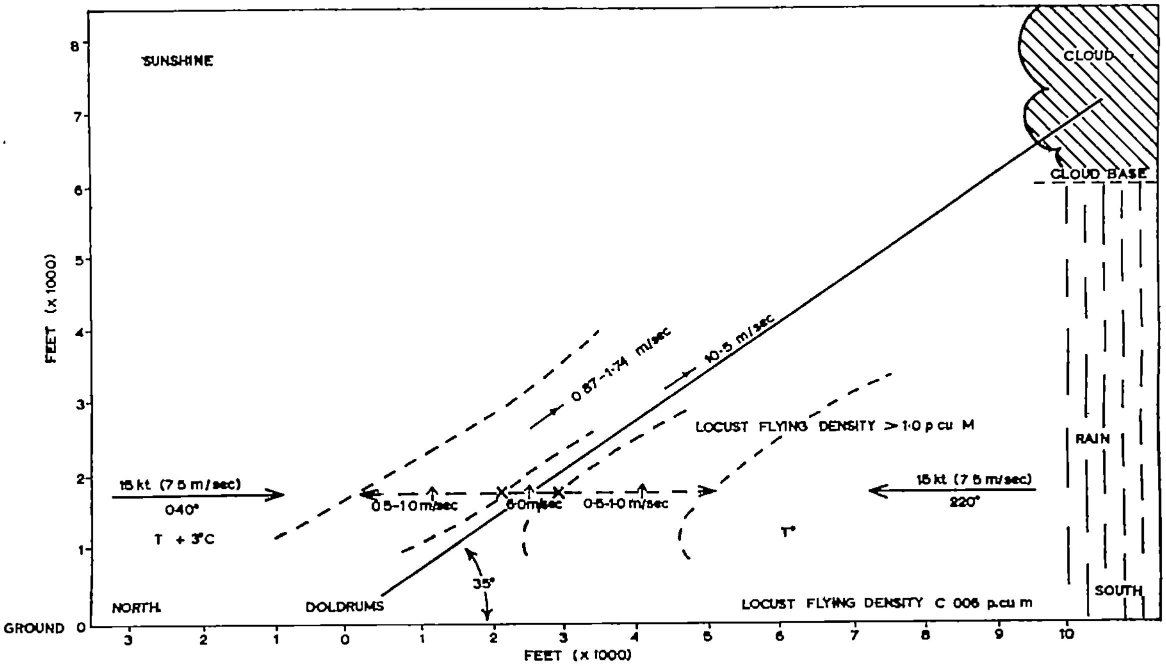

Fig. 2. Typical north–south cross-section of the inter-tropical front as found around Borama, Somali Republic, during June–August, 1960

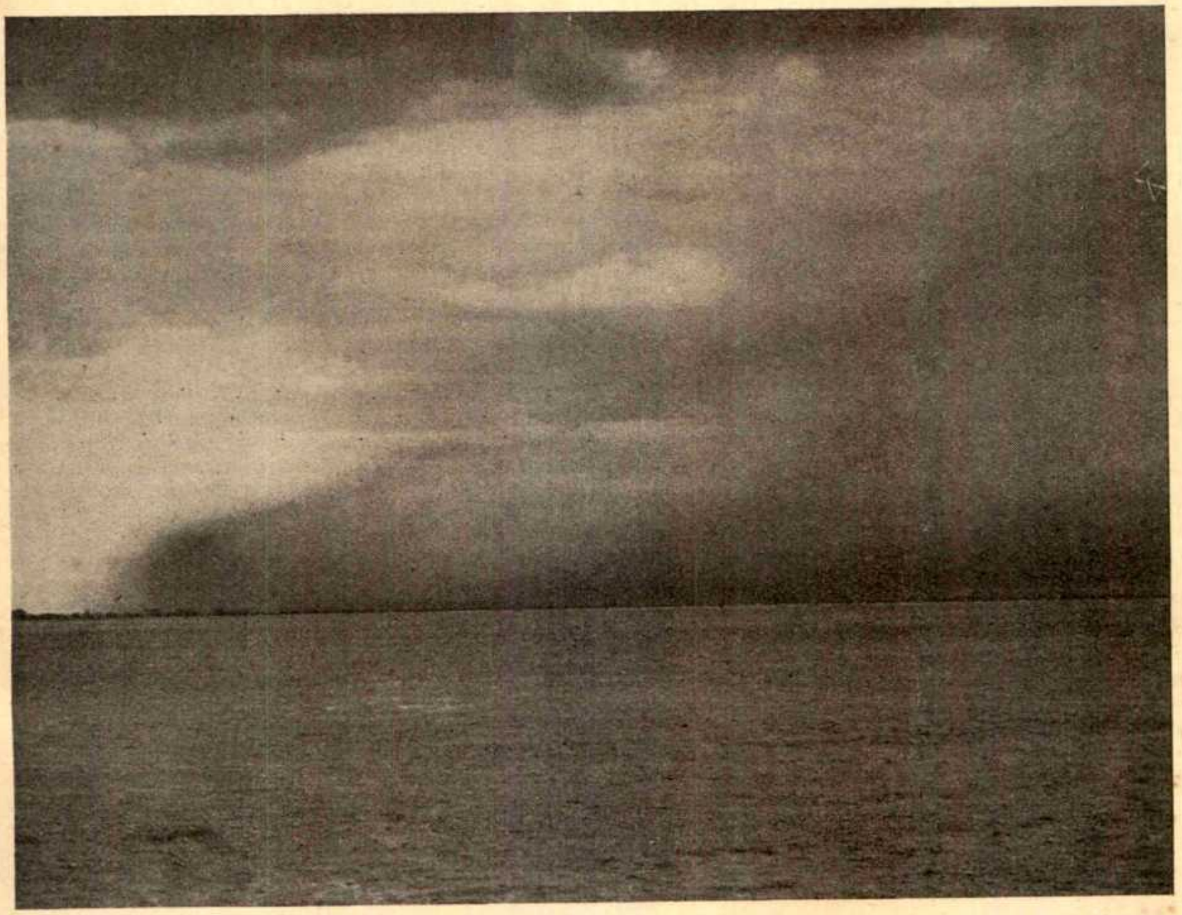

Fig. 3. Large swarm showing typical association with convergence. The camera is pointing approximately towards the north-east from Hargeisa airport (Northern Region, Somali Republic, August 3, 1960). The photograph was taken in the late afternoon and the density of the swarm when it passed over the airfield was such as to prevent aircraft taking off. Photograph by A. J. Wood, Desert Locust Survey

which flapping flight can take place; and there is every reason to believe that, at this altitude, the locusts stop flapping flight and adopt a gliding posture. The gliding locusts will tend to concentrate at an altitude at which a balance is reached between the rate of gliding descent and the rate of air ascent. The highest point of balance will be where the up-draught is greatest near the centre and, thus, the curved umbrella-shaped streaks at the top of the swarm would indicate the decreasing velocity gradient, due to the turbulent mixing and entrainment of more air from the surfaces inwards. Those gliding locusts which, in their various random courses, reach the south face of the discontinuity, will drop down into the south-westerly wind, either to be brought back into the system at a lower level or to reach the ground. Those reaching the edge on the northern face will drop down into the up-draught again. Thus, the northern surface will tend to be more sharply defined than the southern, the system, by virtue of the slope, showering gliding locusts towards the ground on the southern side.

Wind convergence, therefore, together possibly with the adiabatic lapse rate, acts as a concentrator of flying locusts which contrasts with the state existing during the post-frontal movement of swarms, wherein the wind, by virtue of turbulence, both convective and flow, is operating as a disperser of locusts; and wherein the only cohesive factor is the gregariousness of the locusts which are counteracting the wind dispersion and maintaining the identity of the swarm. In frontal flight, gregariousness and meteorological conditions can thus act together to concentrate the locusts, producing some of the highest flying densities ever to be recorded. Thus very high flying densities, averaging about 1 locust per m.^{3}, were recorded photographically in a swarm near Asmara in August 1960, which was seen to exhibit rapid ascent between opposing winds separated by no more than 3 km. Along the length of the front, the third dimension, there is no constraint on the locusts' movement except gregariousness, and, in time, swarms extend along the front, often coalescing with other swarms so that by the end of the day there may be an almost continuous line of flying locusts, two or three miles north–south and tens of miles east–west, as was repeatedly seen to occur.

The winds on each side of the discontinuity near Borama were almost diametrically opposed, and the diurnal movement of the front would be expected, therefore, to produce a negligible net daily displacement of swarms. However, there was always a net movement eastwards from Borama, as can be seen in the 1953 swarm trajectories (Fig. 1).

Elsewhere, such as near Dessie and Asmara to the north-west in neighbouring Ethiopia, a northward displacement of swarms has been observed along the northward inflexion of the front. Two possible

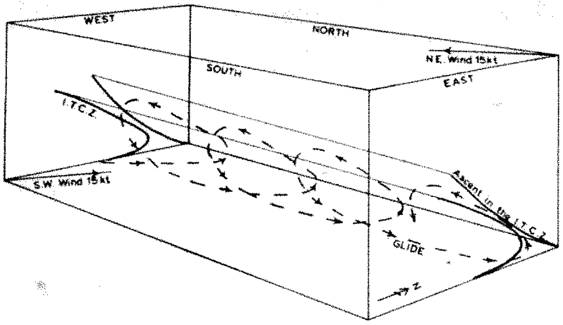

Fig. 4. Suggested average frontal track of locusts. The approach path is along the south-west wind which is followed by a rapid ascent in the updraught at the interface. Release from the convergence occurs by gliding down into the south-westerly wind resulting in a net easterly gain after the return to the front

explanations of this lateral frontal displacement are suggested. All the pilot balloon ascents through the front made at Borama showed that the air in the front was moving eastwards, the wind veering through north-west at the interface. The frontal air, therefore, besides having a vertical motion, was also moving eastwards. The second explanation is that the slope of the front causes locusts to be showered into the south-wester underneath, thus continuously adding an easterly movement to the body of the locusts from the steadily returning locusts in the south-wester (Fig. 4). This suggests that it is the wind beneath the front, the southern side, which largely determines the frontal displacement, the wind from the northern side having a negligible influence in this respect. The two effects may operate together as during these observations at Borama, or sometimes may oppose each other. As a general rule, it appears that locusts in the front are displaced along the front in the direction opposite to that from which the southern hemisphere wind approaches the front. There appears to be a point at or about the inflexion of the intertropical convergence zone near Diredawa, where swarms can arrive at the front within a few miles of each other and yet move along it in opposite directions to become hundreds of miles apart in a week or so. At this point of dispersion or instability, swarms have a small net daily displacement, but farther away along the front, the net daily displacement appears to increase. From these and other observations of locust movements at the front it is reasonable to assume that along the intertropical convergence zone across Africa there are several points of dispersion such as that found near Diredawa, which are also interspersed with points of accumulation (or stability).

The effect of the intermediate order of movement of the intertropical convergence zone, the surge about a mean position lasting several days, superimposed on the daily cycle, complicates considerably the relatively simple picture of the daily swarm movement given above. During the northward movement, the intertropical convergence zone, in this area and season, generally appears sharp and well defined, the cloud-line formed by the middle of the afternoon being fairly narrow and clear, whereas during the southward surge, it is usually associated with a much wider belt of bad weather, the front appearing extremely broken and ragged. A rapid northward movement of the front means that the locusts will have farther to go from the night-roost site, which is generally to the south, in the south-westerly wind to the front, and, consequently, will arrive in the front much later in the afternoon. The time of arrival at the front will also be influenced by the time of departure from the roost site and the wind speed, which are both affected by temperature and, therefore, by the altitude of the ground above sea-level and its distance from the coast. The build-up of clouds at the front was usually seen to start at about mid-day, and by the middle of the afternoon rainstorms were beginning to appear, the rain being a few miles south of the ground-level meeting point of the winds owing to the slope of the discontinuity.

Locusts approaching the front in the late afternoon will, therefore, often have to pass through a belt of rain-storms, which may not necessarily be continuous. Swarms become disintegrated by this storm belt, some locusts ceasing or reducing flying because of the rain, while others may have a rain-free path to the front and be carried many miles away from the locusts affected by the rain. Under such conditions, large swarms become disintegrated and break up into several small swarms connected by a thin, scattered population. Thus, over a period, the front can act at one time as a concentrator, producing a massive, dense swarm containing all the locusts in the area, and at another as a disintegrator producing many small swarms tenuously connected by a thin population. In contrast to post-frontal flight, where individual swarms maintain their identity by means of gregariousness, locusts at the front can only be considered as part of a general population, individuals being successively concentrated and dispersed, and being constantly redistributed by the frontal meteorological conditions.

The results of the effect of convergence can be exploited for aerial control operations, both from the point of view of the daily tactics and from the overall strategy. The indications that by the late afternoon flying locusts may be held balanced at high density in the wind discontinuity suggests that a cloud of insecticide spray droplets of suitable size and therefore terminal velocity, would be held similarly, spraying being envisaged as taking place from the same point of supply as the locusts, that is, underneath. The spray would be dispersed only along the front, and, provided that a suitable non-volatile formulation were used[1,2], thus ensuring efficacy over a long period, the frontal balancing and concentrating conditions affecting both locusts and spray would give a considerably increased chance for spray collection by the locusts, compared with previous post-frontal air-to-air spraying experience.

Aerial reconnaissance for swarms in the past has been based on a knowledge of the post-frontal movement of swarms in an established air-stream, and has been designed to search, usually on a triangular or rectangular course, the air-stream approaching the operational base. Frontal reconnaissance, however, can be designed to search more or less linearly along the front (easily identified by a cloud line at the commonly adopted reconnaissance period, early afternoon). Such linearly directed flights could considerably increase the range and effectiveness of reconnaissance, besides eliminating pointless search in areas where swarms are unlikely to be found. Table 1 shows the approximate time-periods of the various stages of the desert locust life-cycle, together with their displacement rate and, in the case of adults, their flying density. It will be seen that the longest period for the whole cycle is that in which the adult locusts are associated with frontal convergence, and

Table 1. APPROXIMATE PERIODS OF THE LIFE-CYCLE OF THE DESERT LOCUST

	Period (months)	Rate of movement (miles per day)	Representative flying density (locusts per m.^{3})
Egg	0·5–1·5	nil	
Nymph	1·0–2·0	0–1·0	
Total adult	0·5–4·0		
(*a*) Under post-frontal conditions	0·5–1·0	20–100	0·01
(*b*) Under frontal conditions	1·0–3·0	0–20	1·0

this is also the displacement period for adults which gives rise to regularly occurring high-density flight. The efficiency of killing locusts, measured in terms of locusts killed per gallon of insecticide, by air-to-air spraying (which can be the most consistently efficient of all locust control methods) has been shown to be almost linearly proportional to the locust flying density[1], and thus the conditions which can occur in the late afternoon when locusts fly densely in the intertropical convergence zone are, if they can be exploited, of very great importance. The small net daily displacement at the front means that aerial operations can take place against the same locusts from the same air-base for a period of several days, in contrast to post-frontal operations, wherein, in order to apply in the shortest possible time the quantity of insecticide which would be required to control large and therefore economically potentially dangerous swarms, it would often be necessary to move the operational air-base almost daily—an onerous and costly task.

The movement of locusts latitudinally along the front from areas of dispersion to areas of accumulation suggests that these are worth identifying and assessing for control operations, since both may be areas of slow swarm displacement. The convergence zone across Africa, acting as a major concentrator of locusts, appears to produce transient longitudinal inflexions during its annual cycle, associated usually with significant geographical features such as large mountain masses. These frontal inflexions appear to have a retarded movement as compared with the normal annual cycle of movement over relatively flat desert or bush and may therefore be of strategic control value. The greatest surging and the greatest fluctuation around the mean position can be expected where the annual cycle has the largest range and where the geographical background is most homogeneous. On the other hand, preferred positions for the front may be provided where orographic features in themselves tend to produce a front. Thus, in northern Somaliland the coast-line runs roughly east–west with a range of mountains running parallel to the coast a few miles inland, both tending to give rise to a convergence zone a few miles inland in the late afternoon due to the sea-breeze effect and an anabatic wind Such fixed orographic zones of convergence can trap and reinforce the intertropical convergence zone for periods greater than normal, giving rise subsequently to a higher rate of movement across the plains. The two periods of the intertropical convergence zone's annual cycle during which it is relatively static are at the solstices, when the Sun's latitudinal movement is slow, and these two seasons (June, July, August and December, January, February) may be expected to be the most important from the point of view of adult control. Places where the intertropical convergence zone becomes virtually stationary for periods of two or three months are major accumulator points for locusts, and are reflected as maxima in swarm frequency maps.

The swarm movement latitudinally along the front will, over a period, tend to bring locusts to the eastern and western seaboards. Further slow frontal displacement out over the sea would result in exceeding the flight endurance of the locusts with subsequent drowning. Although from time to time large numbers of locusts have been recorded as drowned, the general pattern of coastal movement is the survival of a significant proportion of the arriving locusts. This low overall loss at the seaboards is apparently due to the sea-breeze effect which is strongest in the afternoon at the same time as the locusts are usually flying most actively. The sea breeze at this time of the day regularly produces convergence zones along the coast of Africa which not only tend to stop further seaward movement of the locusts but can also move them along the coastal belt[11] in a way similar to the movement along the intertropical convergence zone. Owing to the differences of surface heating and radiation over the land and sea and consequent differences of latitudinal displacement of the intertropical convergence zone, it is often deflected along the coast, reinforcing the sea-breeze effect at these places. The limits of the area which the desert locust regularly infests do not coincide with the limits of the intertropical convergence zone for the northern solstitial position although very good agreement is shown by the southern limit of the intertropical convergence zone and the known locust distribution. At the same time, the distribution area and the intertropical convergence zone limits are not evenly placed in relation to the equator, both being displaced towards the northern hemisphere. This northern bias is explained by the greater amount of ocean and the lower temperatures over Antarctica giving rise to stronger winds in the southern hemisphere, which penetrate across the equator into the northern hemisphere[12]. Over most of Africa, the southern limit of the intertropical convergence zone is mainly north of the equator except for a deep inflexion over eastern Africa, and the northern limit is generally over 15° north. This suggests that the desert locust has more chance of being involved in subtropical weather in the northern hemisphere than in the south, as may be found reflected in the distribution maps.

Lastly, it is of interest to consider the annual cycle of movement of the intertropical convergence zone and its possible effect on breeding. The solstitial movement of the convergence zone over Somaliland keeps the locusts in front of the rain-belt and therefore over relatively dry terrain. The southerly movement of the front, which occurs in October, November and December, brings the locusts over an area which normally has had recent heavy rain. Since the desert locust requires moisture for successful hatching of its eggs, and it is thought that moist conditions encourage maturation, the southward movement of the front might be expected to be the most conducive to successful breeding. It is perhaps significant that during this period of the year, in the Somali peninsula which has long been recognized as a major high-frequency breeding area, breeding recorded during the southerly movement of the front is often more extensive than during the northerly movement.

I thank Mr. P. R. Stephenson and Mr. R. J. V. Joyce, director and deputy director of the Desert Locust Survey, Nairobi, for making this work possible, and for their helpful encouragement, and also Drs. T. H. C. Taylor, P. T. Haskell and R. C.

Rainey, and Miss Z. Waloff and Miss E. Betts (all of the Anti-Locust Research Centre, London) for their considerable help in producing this article.

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NEWS and VIEWS

French Academy of Sciences

Mlle. M. Perey has been elected the first woman corresponding member of the French Academy of Sciences. She succeeds the late Emile Henriot. L'Institut du Radium writes, through Prof. A. Haddow: "L'élection de Mademoiselle Marguerite Perey à l'Académie des Sciences de France est venue récemment couronner sa brillante carrière de radiochimiste. Entrée en 1929 au Laboratoire de Madame Curie, Mademoiselle Perey s'était spécialisée dans la chimie des éléments de la famille de l'Actinium. La qualité remarquable de ses travaux avait permis de mener à bien de nombreuses recherches physiques qui nécessitaient l'emploi de sources radioactives très soigneusement préparées. L'aboutissement de ces travaux a été, en 1939, la découverte par Mademoiselle Perey d'un radio-élément nouveau, le Francium (Actinium K,Z = 87, période 21 minutes). Au cours des années suivantes Mademoiselle Perey s'est attachée à l'étude de la préparation et des propriétés physiques et chimiques du Francium. Ces travaux ont fait l'object de sa thèse de Doctorat soutenue en 1946.

"Nommée en 1946 Maître de Recherches au Centre National de la Recherche Scientifique, puis, en 1949, Professeur à la Faculté des Sciences de Strasbourg, Mademoiselle Perey devait, en 1951, faire une autre découverte importante, celle des propriétés biologiques du Francium, et leur application à des problèmes de cancérologie. Le Francium est, en effet, un alcalin (homologue supérieur du Césium) et malgré la courte période de cet élément, Mademoiselle Perey a pu étudier sa répartition dans les tissus biologiques et constater sur des rats porteurs de sarcomes qu'il se fixe 2 à 4 fois plus vite dans les tissus cancéreux que dans les tissus sains. Mademoiselle Perey a ainsi trouvé un moyen nouveau pour établir le diagnostic précoce du cancer, et plusieurs de ses travaux récents sont relatifs aux propriétés biologiques des radioéléments alcalins. Lauréate de l'Académie des Sciences, Mademoiselle Perey a reçu en 1961 le Grand Prix Scientifique de la Ville de Paris. Malheureusement sa carrière a commencé à une époque où les dangers des radiations étaient mal connus, et malgré le soin avec lequel elle travaillait, elle n'a pas échappé aux atteintes du rayonnement, et elle en subit actuellement les douloureuses conséquences. Nous souhaitons vivement qu'elle en soit rapidement guérie".

Pharmacology at Cambridge :
Prof. E. B. Verney, F.R.S.

Prof. E. B. Verney has retired from the Sheild chair of pharmacology in the University of Cambridge. He went to Cambridge in 1934 from the chair of pharmacology at University College, London. A pupil of E. H. Starling, his early work was on the isolated perfused kidney. This led to his classical studies (with F. R. Winton, Mary Pickford, G. W. Theobald, Marthe Vogt, W. J. O'Connor and P. A. Jewell) on the nervous, hormonal and vascular factors controlling urinary excretion and water diuresis, to the demonstration that the secretion of antidiuretic hormone depends on the osmotic pressure of the blood, and to the identification of the osmoreceptors in the neurohypophysis. He also worked on the reflex regulation of the heart-rate, and on central nervous control of ovulation.

Prof. A. S. V. Burgen

Prof. A. S. V. Burgen, professor in the Department of Physiology, McGill University, Montreal, has been appointed to succeed Prof. Verney. After qualifying in 1945, he had been in Prof. C. A. Keele's Department of Pharmacology at the Middlesex Hospital Medical School, and then joined Prof. F. C. MacIntosh at McGill in 1949. His first major work was on the general pharmacology and kinetics of the anticholinesterases hexaethyl tetraphosphate and tetraethyl pyrophosphate, which led to work on the distribution of cholinesterase in the brain and on factors governing uptake of inhibitors into the brain. With F. Dickens and L. J. Zatman, he has made the only direct demonstration of the mode of action of botulinum toxin. In Montreal he showed, with microelectrode techniques, the characteristic action of the cardiac vagal transmitter, acetylcholine, on the action potential of the auricle and that it was due to an increase in potassium permeability. He later returned to the physiology and pharmacology of salivary secretion, work which is summarized in a recent Physiological Society monograph with N. Emmelin. Most recently he has worked on the turnover of fluid in pleural exudates, on reactions to autologous and non-autologous human plasma, and on the fate in the body of labelled magnesium and folic acid.

The 1961 Wolfe Award : Dr. Keith Froome

Dr. Keith Froome, of the Standards Division of the National Physical Laboratory, has been awarded the £500 Wolfe Award for 1961 for two major advances in the field of accurate measurement. This award is the fourth of ten to be made annually, under the terms of the Wolfe bequest, to the research worker in the Department of Scientific and Industrial Research who makes an outstanding contribution to the Department's research during the previous twelve months. One of Dr. Froome's achievements during 1961 was the invention of the 'Mekometer', an instrument using a beam of light to measure distances from a few yards to several hundred with great accuracy (1/100 cm.). He was also responsible for the production of continuous radio-type (or radar-type) electromagnetic waves of extremely short wave-lengths from the "cathode spot" of an electric arc discharge. Using a new arc microwave harmonic generator, wave-lengths as small as 0·4 mm. have been generated, corresponding to a frequency of 700,000 Mc./s. These short waves will be used to measure the velocity of electromagnetic waves to the full accuracy of the new light-wave definition of length, but they have considerable application to wider fields than this, including the investigation of atomic arrangements of crystals, and the study of powerful electric discharge plasmas needed for controlled nuclear fusion. Dr. Froome was educated at Windsor County School and took his first degree in physics in 1941 at University College, London. After the War he spent four years at the Imperial College of Science and Technology, first as Beit Postgraduate Fellow and then as 1851 Senior Fellow, working on the cathode emission process in transient arc discharges. In 1949 he joined the National Physical Laboratory and was assigned to his present work in microwave radiation. He was promoted to principal scientific officer in 1953 and to senior principal scientific officer on special merit four years later.

Fleming Memorial Fund for Medical Research

VISCOUNT TENBY, chairman of the Fleming Memorial Fund for Medical Research since its inception, has been appointed president in succession to the late Lord Birkett. Viscount Caldecote, at present vice-chairman of Council, has been appointed chairman of the Council. Lord Bossom, a member of the Executive Committee, has been elected chairman of that Committee.

Since the Fund's British and International Appeal was launched in March 1961, with an initial target of £1 million, more than £455,000 has been given or firmly promised at home and abroad. The Executive Committee has decided to keep the appeal open—from covenants already given, for example, moneys will continue to be received until 1970—and further British fund-raising efforts are already planned during 1962.

A grant of £13,000 has been made to the University of Edinburgh for its Bacteriology Department by the trustees of the Fleming Memorial Fund. The grant will be used to provide specialized staff and equipment for medical research in relation to studies on parasite–host cell interaction and intracellular parasitism. The research aims at more intensive electron microscopic studies of the interaction between parasite and host at cell-level, with the view of learning more about the phenomena of cell penetration and intracellular parasitism. Parallel investigation of host–parasite interaction will be made, using phase-contrast microscopy, fluorescent staining methods and cytochemical techniques. The first two grants made by the trustees of the Fund were £48,000 to the University of Oxford for the Sir William Dunn School of Pathology (in October 1961) and £15,000 to the Christian Medical College at Vellore, South India, for intensified leprosy research (in December 1961).

Staff Scheme between the Universities of Reading and Khartoum

THE University of Reading has entered into an arrangement with the University of Khartoum for the joint recruitment of staff. The scheme is designed to help the University of Khartoum in filling posts, but its distinctive feature is that it gives the member of staff a guarantee of re-employment in the United Kingdom for a period sufficient to enable him to find a permanent appointment. An advertisement of the first three vacancies (in physics, mathematics and French) has recently been issued. The persons appointed will normally be expected to serve for a period of up to five years, the first four of which will be spent on secondment in Khartoum and the last in Reading. While in Khartoum, each person will have the salary, rank and status appropriate to the post which he holds. The cost of the fifth year, in Reading, will be borne by the University of Reading, although it is hoped that some assistance will be forthcoming from other sources. Indeed, for the first appointment this cost will be met by a generous donation made for the purpose to the University of Khartoum by an oil company. The needs of countries overseas for British staff in their universities cannot be too greatly stressed. The Reading–Khartoum scheme is but one example of what can be done, and it is a good sign that the Department of Technical Co-operation, which supports the arrangement, is discussing the much wider application of this and similar ventures which have long been advocated in these pages (*Nature*, 193, 101; 194, 1 (1962), etc.).

The Pasteur Institute of Southern India

THE Association of the Pasteur Institute of Southern India was founded in 1906 to make available effective means of preventing the occurrence of rabies, to spread the knowledge of such means among the public, and to undertake research work on rabies and other diseases. The Institute is situated at Coonoor in the Nilgiri District of the Madras State and, since its inception in 1907, has served the needs of the Madras and other States of South India. In recent years the Institute has expanded its activities considerably. It is internationally recognized for its research work on rabies. It is the main laboratory for work on influenza and other respiratory viruses in India. It is now serving as an important centre for research on intestinal viruses, vaccinia and smallpox. Studies are also in progress on cholera and syphilis. It is co-operating with the Central and State Governments in various public health activities. The Institute is actively collaborating with international organizations like the World Health Organization and research organizations in India like the Indian Council of Medical Research in several of their research programmes. It serves as a training centre in rabies, other virus diseases and general bacteriology for workers sponsored by the World Health Organization, the Central and State Governments and institutions in India. The Institute has long been recognized by universities for training candidates for postgraduate degrees in bacteriology and virology. The enormous numbers of patients who have been treated with vaccine prepared at the Institute and the numerous papers of scientific interest which have been published during the fifty-four years indicate the important part which the Institute has played in medical and health work in India. Details of its activities in 1960 are set out in its annual report (The Pasteur Institute of Southern India, Annual Report of the Director, 1960. Pp. i+122. Coonoor: Pasteur Institute of Southern India, 1961).

National Research Development Corporation

THE National Research Development Corporation was set up under the Development of Inventions Act, 1948, to secure the development in the public interest of inventions resulting from public research which, in the Corporation's judgment, are not being sufficiently exploited, and to hold and dispose of rights in inventions arising from such research. Its aims and activities have only in recent years become known to many scientific and medical workers through publicity in connexion with some of the major projects with which the Corporation has been concerned, such as electronic computers, hovercraft, fuel cells, cephalosporin *C*, interferon, and the common cold vaccine. As a result, there has been an increase in the number of inventions notified to the Corporation in communications from universities, hospitals and other research institutions. The Corporation's policy, while not encouraging academic research to become primarily orientated towards the development of revenue-earning inventions, aims at encouraging the proper steps to be taken in securing patent protection where patentable inventions are a by-product of research and are of public interest and where they are in the interest of the inventors and their employers. The Corporation already maintains liaison with research workers in a great many of the faculties of universities and with teaching hospitals and other research institutions, but nowadays patentable inventions, particularly in the instrument field, are liable to originate unexpectedly in disciplines such as phonetics, orthopædics, microbiology and psychiatry. In certain instances the Corporation finances development work on an invention which shows promise. The Corporation has made it known that it would be glad to hear of any device or process which could have a commercial application. It warns, however, that it is necessary to take patent action before the relevant work is published in a scientific journal. Further information can be obtained from the managing director, J. C. Duckworth, National Research Development Corporation, 1 Tilney Street, London, W.1.

Security Procedures in British Public Services

IN the House of Commons on April 5, the Prime Minister, Mr. H. Macmillan, announced that the Government accepted the recommendations of the Radcliffe Committee on Security Procedures in the Public Services, and was putting them into effect as rapidly as possible. The report disclosed no radical defect in existing security procedures, but contained valuable proposals for their improvement and intensification. The report was based on a comprehensive and searching scrutiny of British security procedures, but also demonstrated the unique difficulty of maintaining absolutely effective security in a free society. He had discussed the report with the Leader of the Opposition and some of his colleagues, who concurred in the Government's decision that the White Paper published that day contained as much of the report as could safely be published. The Government believed that publication would help to promote the wider public understanding of the importance and the difficulty of the problem of security. Mr. Macmillan gave a full assurance that the method of implementing the recommendations would be discussed with the staff side of the Civil Service National Whitley Council. A similar statement was made simultaneously in the House of Lords by the Lord President of the Council.

Underground Transmission of Electricity in Britain

IN reply to a question in the House of Lords on April 3, Lord Mills said that research into the underground transmission of electricity was the responsibility of the Electricity Council and the Boards, in consultation with the Minister of Power and the Secretary of State for Scotland. Some related research was carried out by the universities and by the Electrical Research Association. The Electricity Council and the Boards were seeking to reduce the cost of transmission underground by using alternative circulating materials and by investigating possible cheaper cables and methods of installation. However, while it was hoped to reduce the wide difference in cost, there was no real prospect that the use of underground cables would ever be as cheap as overhead lines, especially at high voltages. The question of research into this matter was periodically reported on by the Advisory Council on Research of the Minister of Power, and full account was taken of the progress of research in other countries. Even in regard to high-voltage cables, much research was already proceeding.

British Optical Astronomy

IN a written answer in the House of Commons on March 9, the Parliamentary Secretary for Science. Mr. D. Freeth, stated that in its report of 1959–60 the Advisory Council on Scientific Policy had pointed out that British optical astronomy required access to a large telescope of about 120-in. diameter in the southern hemisphere. Various schemes involving international co-operation had been studied, and there had been consultation with other countries. both within and without the Commonwealth, which might be interested in participating in a joint project. Examination of possible schemes was continuing but no decision had yet been reached.

Britain's First Training Reactor for Universities

THE Ministry of Works has let contracts for the construction of an experimental nuclear reactor at East Kilbride in Scotland for a consortium of universities (St. Andrews, Glasgow, Aberdeen and Edinburgh, the Royal College of Science and Technology, Glasgow, and Queen's University, Belfast). The reactor will be made by Advanced Technology Laboratories of California, in association with G. and J. Weir, Ltd., of Glasgow. The laboratory building in which the reactor will be housed was designed by the Ministry of Works and will be erected by J. Crawford, Ltd., of Glasgow. The capital cost of the scheme will be paid for by a grant of £255,000 from the Department of Scientific and Industrial Research to the consortium. The reactor, which will be next to the National Engineering Laboratory, is designed for a continuous power output of up to 100 kW., and will provide the universities with an extremely versatile aid for teaching and training engineers, physicists, chemists and biologists and for research in chemistry, metallurgy, biology, clinical science, etc. It will be the first university research reactor in Britain.

International Commission on Zoological Nomenclature

NOTICE is given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in *Bulletin of Zoological Nomenclature* (19, Part 2; March 23, 1962): (1) Designation of a type-species for *Carcharhinus* Blainville, 1816 (Pisces). *Z.N.*(*S.*) 920. (2) Suppression of W. G. Tilesius von Tilenau, 1814–1818, "Ueber das nächtliche Leuchten des Meerwassers"; validation of several emendations; designation of a type-species for *Metapenaeus* Wood-Mason, 1891; suppression of *Mangalura* Miers, 1878 (Crustacea, Decapoda). *Z.N.*(*S.*) 962. (3) Validation of Xenophoridae Deshayes, 1864 (Gastropoda). *Z.N.*(*S.*) 1483. (4) Designation of a type-species for *Cyrnus* Stephens, 1836 (Insecta, Trichoptera). *Z.N.*(*S.*) 1491. (5) Validation of *Quinqueloculina* d'Orbigny, 1826 (Foraminifera). *Z.N.*(*S.*) 1494. (6) Designation of a type-species for *Lepidopa* Stimpson, 1858 (Crustacea, Decapoda). *Z.N.*(*S.*) 1495. Any zoologist who wishes to comment on any of the above cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7.

British Museum (Natural History)

THE ninth edition of *A Short Guide to the Exhibition Galleries of the Natural History Museum* follows the now accepted lines together with a modern format (Pp. vi+34. London: British Museum (Natural History), 1962). Following a short historical introduction, the various parts of the nearly four acres of exhibition space are described in outline. The *Guide* is devised to help the general visitor and should do much to emphasize the highlights of the exhibition galleries to the non-initiated. The illustrations are chosen to assist the same class of individual, who no doubt will find the geological time scale—brought up to date with the now generally accepted ages in millions of years—to be of service in the elucidation of the names of the systems used in the text. The *Guide* concludes with items of general information and notes concerning the library, lectures and the Children's Centre.

The Lundy Field Society

ANNUAL reports of societies are so often dull and formal that it is interesting to receive that of the Lundy Field Society for 1959–60, which includes much in the way of the results of original work (Pp. 82. Barnstable: John C. A. Dyke, Secretary, Lundy Field Society, 8 Rock Avenue, 1962). A long list, with short notes, of the birds recorded during 1959 is followed by a rather shorter list of migrant birds recorded in 1960. Probably the most interesting one is that of the myrtle warbler (*Dendroica coronata*), which is the first for Lundy Island and only the second for Britain. An interesting paper details the known early maps (prior to 1884) of Lundy, and another deals with remains of the Dark Ages on the Island. The mosses, liverworts and lichens are also tabulated. This Society exists to study and further the conservation of the natural features of Lundy Island, and the thirteenth report gives ample proof that its aims and objects are being carried out by the members.

Art in Nigeria

THE journal *Man* for February contains an article by Philip Allison describing a number of carved stones from the Ekoi country in the River Cross area of Eastern Nigeria. Many regularly shaped natural boulders can be found in the district, and a number of them has been carved so as to show a human face and roughly indicate the body. The carvings stop at the navel and genitalia are not shown. Some small curved lozenges and lines may be intended as tribal marks, but the meaning of a number of spirals, squares and triangles, as well as diamond-shaped figures, cannot be determined. Lists of the sites where these objects occur are given. Nigeria has become famous for its works of art, and mention of these carved stones had been made as long ago as 1903, but the account here given and the excellent plate add a further chapter in the study of Nigerian art. As to dating little can be said, Anything in Africa south of the Sahara before the coming of Europeans has to be classed as prehistoric. These carved stones need not be very old in years, but yet must be said to belong to a prehistoric period.

Awards for Cancer Research

THE Alfred P. Sloan Foundation has announced the establishment of the Alfred P. Sloan Cancer Research Awards, which will initially be confined to members of the professional staffs of the Memorial Sloan–Kettering Cancer Center. To qualify for awards, candidates must have made a significant contribution to basic scientific knowledge relating to cancer, or have advanced the treatment of cancer, or have shown unusual promise of future noteworthy accomplishments in this field. Each award will be worth 10,000 dollars, and the recipients will undertake to engage for one academic year in advanced study or in some other affiliation with a university, research institute, or similar establishment dedicated to research relevant to cancer. Further information can be obtained from the Alfred P. Sloan Foundation, 630 Fifth Avenue, New York 20, N.Y.

Harkness Fellowships of the Commonwealth Fund

THE following have been included in the recent appointments to Harkness fellowships of the Commonwealth Fund: J. B. Adams (Trinity, Oxford), business administration; Miss M. A. Boden (Birmingham), social psychology; J. R. Colbourne (King's, Cambridge), engineering; D. S. Glass (St. John's, Cambridge), organic chemistry; A. L. Rice (Liverpool), marine biology; J. H. M. Thornley (Magdalen and New Colleges, Oxford), physics; Miss P. L. Wells (Girton, Cambridge), sociology; M. D. Yudkin (King's, Cambridge), microbiology.

Heinz Scholarship

A POSTGRADUATE scholarship at the National Vegetable Research Station, Wellesbourne, Warwick, has been endowed by the H. J. Heinz Company, Ltd. The purpose of the scholarship is to enable graduate students to pursue further studies and to obtain training in methods of research at the Research Station. Awards will normally be for two years and be of the value of £500 per annum. It is hoped that the first Heinz scholarship will be awarded this year and will run from October 1. The last date for submission of applications is May 31, and they should be sent to the Secretary, National Vegetable Research Station.

Fritz London Award in Low-Temperature Science

NOMINATIONS for the third Fritz London International Award are being solicited from all low-temperature scientists by the Award Committee. Previous awards were made to Prof. N. Kurti, of the Clarendon Laboratory, Oxford, and Academician Lev Davydovich Landau, of the Institute for Physical Problems, Moscow. The Award is made for outstanding research and advances of fundamental significance in the science of low-temperature physics. Particular attention is directed to distinguishing between research advancing low-temperature science as such, in contrast to research merely utilizing low temperatures as a tool or expediency for advancing science in a different area. The Award is international and selections are made without regard to nationality. Further information can be obtained from Dr. J. R. Pellam, chairman, third Fritz London Award Committee, California Institute of Technology, Pasadena, California.

Announcements

SIR FREDERICK BRUNDRETT has been appointed chairman of the Governing Body of the Houghton Poultry Research Station in succession to Mr. T. H. Turney, who has held this office since 1956. Sir Frederick, who has recently retired from his official position as chairman of the Defence Research Policy Committee and Scientific Adviser to the Ministry of Defence, is chairman and governing director of Sunrise Eggs, Ltd., and is also chairman of the Agricultural Central Co-operative Association.

AWARDS of the Meldola Medal for 1961 have been made to Dr. J. N. Murrell (University of Sheffield) for his work in the field of theoretical chemistry, with special reference to the interpretation of the electronic spectra of organic molecules, and to R. O. C. Norman (University of Oxford) for his work in the field of organic chemistry, with especial reference to the application of modern methods of analysis to the elucidation of mechanisms of reaction of aromatic compounds. The award is made by the Royal Institute of Chemistry with the concurrence of the Society of Maccabaeans.

THE first Gregory Medal, established by the trustees of the Bishop Museum, Honolulu, in memory of Herbert E. Gregory, director of the Museum during 1919–36, has been awarded to Prof. A. P. Elkin, emeritus professor of anthropology, University of Sydney. Prof. Elkin is distinguished by his contribution to anthropological research in Australia and Melanesia, and by his active role in the development of institutions supporting Pacific research.

A SYMPOSIUM on "Methods of Separation of Subcellular Structural Components", under the auspices of the Biochemical Society and the Société Belge de Biochimie, will be held in the University of Louvain during May 11–12. Further information can be obtained from Dr. H. R. V. Arnstein, The Biochemical Society, 20 Park Crescent, London, W.1.

A CONGRESS of the South-Eastern Union of Scientific Societies will be held in Horsham, Sussex, during May 25–27. Further information can be obtained from Mr. F. Edwards, 53 The Drive, Shoreham-by-Sea, Sussex.

THE Laboratory Apparatus and Materials Exhibition, organized by United Trade Press Exhibitions, Ltd., will be held in the Exhibition Hall, Harrogate, during May 14–18. A predominant theme of the Exhibition will be automation in the laboratory. Further information can be obtained from the press officer, Miss Janet Wheaton, U.T.P. Exhibitions, Ltd., 3 Racquet Court, Fleet Street, London, E.C.4.

THE eighth international meeting on "Biological Standardization", organized by the Permanent Section of Microbiological Standardization of the International Association of Microbiological Societies, will be held in Berne during June 18–21. Further information can be obtained from the Committee of Organization, Post Office Box 1560, Berne 2—Transit.

THE third international congress on "Catalysis", organized by the Royal Netherlands Chemical Society, will be held in Amsterdam during July 20–25, 1964. The theme of the congress will be "The Mechanism of Heterogeneous Catalysis", and the topics under discussion will be the molecular description of the catalytic reaction and its intermediate states and selectivity in heterogeneous catalysis. Further information can be obtained from Dr. D. M. Brouwer, P.O. Box 3003, Amsterdam.

THE third international course on "Freeze-Drying", sponsored by the International Institute of Refrigeration in conjunction with the International Association of Microbiological Societies, will be held in Lyons during July 17–28. The subjects under discussion will include: physical aspects of freeze-drying; theory and design of freeze-drying apparatus; pharmaceutical, medical and biological applications of freeze-drying; freeze-drying in foodstuffs industry. Further information can be obtained from the chairman, Prof. L. Rey, Cours Internationaux de Lyophilisation, 97 rue de Vaugirard, Paris 6e.

A SYMPOSIUM on "Enzyme Models and Enzyme Structure", arranged by the Biology Department of the Brookhaven National Laboratory, will be held in the Laboratory during June 4–6. The programme will include: general perspectives; transferases; oxidative enzymes; decarboxylation; condensation and isomerization. The symposium lecture, entitled "Side-Chain Interactions in Protein Structure", will be delivered by Dr. J. C. Kendrew (University of Cambridge). Further information can be obtained from Mr. C. H. W. Hirs, Department of Biology, Brookhaven National Laboratory, Upton, Long Island, New York.

THE Ludwig Maximilians University of Munich, the Bavarian Academy of Sciences, and the International Union of Crystallography are jointly organizing a commemoration meeting on "Fifty Years of X-ray Diffraction" on the occasion of the fiftieth anniversary of the discovery of X-ray diffraction. It will be held at the University of Munich during July 25–27, 1962. In connexion with the commemoration meeting, the International Union of Crystallography and the Section of Crystallography of the German Mineralogical Society will jointly organize a symposium entitled "Recent Advances in the Experimental and Theoretical Methods of Crystal Structure Research", which will be held during July 27–31. Further information can be obtained from Prof. F. Bopp, Institut für Theoretische Physik der Universität München, Schellingstrasse 4–8, München 13, Germany.

THE NATIONAL SCIENCE FOUNDATION

THE eleventh annual report of the National Science Foundation*, covering the year ended June 30, 1961, records grants for research totalling 69 million dollars, compared with 62 million dollars in 1960, requests for support increasing, however, much more rapidly, from 163 million dollars to 256 million dollars. Some 15 million dollars were also made available for research facilities, including 8·5 million dollars for construction and modernization of graduate research laboratories and a further 8 million dollars were awarded to the three national research centres: the National Radio Astronomy Observatory; the Kitt Peak National Observatory; and the National Centre for Atmospheric Research. In the biological and medical sciences support has been afforded in molecular, genetic, developmental, metabolic, regulatory, environmental and systematic biology and psychology. In these fields significant research developments noted in the report are: the determination of the amino-acid sequence of tobacco mosaic virus protein; the successful photographing of unbroken deoxyribonucleic acid; the synthesis of a substance containing 23 amino-acid groups with all the biological properties of adrenocorticotropic hormone; and of luciferan, the light-emitting compound in fireflies. It has also been established that nucleotides play a key part in cell differentiation, that cobalt is essential for the growth of soya bean plants under symbiotic conditions and for the growth of the symbiotic bacteria, that even a few alder trees can play a major part in providing nutrient material for lakes and leaf-fall from alders contains four times as much nitrogen as defoliation from other species, and that carotenoids protect the plant cell from chlorophyll-catalysed photoxidation damage, while further information has been obtained on the structure and function of mitochondria.

In the mathematical, physical and engineering sciences, Project Mohole, a joint venture with the U.S. National Academy of Sciences, was probably the most spectacular project supported by the Foundation during the year, and this deep penetration of the Earth's sub-oceanic crust has established the feasibility of deep-ocean drilling (*Nature*, 184, 140; 1959).

Extra-galactic cosmic particles with an energy 500 million times that generated by the largest accelerator were detected during the year, and an atomic hydrogen maser constructed with an accuracy 100,000 times that of the best time standard yet known. Quantization of magnetic flux in super conductors was confirmed and 17 new exploding supernovæ were discovered during the year.

For the programmes of its Division of Scientific Personnel and Education, the Foundation allocated some 70 million dollars during the year. The 4,196 fellowship awards, totalling 14·1 million dollars, brought the totals since the inception of the programme in 1952 to some 17,000 Fellows and 57 million dollars. The Foundation also supported 771 institute programmes during the year. Of those, 396 were summer institutes attended by 21,000 teachers; 33 were academic year institutes, attended by 1,534 teachers of science and mathematics; 191 were in-service institutes attended by 8,900 secondary school teachers, with a further 13 institutes for 400 participants from elementary schools, all on a part-time basis. Besides containing programmes for secondary schools, such as visits from outstanding scientists and travelling science libraries, and a programme designed to develop co-operation between colleges and secondary schools in improving the quality of education in the sciences, the Division promotes programmes for advanced education in science, including supplementary training for science teachers and studies to improve the extent of courses in science and engineering, the design and development of prototypes of new equipment for teaching science. For this 57 grants were awarded, and since 1958 the Foundation has awarded grants totalling 4 million dollars to the School Mathematics Study Group for projects aimed at improving the teaching of mathematics.

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* National Science Foundation. Eleventh Annual Report for the Fiscal Year ended June 30, 1961. Pp. xxxi+325. (Washington, D.C.: Government Printing Office, 1962.) 1.25 dollars. (See also p. 319 of this issue.)

TECHNOLOGICAL IMPORTANCE OF INDUSTRIAL DIAMOND

THE name 'diamond' conjures up to many only beautiful costly gemstones, yet diamond is a technological hard material of the greatest importance; indeed, it can be now regarded technically as a fundamental strategic raw material. Of the annual world output of mined diamond, which is some 25 million carats, that is, 5 tons, only 1 ton is used for gemstones, the remaining 4 tons being eagerly consumed in industry largely as a hard abrasive or a cutting material. This 4 tons of crystalline matter, as raw material alone, fetches something like £20 million, and its extraction involves the formidable problem of sorting from something like 125 million tons of diamondiferous ore, the diamond being very thinly distributed indeed.

The technological importance of diamond lies in its immense hardness, which surpasses that of al other materials. The uses of diamond are growing rapidly in two broad categories: (1) single individual diamonds; (2) crushed grit. The range of use is enormous, from lathe-cutting tools to gramophone needles, from the truing and shaping of precision grinding wheels to the cutting and drilling of glass. Then again diamond rock-saws, diamond rock-drills, diamond wire-drawing dies, indeed even diamond microtome cutting-knives, all these are but a few of the multifarious industrial uses of singly mounted diamonds.

The strategic importance of the material, even to the military as well as to industry, is immediately apparent when one considers, for example, such aspects as mass-produced engines, automobiles, etc; for many basic components are rapidly shaped by accurately made silicon–carbide grinders of precise form and dimension. But, of course, the grinders wear and they must be continually reshaped and trued, and with what? In fact, diamond is the only material hard enough to re-true the hard carbide grinders, so a handful of diamonds becomes of strategic importance in these heavy industries.

Of equal importance to industry has been the rapid extension of the use of diamond grit bonded into wheels. Such bonded wheels have a fierce cutting and abrasive action and are extensively used for polishing, grinding, cutting and shaping; for gear wheel teeth, manufacture of hard tools, sawing of rocks, etc. These are but a few of the extensive uses of the newer bonded wheels.

Now it is evident that so ubiquitous a use of a material must have led to development of an extensive technical literature and to an equally extensive industrial patent bibliography.

It was in order to assist technologists in this rapidly expanding field of technical publication that more than twenty years ago the de Beer's South African diamond producing and marketing organization took two steps. First, it established in London the Industrial Diamond Information Bureau (2 Charterhouse Street, London, E.C.1), which offered gratuitous technical information on production and use of diamond; secondly, it founded the journal, *Industrial Diamond Review and Abstracts*. Under its first editor, Dr. P. Grodzinski, this journal made rapid strides and was quickly recognized as the most authoritative monthly periodical dealing not only with diamond and its uses but also with the use of other hard materials, such as the hard carbides.

For some years now the journal has been most competently edited by Mr. Patrick Greene, and, early this year, after remaining unchanged for twenty-one years, it appeared in a new format, which is not only attractive in appearance but also essentially practical. The international *A*4 size has been adopted and colour is introduced. The quality of the reproductions is now admirable, and advertising matter has been removed from the main body to the covers and back pages only.

The principal innovation has been the separation of the *Industrial Diamond Abstracts* into a separate companion journal to the *Review*. These abstracts are effectively culled from four hundred journals, and especially those referring to patents are well and clearly illustrated. The classification of the abstracts is made into no less than twenty-one distinctive categories, typical examples being: *B*, properties of diamond; *K*, wire drawing; *L*, rock drilling; *R*, powder metallurgy; and so on. The cross-reference system is very helpful. Indeed, to anyone interested in either the behaviour, properties or applications of diamond and other hard materials these abstracts are a boon. Linked to the publication is a useful loan photostat and reprint service. The subscription is a mere 30*s*. a year for the two companion journals, which is very little for the monthly service rendered.

There is no doubt that these two publications are performing a very real service, and even if it be admitted that one of their purposes is to encourage increased consumption of diamond, this is in no way a detraction, for diamond is a unique material and its unique physical properties deserve to be used as much as possible to help promote industrial production.

S. TOLANSKY

RECENT NUCLEAR EXPLOSIONS AND EARTH TIDES

ELASTIC deformation of the solid Earth, independent of the marine surface, is called an 'Earth tide'. Because of its rigidity, the Earth will not deform as would a case of the same size but of liquid content. It will assume an intermediate degree of deformation depending on the density and elastic properties of the materials that constitute the solid Earth. This change in shape will cause a change in gravity, value *g*, which is also dependent on the latitude, local geological structure and physical properties of the materials. Thus, the variation in tidal forces caused because of the changes in the position of the Sun and the Moon with respect to the observation station on the Earth will indirectly affect the gravity–time variation curve. The maximum of the tidal action corresponds to a measured minimum of gravity.

Continuous recording of the variation of *g* value with time is being made in the Geology Department, Osmania University, Hyderabad, by S. Balakrishna (*J. Sci. and Indust. Res.* (India), 20 A, No. 12; December 1961). The influence of the oceans on the tidal values on continental stations shows that, at a distance of 66 km. from the seashore, the effect of the oceanic tides can be minimized to a large extent. The Osmania observation station has this advantage besides being located on the so-called stable peninsular shield.

When an earthquake occurs, a certain stress energy is usually released which causes an extra, temporary deformation other than the long-period deformation of the Earth-tide type. This temporary deformation is of short duration and the whole process of disturbance can be observed in the normal gravity–time curve. Many such records have been obtained during the past few months. The most intense earthquake which occurred in the year 1961 was that of Kyushu on February 26, 1961. The gravity record showed a violent disturbance at exactly the same time, but the disturbance was of a few hours duration. In cases where the energy is of high magnitude, as in the case of the Chile earthquake of May 1960, the Earth is thrown into its own free oscillation, the period of which is estimated to be 53 min. 47 sec.

During September and October of 1961, disturbances of a different nature were noticed. The characteristic features of these disturbances are longer duration and constant high amplitude. Nearly thirty disturbances of this nature have been recorded, the first being on September 10, 1961. From the records of the gravity–time curve during these disturbances, it appears that they are not due to the energy that is supplied after an intense earthquake, but are caused by the release of enormous stress energy and are possibly due to a different mechanism. The records of September 10, September 25, October 18, October 23 and October 30, 1961, stand out prominently in that the duration of the disturbances was often for a period of more than 24 hr.

The record on October 23, 1961, when the reported megaton bomb was exploded by the U.S.S.R., showed a minor disturbance at about 0700 hr.; this suddenly became intense at about 1000 hr. and reached its maximum amplitude at about 1430 hr. The disturbance in its intensity, duration and amplitude was of a special nature and quite unprecedented. Such disturbances, it is claimed, have not been recorded in the laboratory previously. On October 30, 1961, when the U.S.S.R. exploded a 50-megaton bomb, there was an intense disturbance at 1430 hr., but the nature of this disturbance was different from that of October 23 or earlier explosions. There was little continued high amplitude, but a series of successive disturbances throughout the night of October 30, each one occurring at an interval of about 2 hr. and corresponding to a miniature earthquake. This type of record may be due to the possibility that the explosion was high in the atmosphere and there was consequently a poor transmission of energy to the epicentre.

The diagnostic features of the disturbances caused in the gravity–time curve which differentiate them from those caused by earthquakes are their longer duration, higher amplitude and sometimes the free oscillation of the Earth which follows them. The period of the Askania gravimeter pendulum is 1·5 sec. and the recording galvanometer has a period of 17 sec. The gravimeter is a static helical spring type without astatixation and damped aperiodically. The sensitivity of the instrument is about 10 cm. per milligal. This means that the response of the pendulum to the disturbances in the Earth's crustal regions is magnified nearly 400,000 times by the gravimeter, which is much more than the most sensitive seismometers used. This is why disturbances caused even in mid-air (pressure waves) have been transmitted into the Earth's crust and are recorded by the gravimeter although they may be of a magnitude that a conventional seismograph of the Milne–Shaw type would normally miss or record feebly.

FROTH-FLOW LAVAS RESEMBLING IGNIMBRITES IN THE EAST AFRICAN RIFT VALLEYS

By Dr. JOSEPH McCALL

University of Western Australia, Nedlands

THE communication by Harkin and Harpum[1] of the Tanganyika Geological Survey was of considerable interest to me. The existence of extensive developments of volcanic rocks, which show eutaxitic structure and a fragmental structure giving them the appearance of welded pyroclastic rocks, that is, ignimbrites, has long been known from a field situated to the north of the field newly discovered by the Tanganyika geologists, and within the same anorogenic zone—namely, the central sector of the Eastern Rift Valley in Kenya. A brief mention of these occurrences was made by me in two introductory accounts of the Menengai volcano[2,3]; at the time of writing these rocks were interpreted as welded tuffs. Since that date, however, much further work has been carried out: a detailed account of many occurrences of these rocks is given in a report awaiting publication by the Geological Survey of Kenya[4] and in addition to this I have, during the past year, been engaged on the preparation of a draft manuscript dealing specifically with the problem of derivation of these unusual fragmental and eutaxitic effusive rocks. It is hoped shortly to publish this work. Meanwhile, it might be of interest to the Tanganyika geologists and others to have a brief summary of the more important conclusions reached.

In Kenya the eutaxitic and fragmental rocks occur in a field within and adjacent to the central sector of the main or Gregory rift valley. They occur not in the oldest volcanic sequence but in the second major sequence of eruptives which post-dated the first major episode of faulting. The age of the greater part of these rocks is probably late Pliocene (ref. 4, Table 2), but there were late eruptions of similar effusives, probably during the Pleistocene. The main sequence can be shown to be much older than the artefact-bearing lake beds of Kariandusi[5], being separated from them by two episodes of faulting. They were associated with broad and extensive shield volcanoes, almost devoid of crater structures, and seem to have been largely fissure erupted. Though cauldron subsidence resulting in the formation of large subsidence calderas has affected many of these shield volcanoes, it is eruptions of pumice lapilli and not of coherent fragmental flows which were directly connected with the caldera formation: the fragmental eutaxitic lavas seem to have formed part of the main sequence of intermittent eruptions which built up the volcanic piles.

The caldera of Menengai[2,3] has, in particular, been the subject of detailed investigations, which have revealed unusual flows of a structurally composite nature: these contain both normal lava (well-crystallized and banded partly vitreous types) and fragmental eutaxitic types within the limits of single flow units. Laterally the structurally composite flows pass into complete flows of normal lava or into complete flows of fragmental and eutaxitic character, and the latter might easily on their own merits be taken for ignimbrite. In fact, entire flows of this character have been loosely termed ignimbrites by several geologists working in Kenya, and attributed to processes of welding. The Menengai rocks are phonolitic trachytes, and while their structural characteristics are remarkably similar to those described from the rhyolite–dacite ignimbrite fields, they possess a much greater degree of crystallization than rhyolitic ignimbrites. What is often called the vapour-phase crystallization[6] has its parallel in these rocks: but the fringing growth of crystals around vesicular cavities drawn out by flow (a form of lithophysal structure) is quite inseparable from the primary crystallization in the unquestionable lava which forms part of the same flow: the development of this lithophysal structure and the fragmental structure, which gives to the rock the appearance of a welded tuff, can be traced outwards from its origin in planar zones of drawn-out lithophysæ within a continuously banded lava : the fragmental character appears when distortion has been sufficient to draw out the laminæ with their included lithophysæ or the laminæ which separate the lithophysal layers into a series of disconnected shreds.

It is thought that the fundamental process involved in the development of the fragmental eutaxitic structure is vesiculation in a glassy lava, expanding as the contained volatiles exsolve. Frothing on the sudden release of volatiles consequent on the rupture of the pressure seal to the magma chamber is envisaged. Locally exsolution is retarded, due to physical controls which are not as yet fully understood, and the structures seen reflect segregation of volatiles but only incipient exsolution, and a high fluidity due to retention of the volatiles in solution. In contrast, where exsolution has been complete, a ruptured product is often produced, a collapsed bubble-froth flow, and there are localized areas in the flow where rupturing rather than a process of drawing out has occurred. The basis of the complex structure so developed and which gives the fragmental character to the rock would seem to lie in Shepherd's discovery[7] that a glass from which volatiles have entirely exsolved is brittle, while a glass which has retained even a small fraction of its volatiles in solution tends to retain its plasticity for a long time: and that it requires considerable time, and a much higher temperature to return a brittle glass to the plastic state. The presence of numerous well-crystallized enclaves in all the Kenya fragmental eutaxitic flows—enclaves formerly explained as accidental xenoliths in welded tuffs—is attributed to early patches of crystallization in the flow: these and the glass areas from which volatiles have been entirely lost have responded in a brittle manner to the onsurge of the still partly mobile flow, while the remainder of the glass has responded plastically. The enclaves are neither accidental nor truly xenolithic.

The combination of autoclastic and plastic deformation structures superimposed on a heterogeneous vesiculating lava which give the effect of a welded tuff can, in my view, be adequately explained as due to the effects of brittle and plastic response to deformation in various parts of the flow, and no recourse to any process of welding is necessary. It is felt that the structurally composite flows, which so closely resemble ignimbrites described from other fields, even to the presence of the remarkable *flamme*, are significant in that they throw some doubt on the necessity to invoke welding to explain such structures in the much more glassy coherent and fragmental flows of rhyolite called ignimbrites. Kennedy[8] and Boyd[9] have suggested an alternative process to the orthodox welding hypothesis using the term froth-flows for the coherent effusive types and collapsed bubble-froth flows for the ruptured effusives, represented by pumice and crystal fragments, and showing a uniformity of fragment size reflecting the size of the vesicles at the point of rupturing. I have applied this hypothesis to the rocks of the Kenya field[10] and am of the opinion that it explains the many anomalous eruptive types far better than the orthodox theory of welding. No recourse to the process of segregation of immiscible liquid fractions as advocated by Steiner[11] to explain similar rocks in New Zealand seems necessary in the case of the Kenya rocks, and indeed, with their sodic-alkaline intermediate composition, they must be considered, on the basis of experimental evidence, as most unlikely subjects for such a process.

The late flows of probable Pleistocene age tend to be very thin and to follow ravines as narrow tongues. The structure is fine, and the name 'claystone' has been locally used. These seem to be extremely watery flows: why they should show the effects of segregation and only limited exsolution giving them abnormal fluidity is not known, but it seems that their flow from source to resting place must have been extremely rapid. Similar types occur in the main (Pliocene ?) sequence to a limited extent.

It seems possible that the Tanganyika rocks in the newly discovered 'ignimbrite' field may, on detailed study, reveal similarities to the Kenya rocks and the first interpretation as lavas prove the correct one, in spite of the obvious resemblance to described ignimbrites.

I thank Dr. W. Pulfrey, commissioner of mining and geology, Kenya, for permission to publish this account, based on work done while employed by the Geological Survey, Kenya, and also for constructive criticism.

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[1] Harkin, D. A., and Harpum, J., *Nature*, 193, 57 (1962).

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ORIENTATION IN OCTAHEDRAL BASIC HYDROLYSIS

By Sir CHRISTOPHER INGOLD, F.R.S., Prof. R. S. NYHOLM, F.R.S., and M. L. TOBE

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THREE reviews[1] on octahedral substitution appeared in 1958–59. However, in several of the main departments of that subject, the accumulating observations have since been fitting themselves into clearer patterns. This was shown for octahedral aquation, that is, the replacement in aqueous solution of octahedral ligands by water molecules, in an article[2] of 1960. The present article, essentially a continuation, deals in a similarly collative way with the replacement of octahedral ligands by anions in water, in particular, with basic hydrolysis, the best-studied form of such anion substitutions.

(1) *Experimental data.* In order to elucidate the factors which control the rate and direction of any form of octahedral substitution it is necessary comparatively to study the substitution with reference to rates and products over a suitably extensive series of systematically varied original structures.

The one series which has yet been thus usefully studied comprises bisethylenediamine-colbaltic complexes of the general formula $[\mathrm{Coen}_2AX]^+$.

In the examples to be discussed, the 'replaced ligand' X is chlorine or bromine. As the result of an attack by hydroxide ions, it becomes replaced by a hydroxo-ligand. A variable unreplaced ligand A is present, which we call the 'orienting ligand', because its nature and position has a profound effect on the rate of the substitution and on the proportions of its stereoisomeric products. We work with seven orienting ligands which, between them, illustrate the chief recognized types of electronic interaction between a ligand and its transition-metal. These effects of a ligand, untouched in the substitution, on the rate and direction of the substitution, constitute the matter of interest. The data are thus of two kinds, namely, rates of substitution by aqueous hydroxide-ion, and stereoisomeric product-compositions.

The general equation of the substitutions is:

$$[\mathrm{Coen}_2AX]^+ + \mathrm{OH}^- \rightarrow [\mathrm{Coen}_2A\mathrm{OH}]^+ + X^-$$

All these substitutions are kinetically of second order, first in the factor-complex, and first in hydroxide ion. Hence we compare rates by comparing second-order rate-constants. Arrhenius parameters have been determined, but they are not discussed in this article.

For a given A and X, the factor-complex may be either *trans* or *cis*, and, in the latter case, may be either of the two optical enantiomers. The same possibilities of geometrical and optical isomerism are open to the product-complex. In general, any one stereochemically individual factor will, on substitution, give a mixture of all the possible stereoisomeric products. However, the proportions in which the different isomers appear will depend on the stereochemical identity of the factor-complex. Care has been taken to ensure that no reactions other than the single second-order hydroxo-substitution disturbed the determined rates or product-compositions.

The available second-order rate-constants (at 0°), and the product compositions for hydroxo-substitutions of the type formulated and described above are presented in Table 1. Some explanation may be given of the tabulation of product-proportions. *Trans*-factors can yield only *trans*- and racemic *cis*-products. *Cis*-factors were all at first examined in racemic form, and then some of them were re-examined in an optically enantiomeric form. A racemic *cis*-factor can give only *trans*- and racemic *cis*-products; and it will be clear from the tabulation which *cis*-factors were examined only in racemic form. Those *cis*-factors which were further examined in an optically enantiomeric form gave, of course, as much *trans*-product as before, and as much total *cis*-product. But this last was now unequally split between the enantiomers; and therefore, in the tabulation of these results, the proportions of each enantiomer, rather than their sum, is entered. Moreover, these proportions are tabulated, not according to their sign of rotation at a given wave-length, but according to configuration: it is shown which retains a configuration corresponding to that of the factor, and which appears with an inverted configuration. We can do this because our stereochemical rules for aquation are so definite[2], and because aquo- and hydroxo-complexes can always be interconverted without configurational change. Thus, there is never any doubt about which halogeno-, aquo-, and hydroxo- complexes have corresponding configurations.

Table 1. RATE-CONSTANTS (k_2 IN SEC.$^{-1}$ MOLE^{-1} L.) AND ISOMER PROPORTIONS (PER CENT) OF PRODUCTS FORMED IN THE AQUEOUS REACTION:

$$[\mathrm{Coen}_2AX]^+ + \mathrm{OH}^- \rightarrow [\mathrm{Coen}_2A\mathrm{OH}]^+ + X^-$$

X	A	k_2 in H_2O at 0°	Product proportions				Ref.
			Trans	Rac. *Cis*	Act. *Cis*, Config. Retnd.	Act. *Cis*, Config. Invert.	
trans-Cl	Br	110	100	0	—	—	8
,,	Cl	42·6*	95	5	—	—	8
,,	N_3	0·41	87	13	—	—	7
,,	NO_2	0·080	94	6	—	—	5
,,	NH_3	1·25	24	76	—	—	4
,,	NCS	0·84	24	76	—	—	3
,,	OH	0·017	6	94	—	—	8
trans-Br	Cl	269	95	5	—	—	8
,,	NCS	1·95	19	81	—	—	3
,,	OH	0·168	10	90	—	—	8
cis-Cl	Br	22·9	60	40	—	—	8
,,	Cl	7·6*	63	—	21	16	8
,,	N_3	0·17	49	51	—	—	7
,,	NO_2	0·032	34	—	46	20	5, 9
,,	NH_3	0·50	16	—	60	24	4
,,	NCS	1·40	20	—	56	24	4, 7
,,	OH	0·37	3	—	61	36	8
cis-Br	Cl	71	70	30	—	—	8
,,	NH_3	3·3	15	—	59	26	4
,,	OH	2·7	4	96	—	—	8

* The tabulated rate-constant is one-half of the measured rate-constant for the hydrolysis of the dichloro compound.

(2) *Substitution mechanism.* The kinetic form of the substitutions delimits the possible mechanisms to two. One is the mechanism, labelled S_N2, in which the entering anion attacks the central metal, so aiding release of the ligand being replaced. The other mechanism is that, labelled S_N1cb, in which a pre-equilibrium extraction of a proton from some ethylenediamine ligand by the hydroxide reagent is followed by a rate-controlling dissociation of the replaced ligand, and thereafter by the rapid combination of another hydroxide ion with the metal, and then a final re-protonation of the despoiled ethylenediamine ligand. The arguments for and against each of these interpretations have been presented[1], and, for reasons which need not now be repeated, we favour the former, which we shall use in order to interpret the results in Table 1.

Table 1 discloses that the rate pattern in basic hydrolysis is more complicated than that applying to aquation; and, furthermore, that extensive configurational changes are the rule in basic hydrolysis rather than the exception as in aquation. We did suggest[3] that the kinetic and stereochemical pattern of aquation owes its orderliness to the circumstance that the reagent, water, is ubiquitously available all over the surface of the complex ion, with the result that we have to consider chiefly the internal electronic mechanisms that determine how easily exchange of bonding can occur between the replaceable ligand and neighbouring water, or between the ligand and an adjacent ethylenediamine 'end' internally loosened enough to compete with water.

The situation in basic hydrolysis is evidently different, and we take the first question for consideration to be that of where the substituting anion will present itself, or, in other words, what is the distribution of its potential in the close field of the complex ion, as modified from case to case by the variable orienting ligand. In general, this is an electrostatic question. However, the fact that our reagent is the hydroxide ion is relevant. Transport by Grotthus chain-conduction is allowed to a lyate ion, and concerted proton-transfers to, and through, the solvation shell of the complex, and ending on some particular inner water molecule, will be a main mode of transport of the attacking hydroxide ion. When such a chain-terminating water molecule is firmly localized among the ligands, as by hydrogen bonding to one of them, its position may well determine that of the hydroxide entry.

As to the second question, that of the ease of the eventual exchange of bonding, certain differences between basic hydrolysis and aquation have to be appreciated. One is that, in basic hydrolysis, an anion, expanded by its charge, has to be accommodated in the transition state, wherefore a larger 'hole' in the residual electronic system must be provided; and it results that although, and even because, a bimolecular process is in consideration, loosening of the outgoing ligand will constitute the more advanced of the concerted bond-changes that lead up to the transition state, in contrast to bimolecular aquation wherein water entry initially takes the lead. Another point of difference is that, in basic hydrolysis, the substituting agent is much more nucleophilic than in aquation, and is thus more competent to impel stereochemical change by edge-displacement of a ligand[1], if that is needed for the completion of its binding; wherefore special ligand-loosening mechanisms are much less important than in aquation.

(3) *Electronic properties of the ligands.* The foregoing general principles have to be applied with reference to the effects of the orienting ligands, the properties of charge and potentialities of electron-displacement of which are derived from their constitution. These matters were set out in the former article[2], but we shall summarize them, taking the opportunity to develop one point further.

The inductive effects $(+I)$ of the orienting ligands stand in the following order of decreasing electropositivity $(+I)$:

$$\mathrm{OH,\ N_3 > NCS,\ Cl > NO_2 > NH_3}$$

Induction will change the density of the unshared metal electrons in the same sense everywhere, but most strongly about the *trans*-ligand.

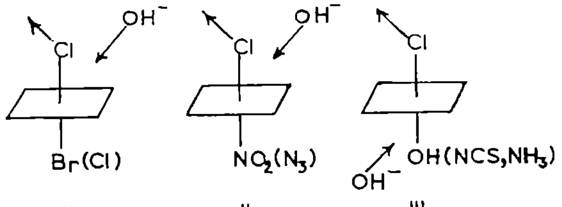
Four ligands, namely, OH, N_3, NCS and Cl, have unshared electrons adjacent to the metal. They are constitutionally capable of an electropositive electromeric effect $(+E)$, which directly loosens ligand bonds, and is *cis*-specific. The order of the groups with respect to this electron-transfer could not be prescribed, either theoretically or even empirically, in the former article[2], because the quantitative data on its most characteristic effect[10], that is, rearrangement in the aquation of *trans*-factors, were then incomplete[11]. But they have now been completed for the replacement by aquation of a chloro-ligand in bisethylenediamine cobalt-III complexes[12]: the *trans*-hydroxochloro-complex thus gives 75 per cent, and the *trans*-dichloro-complex 35 per cent of the *cis*-aquo-product. These results, combined with those reported previously, furnish the complete $+E$-order:

$$\mathrm{OH > NCS > Cl > N_3}$$

Two ligands, namely, N_3 and NO_2, have dipolar canonical structures with an electron-deficiency adjacent to the metal. This constitutional feature allows them an electronegative conjugative effect $(-T)$ by which they can accept metal electrons. Such an electron-transfer will reduce the density of the unshared metal electrons on the remote side of the complex. With respect to this effect, the ligands stand in the well-established $-T$-order:

$$\mathrm{NO_2 > N_3}$$

(4) *Basic hydrolysis of the* trans-*substrates.* The three main types of case are formulated at I–III. The less extreme members of the types are indicated by alternative orienting ligands written in parentheses.



I II III

Orienting halogen is represented in I. The halogens, uniquely, are monatomic and monopolar ligands, and are themselves the only easily replaceable ligands, bromine more easily than chlorine. Therefore, their binding can have only a light covalent content, and thus they must carry a large negative charge, bromine the larger. This will direct the attaching anion *trans*. There, with the loosening halogen, it can form a transition state for bond exchange, wherein both exchanging ligands are only lightly bound, the change in the binding of either from the initial to the transition state being therefore somewhat small. Hence, we can understand the relatively rapid substitutions, the retained configurations, and the minor quantitative differences between the halogens.

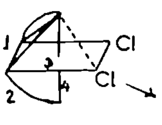
The orienting $-T$-ligands are represented in II. These ligands draw to themselves the cobalt $3d_{\varepsilon}^{6}$ shell during reaction, building up negative charge which directs the attacking anion *trans*, and also tightens the binding of the *trans*-halogen. In order to produce the transition state of the ensuing exchange, a greater loosening of the ligand under release will now be required. Hence, we can understand the relatively slow substitutions, the very largely retained configurations and the minor quantitative differences between the effects of nitro- and azido-ligands.

Included in representation III is the orienting hydroxo-ligand. It is the most electropositive of all the ligands, and, for that reason, as well as because local hydrogen-bonded hydration will cause it to attract a lyate ion (Section 2), it will direct the hydroxide-ion attack adjacently to itself. We see in the result a slow reaction, associated with an almost total change of configuration. The isothiocyanato-ligand stands second in electropositivity, and has a partial positive charge adjacent to the metal. The ammonio-ligand is relatively electronegative, but must still carry a substantial residue of its formal positive charge. It seems that, for these different reasons, these two orienting groups secure hydroxide-ion attack mainly, though not exclusively, near themselves. In both cases, we find somewhat slow reactions, associated with preponderating, but not exclusive, changes of configuration.

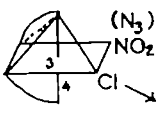
(5) *Basic hydrolysis of* cis-*substrates.* The orienting ligands divide themselves into the same three classes, with broadly distinct properties, though the distinctions are not quite the same as before. Orientation by halogens now leads to high rates and strongly predominating configurational change; by nitro- and azido-ligands to low rates and medium amounts of configurational change; and by hydroxo-, isothiocyanato- and ammonio-ligands to medium rates and minor amounts of configurational change.

The rates for most *cis*-substrates follow the pattern of those of the *trans*-substrates, the *cis*-rates lying below the *trans* by factors of 2·5–5. The few exceptions relate to orientation by the two best electromerically *cis*-activating ligands (Section 3), namely, hydroxo and isothiocyanato. These orienting ligands raise their *cis*-rates above their *trans*- by factors in the expected order, near 20 for the hydroxo-examples, and 4 for the isothiocyanato.

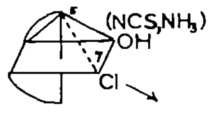
In setting down the stereochemical possibilities, we have to distinguish between the molecular processes of substitution, without and with edge-displacement (to be denoted by *o* and *ed*, respectively), and the macroscopic products of substitution. These are *trans*-products, and stereochemically inverted *cis*-products (to be written inv-*cis*), which are necessarily formed with edge-displacement (*ed*), and *cis*-products with a retained configuration (to be denoted ret-*cis*), which may be formed without or with edge-displacement (*o*-ret-*cis* or *ed*-ret-*cis*)[1]. The hypothesis of edge-displacement implies that all group-shifts are *cis*, and that an end of an ethylenediamine ligand can shift only to a position which is *cis* with respect to both ends. Figs. IV–VI are drawn for one enantiomer in each case.



IV



V



VI

Orienting halogen is represented in IV. A discussion of rates would follow the same lines as in Section IV, and the main new interest is in the product proportions. The halogen being replaced is marked with an arrow. The other is the orienting halogen, and, for reasons explained in Section 4, reagent attack is expected mainly around the point *trans* with respect to it, that is, within the octahedral faces numbered (1)–(4). Attack at the octahedral face (1) marked with three full lines, and on its counterpart (2) below the square, should lead to the qualitative results set out below, where the added numerical illustration results from assuming an exclusive attack equally divided between these faces, and, at either face, leading with equal probability to the possible consequences indicated:

(1) *trans* (2) *trans*, inv-*cis*
(75 per cent *trans*, 0 per cent ret-*cis*,
25 per cent inv-*cis*)

Attack on face (3) completed with a dotted line, and on its counterpart (4) below the square, should lead to the following results, similarly numerically illustrated:

(3) *trans*, *o*-ret-*cis* (4) *trans*, *o*-ret-*cis*, inv-*cis*
(42 per cent *trans*, 42 per cent ret-*cis*,
16 per cent inv-*cis*)

The observed results, bracketed as they are, between these two sets of figures suggest that the four faces considered are attacked to somewhat similar extents.

Orientation by the nitro- and azido-ligands is represented in V. Again, a discussion of the relevant rates would resemble that contained in Section 4, and we may turn at once to the product ratios. The main new factor is that the cobalt $3d_\varepsilon$ shell is swollen towards these $-T$-ligands, a circumstance calculated to widen the angle between the orienting ligand and the departing halogen (the external field of which is wholly negative), so moving the point *trans* to the former towards the latter, and hence concentrating reagent attack, otherwise expected to be spread over faces (1)–(4), particularly towards faces (3) and (4). The results to be expected from attack at these faces are set out above. The observed results for the nitro- and azido-ligands are reasonably similar to the figures illustrated.

Orientation by the hydroxo-, isothiocyanato- and ammonio-ligands is represented in VI. The factors affecting rate are as discussed in Section 4, except for the modification, concerning the hydroxo- and isothiocyanato-ligands, mentioned earlier in this section. As to product proportions, the important consideration is that, again for reasons given in Section 4, reagent attack is now concentrated around the orienting ligand, almost totally around the hydroxo-, and mainly, but not totally, around the isothiocyanato- and ammonio-ligands. Moreover, the relative polarity of the departing halogen should determine that attack on the face (5) marked with three full lines, and on its counterpart (6) below the square, is more important than on the face (7) completed with a dotted line, and its counterpart (8). The stereochemical results to be expected from attack in these positions are given below, and are numerically illustrated with the same assumptions as before:

(5) *ed*-ret-*cis* (6) *ed*-ret-*cis*, inv-*cis*
(0 per cent *trans*, 75 per cent ret-*cis*,
25 per cent inv-*cis*)

(7) *o*-ret-*cis*, *ed*-ret-*cis* (8) *o*-ret-*cis*, *ed*-ret-*cis*, inv-*cis*
(0 per cent *trans*, 83 per cent ret-*cis*, 17 per cent inv-*cis*)

The product compositions observed for the hydroxo-oriented substitutions are fairly similar to the former set of figures, while those observed for the isothiocyanato- and ammonio-oriented substitutions are consistent with the idea that similar directive effects are less exclusive in these cases.

The previous analysis[2] of aquation rates and products not only rationalized them but also disclosed a simple stereochemical rule. Although no all-embracing rule emerges for bimolecular basic hydrolysis, the above discussion of its rates and products does seem to carry a similar qualitative rationality. Though there are other branches of the subject which we must leave for future articles, the general position of orientation theory in aqueous octahedral substitution encourages us to think that we may be on the way to a condition of intelligibility comparable with that of the present theory of orientation and reactivity in aromatic substitution.

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SPECIFIC ELEMENTARY PROCESSES IN THE RADIATION CHEMISTRY OF ORGANIC OXYGEN COMPOUNDS

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ALTHOUGH the primary modes of bond rupture and re-arrangement following the electronic interaction between high-energy radiation and polyatomic molecules in the macroscopic state can often be inferred from kinetic measurements and product analyses, no adequate theory has been developed to enable the prediction of elementary processes as a function of molecular structure; it has been aptly commented[1] that the present status of this subject is the result of empirical growth devoid of a definite theoretical foundation.

It is well recognized that radiation-induced reactions cannot be attributed to a statistical distribution of random bond fractures, and that preferred paths are evident in the reaction sequence. Elsewhere[2], I have argued that reactions of the parent molecule-ion involving low-activation energies probably precede the neutralization process, even in the liquid state; and that the consideration of such reactions affords a simple guide to a qualitative understanding of the radiation chemistry of liquid alkanes as a function of molecular structure. In this article I am adopting the same premise to a discussion of the radiation chemistry of some organic molecules possessing functional groups with oxygen atoms.

A fundamental distinction between the ground-state electronic structure of saturated hydrocarbons and organic oxygen compounds is that whereas the occupied molecular orbital of highest energy (that is, corresponding to the lowest binding energy) in the former is σ bonding, in the latter it is non-bonding (n) and can be considered to be largely derived from the lone-pair $2p$ electrons of the constituent oxygen atom. It follows that the threshold ionization of an organic oxygen compound refers to the loss of a non-bonding electron with the result that the unpaired electron is highly localized on the oxygen atom; thus Mulliken[3] regards $\text{CH}_3\text{—}\overset{+\cdot}{\text{O}}\text{H}$ as the major valence bond resonance structure in the ground-state of the methanol positive ion. Cummings and Bleakney[4] made the original suggestion that ionization in this manner confers trivalency on oxygen, and they proposed that additional bonding between carbon and oxygen could account for the low appearance potential and relative abundance of the major fragment ions in the mass spectra of methanol and ethanol (*vide infra*). In more general terms, it can be stated that the ensuing reactions of the parent molecule-ion are strongly directed by the tendency of the oxygen atom to complete its valence shell of electrons. It is proposed that this factor also represents a key to the interpretation of radiation-chemical changes in many oxygen compounds, but first let us indicate the assumptions on which this approach rests.

When the energy of the incident electron far exceeds the binding energies of the electrons in the molecule, some ionization events would be expected to occur through the removal of electrons below the highest occupied level and also by the possibility of a dielectronic process involving electron promotion to anti-bonding orbitals in the ion. For these electronically excited ions, it is postulated that there follows a radiationless electron re-organization within about 10^{-13} sec. which leaves the electron vacancy in the orbital corresponding to the highest occupied level in the ground-state; and that the excess electronic energy is internally converted to vibrational energy in the molecule-ion. This latter assumption constitutes a pillar of the quasi-equilibrium rate theory[5] of the origin of mass spectra. It is also generally accepted[6,7] that the higher excited states of neutral molecules undergo internal conversion, at least to the lowest excited state of the same multiplicity, in a comparable time (10^{-13} sec.).

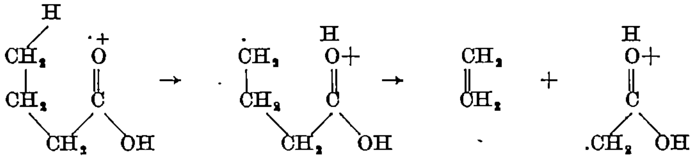
There are two general reactions which can be invoked whereby the ionized oxygen atom in an organic molecule-ion can regain its full complement of valence shell electrons. First, by the inter- or intra-molecular abstraction of a hydrogen atom. An example of the former type is given by the ion-molecule reaction (1) of methanol parent ions[8], ($R = \text{H}$).

$$R\text{CH}_2\overset{+\cdot}{\text{O}}\text{H} + R\text{CH}_2\text{OH} \rightarrow R\text{CH}_2\overset{+}{\text{O}}\text{H}_2 + R\dot{\text{C}}\text{HOH} \qquad (1)$$

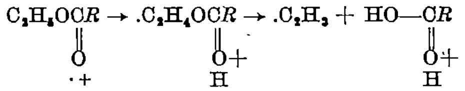
The generality of this reaction (M) for many diverse organic compounds containing oxygen or nitrogen atoms is indicated by repeated observations[9,10] that ions corresponding to a mass = parent + 1 occur in the mass spectra recorded at elevated pressures. The argument for charge localization on oxygen applies with equal force to nitrogen due to the similar property of non-bonding electrons. Since ion-molecule reactions are usually considered to be near-exothermic, it is pertinent to inquire into the factors which determine the energetics of processes akin to equation 1. The exothermicity, $-\Delta\text{H}_r$, of such reactions can be shown to be represented by the equation:

$$-\Delta\text{H}_r = I_M + PA_M - I_H - D_{\text{C—H}} \qquad (2)$$

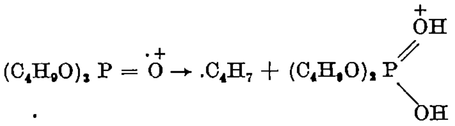
where I_M and PA_M refer respectively to the ionization potential and proton affinity of the original molecule, I_H is the ionization potential of the hydrogen atom (13·6 eV.) and $D_{\text{C—H}}$ is the dissociation energy of the C—H bond involved in the reaction. In a series of compounds where the oxygen functionality remains the same, $D_{\text{C—H}}$ is almost invariant so that $-\Delta\text{H}_r$ in equation 2 is largely determined by the first two terms. Now it can be argued that in any homologous series, I_M and PA_M depend on the condition of the lone-pair electrons in a way that an increase in one of these quantities will be compensated by a decrease in the other, so that their sum remains approximately constant. For example, the decrease of I_M in carbonyl compounds with increasing polarity of the $>\text{C=O}$ bond[11] is attributed[12] to a repulsion of the



(4.1)



$$C_2H_5O\underset{\overset{\cdot +}{O}}{\underset{\|}{C}}R \rightarrow .C_2H_4O\underset{\underset{H}{O+}}{\underset{\|}{C}}R \rightarrow .C_2H_3 + HO{-}\underset{\underset{H}{O+}}{\underset{\|}{C}}R \qquad (4.2)$$



$$(C_4H_9O)_3\,P = \overset{\cdot +}{O} \rightarrow .C_4H_7 + (C_4H_9O)_2\,P(=\overset{+}{O}H)OH \qquad (4.3)$$

lone-pair electrons away from the oxygen atom, which should also increase the basicity (PA_M). Hence, there is some basis for expecting $-\Delta H_r$ to be relatively insensitive to molecular environment. While the assumed exothermicity of ion-molecule reactions has been used to establish PA_M values between definite limits, few independent determinations are available to make a strict calculation according to equation (2). However, for reaction (3):

$$CH_2 = \overset{\cdot +}{O} + HCH = O \rightarrow CH_2 = \overset{+}{O}H + \overset{\cdot}{C}H = O \qquad (3)$$

which represents the prototype for carbonyl compounds, PA_{CH_2O} can be deduced from measurements on the appearance potential of the $CH_2 = \overset{+}{O}H$ ion derived from methanol; from the values[13,14] $I_{CH_2O} =$ 10·9 eV., $D_{C-H} = 3{\cdot}3$ eV. and $PA_{CH_2O} = 7{\cdot}0$ eV., we obtain $-\Delta H_r = 1{\cdot}0$ eV. Similar energetic considerations apply to the intra-molecular transfer of a hydrogen atom, and this process (R) is often favoured where a 'quasi transition-state' can be formed corresponding to a five- or six-membered ring[15]. In many instances the transfer process is accompanied or followed by re-arrangement, as shown in reactions (4.1), (4.2) and (4.3).

The re-arranged even-electron ion formed in (4.2) is a protonated carboxylic acid; an analogous reaction (4.3) for phosphoric acid esters[16] further underlines the important role of the oxygen atom for the occurrence of these reactions.

The completion of the electron octet on the initially ionized oxygen atom can also come about by a second general process (F) in which a π bond is formed between oxygen and the adjacent carbon atom. This step is facilitated by the homolytic scission of a C—H or C—C bond so as to leave a free valence on carbon to couple with the unpaired electron on oxygen. Following Cummings and Bleakney[4], the results of many investigations[17–20] show that reaction 5, for example, makes a major contribution to the fragmentation pattern of alcohol parent-ions in mass spectra.

$$R_1R_2R_3C{-}\overset{\cdot +}{O}H \rightarrow R_1R_2C = \overset{+}{O}H + R_3. \qquad (5)$$

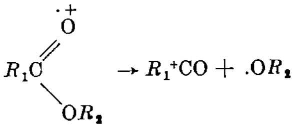
$R_1 = R_2 = R_3 = H$; $\Delta H_r = 1{\cdot}0$ eV. (5.1)

$R_1 = R_3 = H$; $R_2 = C_2H_5$; $\Delta H_r = 0{\cdot}9$ eV. (5.2)

$R_1 = R_2 = H$; $R_3 = CH_3$; $\Delta H_r = 0{\cdot}6$ eV. (5.3)

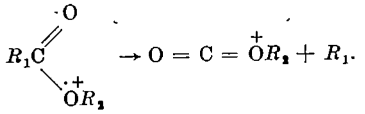
$R_1 = R_2 = H$; $R_3 = C_2H_5$; $\Delta H_r = 1{\cdot}4$ eV. (5.4)

It has been demonstrated by using deuterated alcohols[19,20] that fragmentation occurs by simple bond scission in the manner indicated. To illustrate the general nature of this reaction, examples are given here for carboxyl and carbonyl compounds; in each case the process is known to be of primary importance in the interpretation of the mass spectra[21].



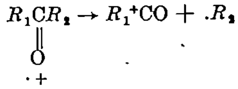
(6)

$R_1 = R_2 = H$; $\Delta H_r = 1{\cdot}1$ eV. (6.1)



(7)

$R_1 = R_2 = H$; $\Delta H_r = 1{\cdot}8$ eV. (7.1)



$$R_1\underset{\overset{\cdot +}{O}}{\underset{\|}{C}}R_2 \rightarrow R_1{}^+CO + .R_2 \qquad (8)$$

$R_1 = R_2 = CH_3$; $\Delta H_r = 1{\cdot}2$ eV. (8.1)

$R_1 = CH_3$; $R_2 = H$; $\Delta H_r = 0{\cdot}8$ eV. (8.2)

Whereas the ion-molecule reactions (M) tend to be exothermic and the measured rate constant[8] for equation (1) suggests the occurrence of reaction on every encounter, the dissociation reactions of type (F) must have activation energies corresponding at least to the endothermicity (< 2 eV.). However, for the n-propanol molecule-ion, there is quantitative evidence[22] that the vibrational energy is stored in only a few degrees of freedom. This finding supports the concept of directed as opposed to random fracture and it has important kinetic repercussions. From the simplified form of the unimolecular rate expression[5]:

$$k = \nu\left(1 - \frac{\varepsilon_0}{E}\right)^S \qquad (9)$$

and assuming ν, the frequency factor for dissociation, to be 10^{14} sec.$^{-1}$ and $S = 12$ effective oscillators, it may be calculated that for parent molecule-ions with initial vibrational energy $E = 3\varepsilon_0$, where ε_0 is the activation energy, the rate constant k for dissociation is 10^{12} sec.$^{-1}$. On the basis of the reasonable values used in the calculation, it follows that (F), as well as (M), reactions can logically be expected to occur before neutralization or thermal quenching intervenes in the liquid state[2]. Moreover, there is no reason to suspect that solvation would adversely affect the rate of reactions (M), (R) and (F) since only homolytic bond rupture is involved and the locus of positive charge remains unaltered. Other fragmentation processes not included in our classification are characterized by high activation energies or very low frequency factors and are therefore of secondary importance, particularly for consideration in the liquid state.

The large majority of the product and fragment ions formed in the above reactions are even-electron ions and are likely to adopt the most stable structure in which all the valence orbitals are filled. Thus, $\mathrm{CH_3\overset{+}{O}H_2}$, $R_1R_2\mathrm{C{=}\overset{+}{O}H}$, and $\mathrm{O{=}C{=}\overset{+}{O}H}$ are protonated molecules while $\mathrm{H^+CO}$ and $\mathrm{CH_3^+CO}$ can be identified as the union of a Lewis base, carbon monoxide[23], with a proton and a carbonium ion, respectively. It is improbable that neutralization of these even-electron positive ions occurs by thermal electron attachment. Eyring, Hirschfelder and Taylor[24] have concluded from the work of Bradbury[25] that, for electrons with low velocities, electron capture only occurs readily for molecules with free valencies. There seems to be no reason to alter this view for the ions discussed above. A similar conclusion appears to follow from an application of the Franck–Condon principle[26]. In order for thermal electron capture to proceed, the potential energy surfaces corresponding to the initial ion and the resultant neutral aggregate must cross. Among typical processes which might be envisaged are the formation of a radical by electronic rearrangement as in equation (10), or of a radical plus neutral molecule as in equation (11):

$$R^+\mathrm{CO} + e^- \rightarrow R\dot{\mathrm{C}}{=}\mathrm{O} \qquad (10)$$

$$R\mathrm{\overset{+}{O}H_2} + e^- \rightarrow R\mathrm{OH\ H} \qquad (11)$$

In general, processes of this type require the gain of vibrational energy by the ion to give the appropriate nuclear configuration of the products before the thermal electron can be captured. It is surmised that while the electron will be polarized in the field of the positive ion, there remains an energy barrier to direct neutralization by attachment. In these circumstances the most appealing possibility is proton or carbonium ion release from the even electron ion, brought about largely by the electrostatic field of the 'free' electron. This type of reaction is illustrated by equations (12–15), and has much in common with the mechanism of discharge processes at electrodes in the electrochemistry of solutions.

$$\mathrm{O = C = \overset{+}{O}H} + e^- \rightarrow \mathrm{O = C = O + H} \qquad (12)$$

$$R_1R_2\mathrm{C = \overset{+}{O}H} + e^- \rightarrow R_1R_2\mathrm{C = O + H} \qquad (13)$$

$$\mathrm{CH_3\overset{+}{O}H_2} + e^- \rightarrow \mathrm{CH_3OH + H} \qquad (14)$$

$$\mathrm{OC^+}R + e^- \rightarrow \mathrm{OC} + R. \qquad (15)$$

The occurrence of equations (12–14) in the liquid state involves a thermochemical change given by:

$$-\Delta \mathrm{H}_r = I_H - PA_M - S_{MH^+} \qquad (16)$$

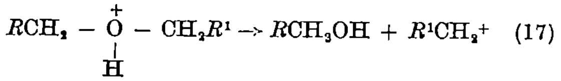
where S_{MH^+} represents the solvation energy of the protonated molecule. In the discharge of a hydrogen atom from a fully solvated hydronium ion $\mathrm{H_3O^+}$ in water, $-\Delta\mathrm{H}_r = 1{\cdot}4$ eV., and it is to be expected that comparable values obtain for the other reactions depicted above. Thus neutralization by discharge as represented here is not a process accompanied by high-energy release, in contrast to the situation that exists if the parent molecule-ion is neutralized before ionic reactions can take place.

It is probable that the discharge processes involve small but necessarily finite potential energy barriers. Therefore, these reactions are unlikely to occur instantaneously but the electron and positive ion can remain associated for sufficient time to represent a distinct and kinetically significant intermediate. It also follows that proton discharge, as in equation (14), would tend to occur more readily by tunnelling, compared to $\mathrm{CH_3^+}$ discharge from $\mathrm{CH_3\overset{+}{O}H_2}$, despite the more favourable overall thermochemical change in the latter case. It is proposed to discuss these reactions more fully in a future publication.

In terms of the foregoing discussion it is now possible to seek an interpretation of the radiation chemistry of representative compounds. McDonell and Newton[27] have shown that the major liquid phase products from the radiolysis of aliphatic alcohols are glycols, aldehydes and ketones. Glycol formation from primary alcohols is consistent with the coupling of $R\dot{\mathrm{C}}\mathrm{HOH}$ radicals, which are formed according to equation (1) and also by abstraction due to atomic hydrogen liberated in the discharge process. The formation of carbonyl compounds is readily accounted for by equation (5), which also explains the higher proportion of ketone to aldehyde with alkyl substitution on the α-carbon atom. On this basis, the results show that hydrogen atom loss in equation (5) occurs with higher probability than the corresponding loss of an alkyl group, and while this trend agrees with the lower activation energy of equation (5.2) relative to equation (5.4), it is doubtful if such fine energy differences can always give reliable predictions regarding the predominant mode of scission. Except in the case of *t*-butyl alcohol, the yield of hydrogen is consistently greater than methane, particularly for the primary alcohols, which again suggests that hydrogen atom loss by equation (5) and in the discharge equation (14) occurs preferentially over the removal of an alkyl group. Although the overall pattern of products is clearly consistent with the specified reactions, it might be thought that other, purely empirical, schemes utilizing free radical intermediates without intervening ions could also account for the products. However, a re-interpretation of Burr's work[19] on hydrogen formation from deuterated ethanols provides strong support for the present mechanism. The total deuterium content of the radiolytic hydrogen gas from $\mathrm{CH_3CD_2OH}$ and $\mathrm{CH_3CH_2OD}$ was found to be 47 and 39 per cent respectively. Burr assumed that all the hydrogen (or deuterium) atoms originate by loss from the α-carbon atom in a primary process (as in equation 5); and hence concluded that hydrogen atom abstraction occurs mainly from the OH (OD) group. Now this latter step must be regarded as intrinsically improbable both in view of the greater bond strength of O—H relative to C—H and the experimental fact that hydrogen abstraction processes proceed most readily on the carbon atom adjacent to an electronegative group. This difficulty is avoided in the present mechanism since the liberation of a hydrogen (or deuterium) atom from the hydroxyl position follows from the discharge reaction analogous to equation (14), and therefore provides a more acceptable route to the formation of HD from $\mathrm{CH_3CH_2OD}$.

The formation of carbon monoxide and carbon dioxide as major products from compounds with carboxyl groups[28] (acids, esters), and of carbon monoxide from carbonyl compounds are described by the (*R*), (*F*) and (*N*) reactions we have considered earlier. According to this mechanism, the formation of carbon oxides and of any molecule, such as the carboxylic acid in equation (4.2), produced in a protonated form prior to discharge should be unaffected by the presence of a free radical scavenger in the system. Careful experiments by Hummel[29] on methyl acetate concur with this theoretical expectation. Since hydrogen atoms and alkyl radicals are also released in the above reactions, the kinetic interplay of abstraction and combination processes, dependent on the initial geometry of formation, will result in hydrogen, methane, etc. It is clear that high yields of methane are expected, and found, for those compounds (acetone, acetic acid, etc.) where initial dissociation (F) and/or subsequent discharge (N) can result in methyl radicals. On the subject of carbonyl compounds, it has been frequently said that the radiolysis results are similar to those obtained in photolysis, and this has inadvertently contributed to the neglect of ionic processes. While it would be remarkable if no correspondence existed between the effects of these differing radiations, closer inspection of the results reveals significant differences. In particular, whereas hydrogen is usually a major product of radiolysis, it is much less important in photochemical studies on carbonyl compounds. Further, the interpretation of radiolysis studies by analogy with photochemistry often leads to difficulties of interpretation which are only partially resolved by recourse to additional empiricisms such as 'molecular' processes and the specification of radical reactivity according to comparative degrees of thermal energy[30]. Since many organic compounds do not show significant light absorption in the conventional photochemical range, photochemistry represents at best a limited guide to the effects of high-energy radiation.

The application of the ionization model can also be illustrated by reference to aliphatic ethers[31] and tri-n-alkyl phosphate esters[32]. In the former case, any attempt to rely on a simple correlation with the mass spectrum is handicapped by the presence of a large number of fragment peaks. On the other hand, the theoretical approach we have outlined leads to the expectation that the ions $R\text{CH}_2-\overset{+}{\underset{|\atop \text{H}}{\text{O}}}-\text{CH}_2R^1$ and $R\text{CH}=\overset{+}{\text{O}}-\text{CH}_2R^1$ will be formed by the processes (M) and (F), respectively. There is independent evidence from thermal chemistry[33] and mass spectra[15] that these ions can further rearrange according to equations (17) and (18):



$$R\text{CH}_2-\overset{+}{\underset{|\atop \text{H}}{\text{O}}}-\text{CH}_2R^1 \rightarrow R\text{CH}_3\text{OH}+R^1\text{CH}_2^+ \quad (17)$$

$$R\text{CH}=\overset{+}{\text{O}}-\text{CH}_2R^1 \rightarrow R\text{CH}=\text{O}+R^1\text{CH}_2^+ \quad (18)$$

The discharge of the carbonium ions may be expected[2] to result in olefine and hydrogen atom formation. It is completely consistent with this sequence that compounds with carbonyl and hydroxyl groups, as well as hydrogen gas and olefines, account for the principal products from the liquid phase radiolysis of aliphatic ethers. In the case of tri-n-alkyl phosphates, a rational description of the radiolysis results[32] is provided by (R) reactions similar to equation (4.3).

In conclusion, it would seem that the success of the qualitative theory presented here militates against the thesis that electron capture by the parent ion necessarily precedes any chemical reaction to liquid systems. Since recent work[34] on radiation-induced polymerization has furnished unequivocal evidence for ionic intermediates, it seems appropriate to reiterate the view[35,36] that the reactions of ions should be considered to represent an integral part of mechanism in radiation chemistry.

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CÆSIUM-SODIUM ION EXCHANGE ON CLINOPTILOLITE

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THE interactions between nuclides and the Earth's soils and minerals have received much attention because of the accidental and purposeful release of fission fragments into the environment. Of particular importance are cæsium and strontium ions due to the combination of abundance and relatively long half-lives. A search has been made to determine which soils and minerals will selectively adsorb either the cæsium or the strontium. A high selectivity for strontium is difficult to find when considering ordinary ion exchange because of the chemical similarity of strontium to the calcium which is found in these soils and minerals in their natural state. Some other type of mechanism must be found to segregate strontium. Naturally occurring soils and minerals, however, generally show a selectivity for cæsium over other alkalis and alkaline earths. Montmorillonite, for example, shows a relatively high selectivity for cæsium[1]. Clinoptilolite is a natural zeolite shown by preliminary experiments to have an even higher selectivity for cæsium. The structure of clinoptilolite is discussed in a recent article by F. A. Mumpton[2]. A representative formula for clinoptilolite is:

$$(Na_2O)_{0\cdot70}(CaO)_{0\cdot10}(K_2O)_{0\cdot15}(MgO)_{0\cdot05}\cdot Al_2O_3\cdot 8\cdot5-10\cdot5\ SiO_2\cdot 6\text{–}7\ H_2O$$

The material often contains up to 25 per cent impurities, mostly quartz, feldspar and montmorillonite.

Clinoptilolite has one very distinct advantage over montmorillonite. This is the mechanical stability of the small chunks when exposed to an electrolyte solution. The material − 20 + 40 mesh can be placed directly in a chromatographic column. A steady flow can easily be maintained. There is no tendency for the column to clog even in the presence of sodium solutions. Montmorillonite, on the other hand, cannot be used in a column in the presence of sodium solutions. The clay will disperse and completely clog the column. Even for columns exposed to other electrolytes the montmorillonite must be suspended on large volumes of a filter aid such as asbestos to prevent clogging or its being washed from the column. Clinoptilolite thus has a tremendous advantage in that it can be used directly in the column and will allow a large variation in flow-rate.

The chunky nature of the clinoptilolite means that all the exchange positions are not equally rapidly available to ions in solution. This is a disadvantage because some type of slow diffusion process will be involved to reach the less accessible positions. The experimental implications of this are discussed later.

Cæsium ion sorption. The saturation and elution chromatograms for the sorption of cæsium ions on clinoptilolite were determined at 25° C. This was done by taking a column containing 2 gm. of clinoptilolite and saturating it with 0·02 *N* cæsium chloride with the radioisotope cæsium-134 added. The saturation chromatogram shows the concentration of the effluent as a function of the volume of solution through the column. When the concentration of the effluent rose to and remained equal to the concentration of the input solution the saturation was complete. The exchange capacity of the clinoptilolite is then just equal to the total number of milliequivalents which entered the column and not found in the effluent. This 'uptake' can be determined quantitatively from the area over the saturation chromatogram.

A more exact determination of the amount of cæsium ions adsorbed was obtained when the sorbed cæsium was eluted with a cæsium chloride solution of the same concentration but which carried no radioactive tracer[3]. This is an isotopic exchange, with the clinoptilolite at all times in equilibrium with the cæsium chloride solution. The elution is considered complete when the counting-rate reaches background. The area under the elution chromatogram is equal to the total amount of cæsium adsorbed. The milliequivalents of cæsium in the column free-volume solution, however, must be subtracted. The free volume is determined from the difference between the wet and dry weights of the column.

The exchange capacity of the clinoptilolite was found to be 1·61 ± 0·01 mequiv./gm. Columns which were exposed to 0·02 *N* electrolyte solutions for a period of four months showed a 3 per cent increase in exchange capacity.

The elution chromatogram exhibits decided 'trailing'. This trailing means that certain of the cæsium ions were delayed in their release from the mineral to the solution. It may be that some of the exchange positions are located deep in the chunk of clinoptilolite. The replacing cæsium ion is then required to travel a path through the mineral. This type of a slower diffusion could account for the trailing. Reducing the particle size may greatly reduce the trailing; but this would adversely affect the column behaviour.

Sodium ion sorption. The sodium ion sorption was not found to differ from that of cæsium. The clinoptilolite sorbs an amount of sodium ion equal to its exchange capacity. The chromatograms, however, are markedly different because of sodium's ineffectiveness in displacing other ions. Most other ions are preferred over sodium by the clinoptilolite. The chromatogram for the saturation of clinoptilolite with 0·02 *N* ^{22}NaCl showed almost immediate front leakage of sodium ion and very much more trailing than in the cæsium saturation curves. Moreover, for this concentration of saturating solution only 80 per cent of the exchange sites actually contained sodium ions when the curve showed that saturation should have been complete. This concentration of sodium chloride was inadequate to convert the natural clinoptilolite to the sodium form.

Monoionic sodium clinoptilolite was prepared by saturating a 2-gm. column with 1 litre of 1 *N* sodium chloride. Isotopic exchange curves on this material were similar to the ones obtained with cæsium. An exchange capacity determination using sodium on this material showed the exchange capacity to be identical to that previously obtained with cæsium. The exchange capacity of clinoptilolite is thus independent of which ion, cæsium or sodium, is used for measuring it.

Sorption from a mixed sodium–cæsium solution. The column can just as easily be saturated with a mixture of electrolytes as with a single cation. At saturation the clinoptilolite in the column is in chemical equilibrium with the mixture of sodium and cæsium ions in solution. The fraction of exchange positions held by cæsium, q/q_0, is a function of the relative concentration of cæsium to the total solution electrolyte concentration c/c_0, where q_0 is the total cation exchange capacity. A plot of q/q_0 versus c/c_0 at a given temperature is called an exchange sorption isotherm.

Exchange sorption isotherms were determined for cæsium–sodium exchange on clinoptilolite at two different temperatures, 50° and 75° C. This was done experimentally by saturating a series of columns with different ratios of cæsium and sodium ionic concentrations and then determining the composition of the total sorbed cations by the isotopic elution technique previously described. Cæsium is determined directly and the sodium by difference. Seven columns were used each containing 2·000 gm. clinoptilolite. Saturation solutions contained 5–95 per cent cæsium with a constant total electrolyte normality of 0·02 *N*. The cæsium was traced by adding cæsium-134. The lower the percentage of cæsium

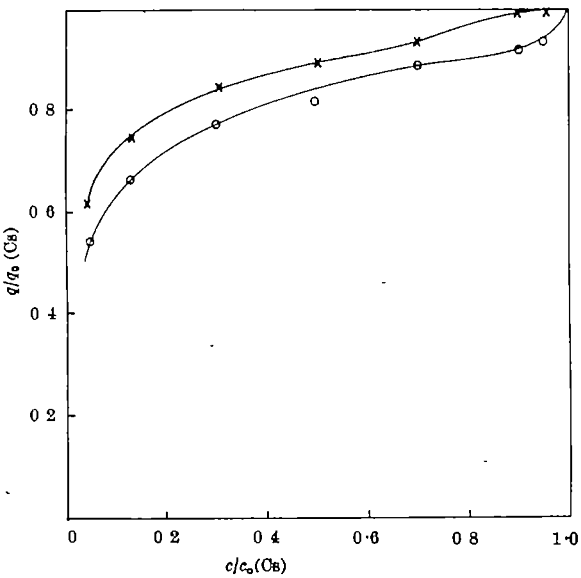

Fig. 1. Exchange sorption isotherm for the sodium–cæsium exchange reaction at 50° and 75° C. ×, 50° C.; O, 75° C.

the larger the amounts of solution which must be passed through the column to bring it to complete saturation. This is due to the high ratio of cæsium in the total amount sorbed even in the case of equilibrium with the low percentage cæsium solutions. A total of seven litres of the 5 per cent cæsium solution was required to equilibrate the column.

The exchange sorption isotherms for clinoptilolite (Fig. 1) show that for a solution of 5 per cent cæsium–95 per cent sodium a mixture of about 60 per cent cæsium–40 per cent sodium is sorbed on the clinoptilolite.

Equilibrium constants for the exchange reaction. From the data used in plotting the exchange sorption isotherms the thermodynamic equilibrium constant can be calculated[4]. For the reaction:

$$\mathrm{Na}^+ + \mathrm{Cs}M \rightarrow \mathrm{Cs}^+ + \mathrm{Na}M$$

$$K = \frac{M_{\mathrm{Cs}} N_{\mathrm{Na}} \gamma_{\mathrm{Cs}} f_{\mathrm{Na}}}{M_{\mathrm{Na}} N_{\mathrm{Cs}} \gamma_{\mathrm{Na}} f_{\mathrm{Cs}}} = K'_c \frac{\gamma_{\mathrm{Cs}} f_{\mathrm{Na}}}{\gamma_{\mathrm{Na}} f_{\mathrm{Cs}}} = K_c \frac{f_{\mathrm{Na}}}{f_{\mathrm{Cs}}}$$

where M equals molarity in solution, N equals mole fraction of ions sorbed on the mineral, γ equals activity coefficient of ion in solution, f equals activity coefficient of ion sorbed on exchanger.

In terms of the experimental quantities:

$$K'_c = \frac{(c/c_0)\,(1-q/q_0)}{(1-c/c_0)\,(q/q_0)} \simeq K_c \,.$$

K'_c is nearly equal to K_c in this case. For a uni-univalent system the quotient of single ion activities in a mixed electrolyte solution is nearly unity. Experimental data on the ion activities at these concentrations in this mixture are, of course, not available. They can be estimated from the Debye–Hückel equations. The only deviation of the quotient from unity in this case would be the contribution due to the slightly different 'a' parameter values for cæsium and sodium.

The thermodynamic equilibrium constant can be determined by integrating K_c over the whole sorbed phase concentration range.

$$\ln K = \int_0^1 \ln K_c \, d(q/q_0)$$

The values of $-\ln K$ and ΔF^0 for the sodium–cæsium exchange reaction at the two temperatures are given in Table 1. Based on these data the standard enthalpy change is $\Delta H^0 = 4{\cdot}92$ kcal./mole and the standard entropy change is $\Delta S^0 = 8{\cdot}98$ e.u.

Table 1. THERMODYNAMIC EQUILIBRIUM CONSTANT AND FREE ENERGY OF THE SODIUM–CÆSIUM EXCHANGE REACTION

T (°K.)	$-\ln K$	ΔF^0
323	3·15	2·02 kcal./mole
348	2·58	1·79

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AN UNSTABLE RIBONUCLEIC ACID IN RAT LIVER NUCLEI

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ACCORDING to a widely accepted view, genetic information for protein structure is coded in the deoxyribonucleic acid (DNA) of the cell nucleus. Thereby DNA does not react directly but rather by an intermediate, believed to be ribonucleic acid (RNA).

Since an unstable RNA-fraction was found in rat-liver nuclei[1,2] and in a bacterial system a similar one (messenger RNA) could be demonstrated, which is more definitely involved in the transfer of genetic information from the DNA to the ribosomal particles (RNP)[3,4], it seems that such a messenger RNA appears in all living cells independently from which species the cells were derived.

This article is concerned with some regulation mechanism by which genetic information might be expressed in the cytoplasm of different organs in the same organism. Certain evidence is presented: (1) that the nuclei of different organs synthesize different kinds of messenger RNA; (2) that the cytoplasm of each organ selects and stabilizes different kinds of RNA molecules offered by the nuclei, depending on the cytoplasm of the organ under investigation.

When rat-liver nuclei, labelled *in vivo* with ^{14}C-orotic acid or phosphorus-32 for differing time-intervals, were incubated *in vitro* in a salt–sucrose medium[1], only about 15 per cent of the total nuclear RNA was lost to the supernatant, when compared with controls. In these experiments the nuclei were left intact[1]. Fig. 1 demonstrates the specific activity of the transferred RNA as a function of the labelling time *in vivo* (time between injection of the label and

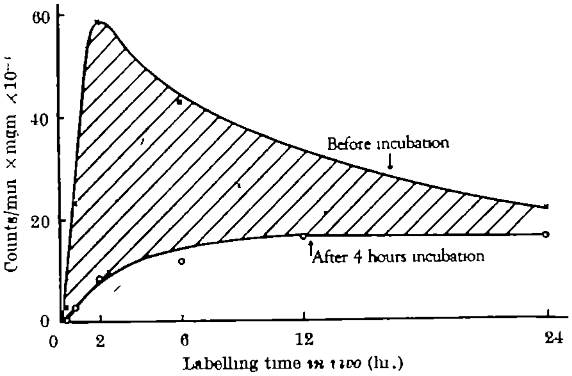

Fig. 1. Changes in the specific radioactivity of the removable nuclear RNA as a function of the labelling time *in vivo*. Rats were injected with ^{14}C-orotic acid and killed at the times indicated on the abscissa. The upper curve shows the specific radioactivity of the RNA of the non-incubated liver nuclei. The lower curve demonstrates the specific activity of the RNA which is stable within the nuclei after 4 hr. incubation *in vitro* in salt-sucrose

death of the rat). The lower curve represents the specific activity of that part of nuclear RNA which cannot be removed during 4 hr. incubation in fortified sucrose (stable RNA). The upper curve shows the specific activity of the nuclear RNA without incubating the nuclei. The removable RNA fraction appears during incubation first in the supernatant medium and is later degraded. The space between these two curves demonstrates the turn-over of the transferable RNA.

This transferable RNA with a high turn-over rate, which amounts to less than 5 per cent of the total cell RNA[1], has the following properties:

(*a*) The oligonucleotide pattern after digestion by RNase is completely different from that of the remaining nuclear RNA (Table 1) and also from the RNA of all other cell components. For the oligonucleotide pattern see refs. 5 and 6. The patterns usually showed variations from one animal to another, but the overall trend was always the same. Therefore, the figures of only one experiment are presented in each case.

Table 1. RADIOACTIVITY IN OLIGONUCLEOTIDES OF NUCLEAR RNA FRACTIONS OF RAT LIVER AFTER DIGESTION BY RNASE, COMPARED WITH URIDINE MONOPHOSPHATE (UMP OR U)

Ratio U/nucleotide	Nuclei after incubation	NPM	NS
C	0·75	1·15	1·00
AC	2·44	2·58	2·43
GC+AAC	1·18	1·41	1·32
GAC+AGC	1·10	1·25	1·32
AAU+AU	3·30	2·72	2·73

The nuclei were labelled *in vivo* by phosphorus-32 for 2 hr. and incubated in a salt-sucrose medium at 30° C. for 20 min. After incubation the nuclei were re-isolated and a postmicrosomal fraction (NPM) was spun down in the Spinco ultracentrifuge from the incubation medium. The final supernatant is called NS (refs. 1 and 2).

C, Cytidine monophosphate (CMP); A, adenosine monophosphate, G, guanosine monophosphate.

(*b*) After 15 min. incubation of nuclei labelled *in vivo* for 2 hr. this RNA appears mainly in a postmicrosomal (NPM) and soluble (NS) fraction[1].

(*c*) In the living animal one finds this RNA first in the nuclei, then in the PM-fraction and afterwards it disappears completely from the cell (Fig. 2). As can be seen from Table 1 the high-turn-over RNA is rich in UMP. For more detailed experiments see ref. 2.

(*d*) This RNA-fraction is very sensitive to degrading enzymes[1,2]. When liver nuclei labelled *in vivo* for 2 hr. were incubated in non-radioactive liver cytoplasm for 4 hr. *in vitro*, less than 5 per cent of the total RNA was degraded, but about 75 per cent of the radioactivity was lost.

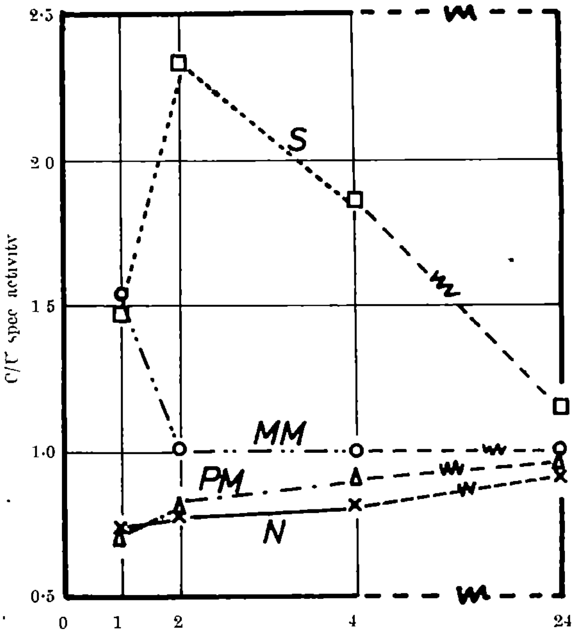

Fig. 2. Changes of the ratios of the specific radioactivities in UMP to CMP as a function of the labelling time *in vivo*. Rats were injected by phosphorus-32 and killed at the times indicated on the abscissa. The livers were homogenized and separated into the subfractions by centrifugation (refs. 1 and 2). *N*, nuclei, *MM*, mitochondria + microsomes; *PM*, postmicrosomes; *S*, final supernatant. The RNA of the final supernatant shows a high end-turn-over of the C-fraction (ref. 2)

(*e*) Its molecular weight is lower than that of the main nuclear RNA[1,2]. This was shown by stepwise degradation with snake venom diesterase. At the beginning of enzyme action the specific radioactivity of the remaining RNA was unchanged. Later, the specific activity dropped to a lower value. This drop was independent of the labelling time *in vivo* (at least from 1 hr. onwards). It seems clear that a highly labelled RNA of smaller molecular weight is used up by the enzyme faster than a rather inert RNA of higher molecular weight.

The foregoing properties of a nuclear RNA fraction of rat liver are very similar to the messenger RNA described by Brenner *et al.*[3] and Gros *et al.*[4] in a bacterial system.

The next question was whether the messenger RNA's of each organ in the same organism were identical or not. For this purpose rat spleen and kidney nuclei were labelled *in vivo* and incubated under the same conditions as the liver nuclei. Table 2 demonstrates that the ratios of the specific activities of CMP and UMP are less than 1 in the non-incubated nuclei of liver and spleen after digestion of their RNA. But the corresponding ratio of the kidney nuclear RNA was higher than 1. In the case of liver and spleen an RNA fraction rich in UMP migrates

Table 2. DIFFERENCES IN THE C/U-RATIOS OF INCUBATED AND NON-INCUBATED NUCLEI OF SPLEEN, KIDNEY AND LIVER

C/U-ratios after digestion by RNase	Spleen	Kidney	Liver
Specific radioactivity of non-incubated nuclei	0·83	1·10	0·74
Radioactivity of incubated nuclei	1·42	2·04	1·17
Radioactivity of supernatant after incubation	0·95	2·62	0·94

The nuclei were labelled *in vivo* for 2 hr. and incubated in salt-sucrose for 15 min. at 30° C.

Table 3. CHANGES IN THE OLIGONUCLEOTIDE PATTERN OF LABELLED RAT-LIVER NUCLEI DURING INCUBATION IN SALT-SUCROSE, NON-RADIOACTIVE KIDNEY OR LIVER CYTOPLASM, RESPECTIVELY

Ratio C/nucleotide	Incubation of rat-liver nuclei labelled *in vivo* for 2 hr. In salt-sucrose	In kidney cytoplasm	In liver cytoplasm
U	1·77	2 06	1·54
AC	3 96	4·92	3·14
GC	1·73	1·74	1 86
AAC	5 55	5 41	4·38

During incubation about 75 per cent of the labelled RNA was broken down The remaining RNA was almost stable. For more details see Table 1.

out of the nuclei during incubation *in vitro*. In contrast to this, the RNA removed during incubation of kidney nuclei contains more CMP[2]. This clearly demonstrates that the messenger RNA's of the three organs under investigation are not identical.

These results are in agreement with the concept that in each organ different genes are active[7], making different kinds of messenger RNA. Pelling[8] showed this directly by his autoradiographic studies on *Chironomus tentans*.

Another point investigated was whether the cytoplasm of each organ is able to select and to stabilize some of the genetic information (RNA) necessary for the function of that organ. Labelled rat-liver nuclei were incubated in fortified sucrose, non-radioactive liver or kidney cytoplasm, respectively[2]. Table 3 shows that although about 75 per cent of the labelled nuclear RNA is broken down during incubation (but less than 5 per cent of the total RNA), the remaining labelled RNA's (almost stable) differ from each other depending on the incubation medium used. The kidney cytoplasm selects a CMP-rich RNA; but the liver cytoplasm an UMP-rich RNA, compared with the RNA of the sucrose incubation. So it appears as if a real selection of some of the messenger RNA by the cytoplasm has taken place.

It is suggested that some messenger RNA is turned over very fast, some has a rather low turn-over rate and again some of the fast-labelled nuclear RNA may not be involved at all in transferring genetic information, but rather in regulation mechanisms. The messenger RNA with a high turn-over rate may be regulated by inducers or suppressors (for example, by hormones[9]), while the stable RNA may be responsible for enzymes or other proteins which cannot be induced or suppressed, but are also characteristic for each organ. A selection of a stable RNA may occur only if a template is present in the RNP particle to conserve the integrity of the messenger RNA. If such a template is not available, the RNA would be broken down. Such a mechanism could help to explain organ specificity and differentiation.

A mechanism, by which an RNA is selected by a pre-existing template, may also explain the carcinogenesis by some chemicals (for example, some azodyes) which do not react with nucleic acids themselves. If it is assumed that those templates consist of proteins and that their synthesis is directed by combination with a messenger RNA, azodyes may react competitively with one special template so that this is lost irreversibly after some time. A special genetic information can no longer be expressed and an irreversible change occurs in the cell without altering the DNA itself[3].

These experiments were performed at the McArdle Memorial Institute for Cancer Research, University of Wisconsin, Madison, and at the Institut für Experimentelle Krebsforschung der Universitat Heidelberg, Germany.

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QUANTITATIVE ESTIMATION OF THE HYBRIDIZATION OF RABBIT ANTIBODIES

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THE major product of the reaction of rabbit antibody with pepsin at pH 4·5 has an average molecular weight of 106,000 and a sedimentation coefficient of approximately 5 S, as compared with 160,000 and 6·5–7 S for the original molecule[1–3]. The pepsin-treated antibody is still bivalent, but is further split into two univalent, 3·5-S fragments, approximately equal in size, on subsequent reduction of a single, highly labile disulphide bond[4]. These fragments correspond closely in a variety of properties[2,5] to the active fractions of a papain digest, described by Porter[6], who first demonstrated the presence of sub-units in the antibody molecule. It was concluded[1–3] that the mechanisms of action of the two enzymes are similar, and that there are one or more regions of the molecule which are particularly susceptible to the action of papain or pepsin. Subsequent to the enzymatic hydrolysis the two univalent fragments are linked by the disulphide bond[2,4]. (Papain, being a sulphydryl enzyme, is used in conjunction with a reducing agent.) Cebra *et al.*[7] have recently degraded the rabbit antibody to 3·5-S fragments by successive treatments with papain, covalently linked to an insoluble polypeptide, and a reducing agent, providing further evidence for a similarity in mechanism of action of the two enzymes.

We recently reported[8] that the active chromatographic fractions of a papain digest, designated I and II[6], do not correspond to two sub-units of an individual molecule, but rather represent univalent fragments derived from different populations of globulin molecules; and that the univalent protein can be further subdivided by variation of the pH of elution. Nevertheless, there is considerable evidence[8] suggesting that the two univalent fragments of an individual molecule may be quite similar, as originally proposed by Porter[6].

The univalent, 3·5-S sub-units of a specifically purified rabbit antibody can be recombined in good yield by oxidation to give 5-S antibody which again has the capacity to form specific precipitates[8,9]. The fact that the sedimentation coefficient agrees very closely with that observed prior to reduction indicated that the re-oxidized, precipitating antibody is bivalent. When a mixture of approximately equal amounts of antibodies of two different specificities was used, the recombined product failed to form a precipitate with either antigen alone, but more than half precipitated when an appropriate mixture of the two antigens was used[10]. This suggested the presence of a large proportion of antibody of mixed specificity.

For random recombination of an equimolar mixture of two antibodies, the proportions of *A–A*, *B–B* and the hybrid, *A–B*, would be 1 : 1 : 2. Since *A–B* is univalent with respect to either precipitating system, it should act as a specific inhibitor. This would account for the failure to precipitate with a single antigen. A mixture of antigens, however, would be expected to form a continuous lattice when added to such a mixture of antibodies.

Although these observations could be explained on the basis of random recombination, the experiments did not show directly that such was the case. The present work was undertaken to provide another type of evidence as to the presence of hybrid antibody and to obtain a quantitative estimate of the degree of randomness of the recombination.

In brief, the method consisted in reducing and recombining a mixture of specifically purified rabbit anti-ovalbumin and anti-*p*-azobenzoate antibodies. The product was tested by treatment with an excess of an insoluble adsorbent specific for anti-ovalbumin. It was expected that hybrid antibodies as well as anti-ovalbumin would be removed by the adsorption, and that this could be demonstrated by measurement of the number of combining sites specific for the benzoate group remaining in the supernatant. The number of such sites remaining was estimated quantitatively by measurement of the capacity to bind labelled *p*-iodobenzoate. In addition, the adsorbent itself was re-suspended after exposure to the antibody preparation and tested for its capacity to bind the same hapten. Controls were carried out with mixtures of the two 5-*S* antibodies (not reduced), and mixtures of the two antibodies which were reduced and re-oxidized separately. The data provide additional evidence for the presence of hybrid antibody and suggest that the recombination is random.

Anti-*p*-azobenzoate antiserum was prepared in rabbits by the repeated injection of an antigen made by diazotizing *p*-aminobenzoic acid in acid solution and coupling to bovine γ-globulin at *p*H 9–9·5 in saline–borate buffer near 0° C.; 40 mgm. of *p*-aminobenzoic acid was used for each gm. of the bovine globulin. The product was dialysed against several 4-litre portions of cold saline–borate buffer at *p*H 8 over a period of about one week. Rabbits were immunized by intravenous injection of 10–15 mgm. portions of the antigen in 1–1·5 ml., administered three times weekly. After 6–8 weeks, weekly partial bleedings were carried out, each followed immediately by an injection of the antigen. A number of antisera of high titre was pooled and purified specifically as described elsewhere[11]. The final steps consisted in dialysing for one week with stirring in ¼-in. tubing against several 4-litre portions of cold buffer, followed by passage through a 7 × 200 mm. column of '*IR*-45' resin and dialysis against cold 0·1 *M* sodium acetate.

Methods used for immunization with ovalbumin are described elsewhere[5]. The antiserum was specifically purified by partial dissociation of a specific precipitate at *p*H 2·5, and fractionation at this *p*H with sodium sulphate[12].

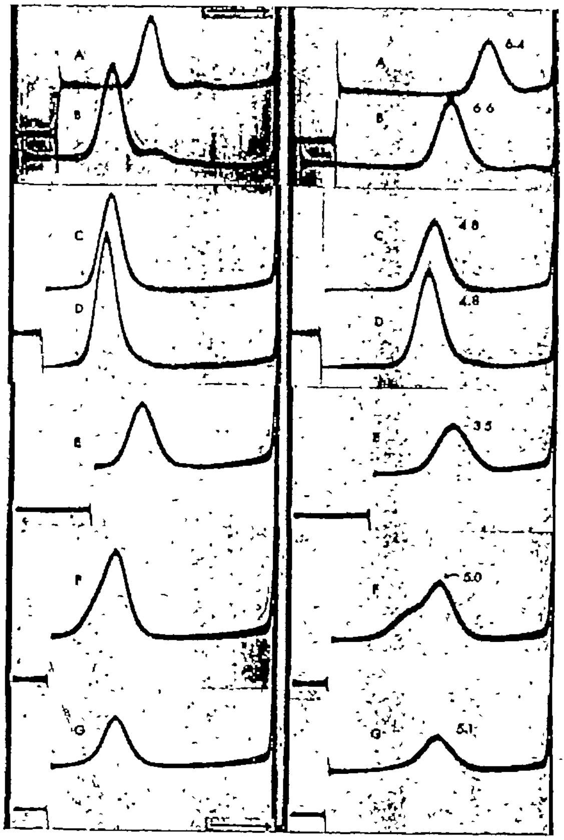

Fig. 1. Photographs of schlieren patterns obtained in the Spinco model *E* ultracentrifuge. Sedimentation is from left to right. Diagrams on left, 48 min. at 59,780 r.p.m.; diagrams on right, 80 min. The numerals are $S_{20,w}$ values. *A* and *B*, specifically purified anti-*p*-azobenzoate antibody and antiovalbumin, respectively; *C* and *D*, above samples, treated with pepsin, precipitated with sodium sulphate; *E*, mixture of equal amounts of above, pepsin-treated antibodies, reduced with mercaptoethylamine; *F*, above neutralized and oxidized; *G*, above precipitated with sodium sulphate. The solvent in each case was 0·1 *M* sodium acetate, *p*H 7·0 except for *E* (*p*H 5·0). (The difference in sedimentation coefficient of 3·5-*S* fragments at *p*H 5 and *p*H 7 is less than 0·2 units)

58 per cent of the antibenzoate and 88 per cent of the antiovalbumin were specially precipitable by a test antigen. The antigen used for testing the anti-hapten antibody was prepared by diazotizing and coupling *p*-aminobenzoic acid to ovalbumin (40 mgm./gm.) and purified by extraction with cold acetone and exhaustive dialysis. Ultracentrifugal patterns of the purified antibodies are shown in Fig. 1, *A* and *B*.

300 mgm. of the specifically purified antiovalbumin and 100 mgm. of the antibenzoate antibody were treated separately with 2 per cent by weight of crystallized pepsin, obtained from the Worthington Co. The reactions were carried out for approximately 6 hr. at 37° C., in 0·1 *M* sodium acetate buffer, *p*H 4·5. The major component was precipitated at *p*H 8·0 with sodium sulphate at a final concentration of 0·18 gm./ml., dissolved in water, and dialysed against 0·1 *M* sodium acetate. Recoveries of the antiovalbumin and anti-*p*-azobenzoate were 137 mgm. and 50 mgm., respectively, of which 72 and 49 per cent were specifically precipitable by an optimal concentration of test antigen. In the ultracentrifuge

at pH 7·0 each preparation migrated essentially as a single, 4·8-S peak (Fig. 1C and D).

20 mgm. portions of each of the pepsin-treated antibodies were mixed and reduced with 0·01 M mercaptoethylamine hydrochloride at 37° C., pH 5·0, for 75 min. In the ultracentrifuge the product now migrated as a single 3·5-S peak (Fig. 1E). After dialysis against saline–borate buffer, pH 8, 800 μgm. of this preparation caused more than 90 per cent inhibition of the precipitation of antiovalbumin γ-globulin with ovalbumin in amounts sufficient to yield 397 μgm. of precipitate in the absence of inhibitor. About 10 per cent inhibition was observed when 1·6 mgm. was present in a mixture containing 470 μgm. of precipitable antibenzoate and an optimal amount of the ovalbumin–p-azobenzoate test antigen. (The 1·6 mgm. corresponds to 0·8 mgm. of antibenzoate antibody.) (We have found that relatively large amounts of 3·5-S fragments are required for specific inhibition of the precipitation of antihapten antibody, as compared with various antiprotein antibodies tested. This is probably attributable to the large number of specific sites on each molecule of the azoprotein test antigen which must be saturated to prevent further reaction with the untreated antibody. A similar explanation may account for the poor inhibition of the homologous reaction by 3·5-S fragments of rabbit antipneumococcus antibody[6].)

The remainder of the reduced material, 35 mgm., was passed through an 8 × 175 mm. column of 'IR-120' resin at pH 4·5 in the cold room in order to remove the positively charged reducing agent. This was done immediately after the treatment with mercaptoethylamine. The bulk of the eluate, containing 31 mgm., was neutralized to pH 8·0 and oxygen was passed over the surface, with stirring, for 2 hr. After dialysis, the sample was treated with oxygen again for approximately 2 hr. and examined in the ultracentrifuge. The solvent was 0·1 M sodium acetate, pH 7·0. It was found (Fig. 1F) that 70 per cent of the material now migrated as a 5·0-S peak (The actual degree of recombination was probably somewhat higher since no correction was made, in measuring areas, for the Johnston–Ogston effect.) After precipitating with sodium sulphate (final concentration 0·16 gm./ml.), washing with sodium sulphate of the same concentration, dissolving and dialysing against 4 litres of 0·1 M sodium acetate, pH 7, the protein migrated essentially as a single 5·1-S peak (Fig. 1G).

To test for hybridization, adsorptions were carried out with an ovalbumin–rabbit antiovalbumin precipitate prepared with an amount of antigen approximately 50 per cent greater than the optimum. The precipitate was first washed repeatedly with saline–borate buffer, suspended in the buffer and dispersed by brief treatment in a tissue grinder. Before removing a sample the suspension was re-dispersed and stirred. Prior to use, aliquots of the suspension were washed three or four times, re-suspended in 1 ml. buffer, agitated for 45 min. with a mechanical shaker, centrifuged, and the optical density of the supernatant was determined at 280 mμ. This process was repeated several times until small and fairly constant amounts of protein were eluted; less than 0·04 mgm. from 8 mgm. of precipitate or 0·1 mgm. from 16 mgm. In determining amounts of protein removed from samples in the subsequent adsorption experiments, corrections were made for the estimated elution that occurred during the shaking period.

The material to be adsorbed was then added to the washed precipitate. The precipitate was re-suspended and the volume of the mixture was adjusted to 1·0 ml. in a graduated tube. After 45 min. of mechanical shaking the mixture was centrifuged, the optical density of the supernatant at 280 mμ was measured, and its capacity to bind p-iodobenzoate labelled[13] with iodine-131 was determined by equilibrium dialysis at 5·0° C. The methods used for measurement of binding have been described in detail[14]. Amounts of radioisotope were determined in a well-type γ-scintillation spectrometer.

The amount of ovalbumin–antiovalbumin precipitate to be used for the adsorption was determined by preliminary experiments, using the decrease in optical density at 280 mμ as a measure of adsorption. It was found that 8 mgm. of precipitate represented an excess of adsorbent for 1·6 mgm. of the re-oxidized mixture. A maximum of 60 per cent of the protein could be removed by the adsorbent.

In the first experiment 8 mgm. of adsorbent was mixed with 1·6 mgm. of the re-oxidized mixture of antibodies in a total volume of 1·0 ml. The mixture was shaken for 45 min., then centrifuged. The effect on capacity to bind p-iodobenzoate is shown in Table 1. Binding experiments were carried out in duplicate with the mean deviations indicated. (A number of samples was dialysed together against a common outer solution so as to permit comparisons with the free hapten concentration constant. Also, the use of

Table 1. REMOVAL OF HAPTEN-BINDING SITES BY AN ADSORBENT SPECIFIC FOR ANTIOVALBUMIN

Samples	Exp. No.	Weight of adsorbent used (mgm.)	Concentration of labelled p-iodobenzoate bound × 10^6 M‡			
			By untreated sample	By supernatant after adsorption	By resuspended adsorbent after adsorption§	By resuspended adsorbent (corrected value‖
Hybridized mixture of antiovalbumin and antibenzoate*	1	unadsorbed	4·8			
	2	8		2 9	1·7	1·4
	3	16		3·2	2·2	1·7
5-S antibenzoate antibody†	4	unadsorbed	5 9			
Mixture of 5-S antibenzoate and antiovalbumin (not reduced)*	5	unabsorbed	5·5			
	6	8		5·9	0·5	0·2
	7	16		5·6	0 5	0
5-S antiovalbumin and antibenzoate, reduced and reoxidized separately, then mixed*	8	unabsorbed	4·3			
	9	16		4 2	0·3	0
8 mgm. adsorbent suspended in 1·0 ml.	10		0·3			
16 mgm. adsorbent suspended in 1·0 ml.	11		0·5			

* Total antibody concentration, 1·6 mgm./ml.

† Antibody concentration, 0·8 mgm./ml.

‡ Equilibrium dialysis at 5·0 ± 0·1° C. Free hapten concentration, 6·4 ± 0·1 × 10^{-6} M. Binding experiments in duplicate, with average value of mean deviations for the concentration bound of 0·14 × 10^{-6} M. A number of samples was dialysed together against a relatively large volume of a common outer solution. The results in the table were obtained in three separate equilibrium dialysis runs. Solutions were buffered with saline–borate, pH 8·0, ionic strength 0·16.

§ The adsorbent was washed twice after the adsorption with small amounts of buffer, then suspended in a total volume of 1·0 ml. of saline–borate buffer, pH 8, ionic strength 0·16, for equilibrium dialysis.

‖ Corrected for binding by untreated adsorbent.

a large volume of outer solution permitted accurate control of the final free hapten concentration.)

With a free hapten concentration of $6·5 \times 10^{-6}$ M, the concentration bound by the unadsorbed, re-oxidized mixture (1·6 mgm./ml.) was $4·8 \times 10^{-6}$ M (Exp. 1, Table 1). The concentration of hapten bound by the supernatant after adsorption was $2·9 \times 10^{-6}$ M, which represents a decrease of 39 per cent (Exp. 2, Table 1).

The adsorbent used was then washed twice with small amounts of saline–borate buffer and re-suspended in the same buffer, added to a final volume of 1·0 ml. Binding of hapten by the suspension, dialysed to equlibrium in the same vessel as the supernatant, was $1·7 \times 10^{-6}$ M (Exp. 2, Table 1). Correcting this for binding by the untreated precipitate ($0·3 \times 10^{-6}$ M), we obtain the value $1·4 \times 10^{-6}$ M for concentration bound by the precipitate due to adsorption of the hybrid antibody.

When 16 mgm. of adsorbent was used (Exp. 3, Table 1), binding by the supernatant decreased to $3·2 \times 10^{-6}$ M and that of the washed, re-suspended adsorbent, corrected for binding by untreated adsorbent was $1·7 \times 10^{-6}$ M.

As a control, a mixture of the pepsin-treated (5-S) antiovalbumin and antibenzoate antibodies, each at a concentration of 0·8 mgm./ml., was similarly adsorbed. In this case a maximum of 46 per cent of the protein was adsorbable. With 8 mgm. of adsorbent, there was a small increase in binding, after removal of the adsorbent, from 5·5 to $5·9 \times 10^{-6}$ M (Exps. 5 and 6, Table 1). This is probably attributable to experimental error since significant increases were not observed in any other experiments. Only a slight increase in hapten-binding capacity was observed when the adsorbent used was re-suspended and tested ($0·5 \times 10^{-6}$ M bound as compared with $0·3 \times 10^{-6}$ M for the untreated adsorbent).

Similar results were obtained when 16 mgm., rather than 8 mgm., of adsorbent was used with the mixture of 5-S antibodies (Exp. 7, Table 1). The concentration of hapten bound after adsorption was $5·6 \times 10^{-6}$ M; the concentration of hapten bound by the washed, re-suspended precipitate used for the above adsorption was $0·5 \times 10^{-6}$ M, which was the same as that obtained with 16 mgm. of the unused adsorbent. The results thus indicate that little or no hapten-binding capacity is removed from a mixture of the two 5-S antibodies by the adsorbent.

The possibility was considered that the removal of hapten-binding capacity from the re-oxidized mixture by an adsorbent specific for antiovalbumin might be due, not to hybridization, but to some non-specific affinity for the adsorbent acquired by anti-hapten antibody during reduction and re-oxidation. To examine this question another batch of specifically purified anti-p-azobenzoate antibody was treated in the same manner as described above to yield in succession 5·2-, 3·5- and 5·3-S antibody ($S_{20,w}$ values). Similarly, 4·8-S antiovalbumin was reduced to 3·5-S, re-oxidized and fractionated with sodium sulphate to give 5·0-S antibody. Equal weights of the two protein preparations were mixed and adsorbed with the ovalbumin–antiovalbumin precipitate. The concentration of each antibody in the mixture was 0·8 mgm./ml. and 16 mgm. of precipitate was used. Measurements of optical density at 280 mμ indicated that 45 per cent of the protein was removed. However, there was a negligible effect on the capacity to bind ^{131}I-p-iodobenzoate. At a free hapten concentration of $6·3 \times 10^{-5}$ M the concentration bound by the mixture of reconstituted antibodies was $4·3 \times 10^{-6}$ M. After adsorption this value was $4·2 \times 10^{-6}$ M (Exps. 8 and 9, Table 1). There was no significant binding by the washed, re-suspended precipitate that had been used for the adsorption. (The concentration bound by the reconstituted antibenzoate alone, 0·8 mgm./ml., without added antiovalbumin, was $4·3 \times 10^{-6}$ M). Also, under the conditions of the above experiments, less than 5 per cent of the optical density at 280 mμ was removed when the reconstituted antibenzoate antibody alone was treated with 16 mgm. of adsorbent.

The evidence is now convincing that antibody of mixed specificity is formed in significant amounts when a mixture of two purified 5-S antibodies is reduced and re-oxidized. When pepsin-treated antiovalbumin and antibovine γ-globulin were mixed and treated in this way, the product failed to form precipitates with a single antigen but did precipitate when a mixture of antigens was used[10]. As indicated in the introduction, this was consistent with the presence of a large fraction of hybrid antibody. It contrasted with the results obtained when a single antibody is reduced and re-oxidized; three different rabbit antibodies have now been tested and precipitating activity found to be restored in each case[5,9]. The present results provide further evidence for hybrid formation and in addition permit an estimate as to the amount produced.

On the assumption that activities are proportional to concentrations, the concentration of hapten bound by a homogeneous group of binding sites should be directly proportional to the total concentration of sites, if the free concentration of hapten is held constant. This will be true of a heterogeneous, as well as a homogeneous population of sites, provided that the distribution of K values is not changed as the concentration of sites is varied[15]. Since it appears unlikely that hybridization, or adsorption by the ovalbumin–antiovalbumin precipitate, would be selective with respect to the combining affinities of the antihapten sites, the condition that the distribution of K values not be changed during the adsorption probably holds. Also the free hapten concentration was essentially constant in the various binding experiments. We will therefore make the assumption that the observed concentration of hapten bound was directly proportional to the concentration of combining sites remaining. On this basis, the fraction of combining sites remaining after adsorption of the reconstituted mixture of antiovalbumin and antibenzoate was 61 per cent when 8 mgm. of adsorbent was used, and 67 per cent with 16 mgm. of adsorbent. No significant reduction in the concentration of hapten-binding sites was observed when a mixture of the 5-S antibodies, or a mixture of 5-S antibodies, that had been reduced and re-oxidized separately, was similarly adsorbed.

After adsorption of the hybridized mixture, a large fraction of the binding capacity removed was found in the washed adsorbent. The total recoveries of binding sites, again assuming direct proportionality with concentration of hapten bound, were 89 and 102 per cent for the experiments in which 8 mgm. and 16 mgm. of adsorbent were used (Exps. 2 and 3, Table 1). The adsorbent did not acquire any significant capacity to bind hapten on exposure to a mixture of the 5-S antiovalbumin and antibenzoate antibodies (Exps. 6 and 7) or to the mixture of separately reduced and re-oxidized antibodies (Exp 9).

No appreciable concentration of faster-moving components was observed in any of the re-oxidized materials. The close correspondence in sedimentation coefficient to that observed prior to reduction strongly suggests that all, or nearly all, the hybrid molecules were bivalent.

It is useful to compare the results with those expected for random recombination of a hypothetical mixture of equal quantities of two completely purified antibodies. As already indicated, the proportions of *A–A*, *B–B* and *A–B* would be 1 : 1 : 2; and if *B* represents an antihapten and *A* an antiovalbumin combining site, half the antihapten sites would be present on hybrid molecules. The same conclusion would hold, regardless of the amount of antibenzoate antibody present, provided that half of the sub-units available for recombination contained an active antiovalbumin site; that is, the fraction of antihapten sites hybridized should depend on the number of antiovalbumin sub-units present. As already indicated, 72 per cent of the 5-*S* antiovalbumin was specifically precipitable. If the remaining 28 per cent represents inactive, but reducible molecules, and if recombination is random, 36 per cent (72/2) of the antihapten sites should have been present on molecules also having a site specific for ovalbumin. This probably represents a lower limit since some of the non-precipitating 5-*S* antiovalbumin may have had an active site; also, the precipitation reaction may not go to completion. The expected upper limit is 50 per cent. The results of the adsorptions, in which 33 and 39 per cent of the hapten binding capacity was removed from the supernatants, are thus in fairly good agreement with those predicted on the basis of random recombination. The results are not precise enough to permit the conclusion that the recombination is completely random. However, they are consistent with this possibility and, in any event, show that there is no pronounced bias toward recombination of sub-units of like specificity.

The fact that a large part of the binding capacity removed on adsorption was found on the adsorbent indicates that there is little steric inference with access of the hapten to the antihapten sites on adsorbed antibody molecules. However, there was no reason to believe, *a priori*, that adsorbed molecules would remain completely accessible or be unchanged with respect to their binding constants. Therefore, the values for the fraction of binding sites removed, used in the discussion above, were based on the binding capacity remaining in the supernatant, rather than on that acquired by the adsorbent.

Deutsch and Morton[16,17] have found that the action of reducing agents on 18-*S* human macroglobulins results in a decrease in sedimentation coefficient to about 6–7 *S* and that these fragments re-aggregate on removal of the reducing agent. (No enzymatic digestion is required in this case.) Also, Jacot-Guillarmod and Isliker[18] have recently obtained evidence for the presence of human macroglobulins of mixed anti-blood group A and anti-B specificity, following reduction and re-oxidation of a mixture of the two purified antibodies. The possibility is suggested that the linkage of sub-units of antibodies may represent a general mechanism for constructing molecules of higher valence. Hsiao and Putnam[19] have demonstrated that the γ-globulins of various species can be degraded to 3·5 *S* by the action of papain, suggesting that important structural features are common to the various γ-globulins.

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ROLE OF THIAMINE AND AMINO-ACIDS IN THE NUTRITION OF *SHIGELLA SONNEI*

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INVESTIGATIONS of the nutritional requirements of *Shigella* grown in chemically defined media have been carried out by Koser *et al.*[1–4], Kligler and Grosowitz[5] and others[6]. Since then chemically defined media for *Shigella* contained inorganic salts, glucose, nicotinic acid and various amino-acids. Erlandson and Mackey[7] studied a large number of amino-acids and inorganic nitrogen compounds for their activity on the growth of *Shigella flexneri* in a synthetic medium. They found that aspartic acid yielded maximal growth of *S. flexneri*. Mizuno and Kosaka[8] reported that glutamic acid was an essential amino-acid for *S. dysenteriae*. Johnson and Mays[9] reported on a strain of *S. dysenteriae* which required the amino-acid proline. In addition to nicotinic acid, pantothenic acid[6], uracil[10], and purines[11] have been reported as growth requirements for certain strains of *Shigella*. Kligler *et al.*[12] reported that thiamine and nicotinic acid allowed better growth of *Staphylococcus aureus* than nicotinic acid alone. The medium used by Erlandson and Mackey[7,13], which also supported the growth of *S. sonnei*, contained thiamine.

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The purpose of this work was to determine whether or not thiamine was an absolute requirement for *S. sonnei* and to examine the role of amino-acids in the nutrition of this organism. The reason we undertook this investigation was that we felt that it is important to determine the minimum requirements for the growth of this organism in a chemically defined medium in order that the medium may be more competently used for: (*a*) assay of chemotherapeutic agents; (*b*) studies on the nutritional requirements of the *Shigellae*; (*c*) the detection of mutants possessing special growth-factor requirements.

Nineteen strains of *S. sonnei* were included in this work. They were constantly checked morphologically, biochemically and serologically for purity. Cells used for inoculation of the experimental tubes were prepared by growing at 37° C. for 48 hr. on nutrient agar ('Difco') slants, washing 3–5 times in buffered saline, and re-suspending to the desired concentration in buffered saline. The cell suspensions were decimally diluted and used for the inocula; initial counts of the inocula were determined by pipetting 0·1 ml. of the diluted cell suspensions on to nutrient agar plates, spreading over the entire surface of the agar with a bent-glass 'hockety stick', incubating aerobically for 24 hr. at 37° C., and counting the bacterial populations on the Quebec darkfield colony counter. Growth of the bacteria in the test media was determined by pipetting 0·1 ml. of the cell suspensions into test tubes containing 9·9 ml. of media yielding a final volume of 10 m. The tubes were incubated at 37° C. for 24, 48, 72 and 96 hr., transferred to cuvettes, and read in the Coleman junior spectrophotometer with the wave-length set at 575 mμ. The second method of enumerating the bacterial populations in the test media consisted of removing 0·1 ml. from the growth tubes, plating on nutrient agar and counting the numbers of colonies that developed. The third method of determining growth of the organisms in the test media containing factors being assayed consisted of placing 0·1 ml. of the washed inocula on solid synthetic medium plates, spreading the cells, incubating and counting the colonies. It was found that all three of these methods must be used to ensure obtaining of competent data.

The chemically defined medium of Erlandson and Mackey[7,13] contained 0·067 *M* dipotassium phosphate, 0·067 *M* monopotassium phosphate, 1 per cent glucose, 0·05 per cent magnesium sulphate, 0·03 per cent thiamine hydrochloride, 0·85 per cent sodium chloride, 0·001 per cent nicotinic acid, and 0·25 per cent aspartic acid. The medium was prepared with distilled, de-ionized water, adjusted to *p*H 7·0 with concentrated sodium hydroxide or phosphoric acid, and autoclaved at 15-lb. pressure for 15 min. The glucose was sterilized by filtration and added aseptically to the autoclaved medium. Studies with solid synthetic media were performed with the synthetic medium to which 1·5 per cent 'Bacto-Agar' ('Difco') was added. The highest-grade chemicals commercially available were used throughout the work. Experiments were performed in the synthetic medium not containing thiamine, in the synthetic medium not containing aspartic acid, and in synthetic media containing various amino-acids tested singly and in combination.

Inocula of varying sizes were used for the experiments. It was found that a minimum of 10^3 cells were required to ensure growth in 100 per cent of the tubes of synthetic media. All experiments were performed in duplicate and repeated 2–3 times. The results presented are average values from all the experiments.

Eighteen of the 19 strains of *S. sonnei* grew well in the absence of thiamine, and could be sub-cultured indefinitely in synthetic media lacking this vitamin. It can be seen from Table 1 that growth, as measured in the spectrophotometer, was not materially affected by the presence or absence of thiamine nor did the concentration of thiamine alter the growth (at 24 and 48 hr.).

Table 1. EFFECT OF THE CONCENTRATION OF THIAMINE ON THE GROWTH OF *S. sonnei* in SYNTHETIC MEDIA*

Concentration of thiamine (μgm./ml.)	Optical density† 24 hr.	Optical density† 48 hr.
0	0·54	0·70
0·3	0·49	0·69
3	0·48	0·74
33	0·52	0·66
330	0·55	0·59
1,000	0·46	0·72
5,000	0·53	0·71
10,000	0·44	0·65

* Data were similar regardless of the size of the inocula.

† Optical density was measured spectrophotometrically, wave-length 575 mμ.

Table 2. EFFECT OF THE CONCENTRATION OF ASPARTIC ACID ON THE GROWTH OF *S. sonnei* IN SYNTHETIC MEDIA

Concentration of aspartic acid (μgm./ml.)	Optical density at 575 mμ at 48 hr. Size of inocula 1×10^2 cells/ml.	1×10^3 cells/ml	1×10^4 cells/ml.
0	0	0	0
155	0	0	0
310	0	0	0·005
620	0·01	0·05	0·345
1,000	0·13	0·49	0·66
2,500	0·32	0·60	0·69
5,000	0·51	0·62	0·59
10,000	0·57	0·61	0·62

Aspartic acid was present in the synthetic medium at a concentration of 2,500 μgm./ml.; however, concentrations of 1,000–10,000 μgm./ml. were tested. Table 2 shows the relationship between concentration of aspartic acid and growth. Clearly, there is a relationship between the two. Up to a point, increasing concentrations of aspartic acid yielded increased growth of *S. sonnei*. Furthermore, when the size of the inocula was small greater concentrations of aspartic acid were required to produce good growth. As the size of the inocula was increased less aspartic acid was required to produce growth. This illustrates the importance of using small inocula in studies dealing with growth-factor requirements of micro-organisms in synthetic media. *S. sonnei* cannot be maintained in cultures by sub-cultivation unless 620–1,000 gm./ml. aspartic acid is present.

Table 3. GROWTH OF *S. sonnei* IN SYNTHETIC MEDIA CONTAINING VARIOUS AMINO-ACIDS

Amino-acids present (1,000 gm./ml.)	Optical density 575 mμ, at 48 hr.
L-Aspartic acid	0·50
L-Asparagine	0·73
L-Glutamic acid	0·82
L-Glycine	0·06
L-Tryptophan	0·06
L-Arginine	0·01
L-Glutamine	0·01
Aspartic acid + glycine	0·49
Aspartic acid + tryptophan	0·51
Aspartic acid + arginine	0·61
Aspartic acid + glutamine	0·59
Aspartic acid + glycine, tryptophan, arginine and glutamine	0·60

The growth of *S. sonnei* in synthetic media containing other amino-acids is tabulated in Table 3. These results were obtained from cultures inoculated with inocula containing 1×10^3 cells/ml. It should be emphasized that since the volume of the inocula was 0·1 ml., the total number of cells inoculated into the tubes was approximately 1 × 10^3 cells. L-Asparagine, L-glutamine, L-glycine, L-tryptophan, L-arginine and L-glutamic acid were studied in addition to L-aspartic acid. L-Asparagine and L-glutamic acid produced better growth than L-aspartic acid when tested in the synthetic medium at comparable concentrations. Glycine, tryptophan, arginine, and glutamine yielded less growth than aspartic acid. This is in conflict with the data of Erlandson and Mackey[7], who reported that asparagine and glutamic acid produced poorer growth compared with aspartic acid when *S. flexneri* was examined. Growth was not materially increased when aspartic acid was combined with the less active amino-acids. We agree with the conclusions of Erlandson and Mackey[7] that combinations of amino-acids, which some workers have indicated as necessary for growth, may function in a stimulatory rather than an essential role in the nutritional requirements of *Shigellae*. We believe that this chemically defined medium is an excellent tool for the study of nutritional variants of *Shigellae*.

Oxythiamine hydrochloride, a chemical analogue of thiamine, did not inhibit *S. sonnei*. Cocarboxylase, one of the probable metabolites of thiamine synthesis, did not materially affect growth of this organism.

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ETHYLENE PRODUCTION BY SUB-CELLULAR PARTICLES FROM TOMATOES

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ETHYLENE has been identified as the main olefinic volatile produced by fruits during ripening[1,2]. However, controversy has developed regarding attempts by many laboratories to prepare cell-free systems capable of ethylene evolution. Early work in this field suffered from lack of a sensitive, specific method for ethylene assay, and also from the possibility of contamination of the tissue with ethylene-producing micro-organisms[3]. More recently[4] this laboratory reported the production of ethylene by mitochondria from tomatoes. Meigh *et al.*[5] were not able to obtain ethylene from sub-cellular fractions from either tomatoes or apples, but they used methods that differed from ours. Lieberman and Craft[6] detected extremely small amounts of ethylene when thiomalic or thioglycollic acid was added to particles (from apples and tomatoes) prepared in an acidic medium. Burg and Burg[7], using gas chromatography for identification, claimed that the volatile substance produced by the 'acidic' particles of Liberman and Craft was not, in fact, ethylene.

We have now been able to detect the sites of ethylene-evolving systems and to isolate sub-cellular fractions which are quite active in ethylene production. Moreover, we have been able to show that its production is associated with disintegration of sub-cellular particles. When these are aged *in vitro*, or sonically treated, the ethylene-producing system is activated. This discovery offers an explanation for the discrepancies in results from various laboratories. It also poses rather interesting questions with respect to the production and the role of ethylene *in vitro*.

A critical point in obtaining active fractions lies in the method of washing the particles and suspending them in the buffer-substrate mixture. In both these steps, in our earlier work[4], according to common practice, we used a Servall Omnimixer at very low speeds (25–30 V. on the powerstat) for 1–2 min. in the cold. We noted that this procedure produced some unavoidable fragmentation of particles. Omission of Omnimixer washing of the particles and use of the instrument only to suspend them in the final reaction mixture gave particulate fractions more active in ethylene production. (Very gentle washing of the particles with the help of a glass rod had no measurable effect on ethylene production.) In the washings by use of the Omnimixer many of the particle fragments were inevitably discarded, and later work showed that these fragments were primarily responsible for the initial ethylene production. It must also be borne in mind that even the most careful use of an Omnimixer or equivalent instrument causes a certain amount of frothing and, possibly, protein denaturation.

In the present investigation the procedures for isolation of different cellular fractions were based on the one we reported previously[4]. In all experiments we used frozen tomato fruit ('*V*121') in the advanced turning stage, since ethylene evolution has been shown to be at a maximum at this time[8]. The initial grinding of the tissue was done in 0·5 *M* sucrose–0·5 *M* potassium dihydrogen phosphate buffer of *p*H 8·0, and this resulted in a homogenate of *p*H 7. Cell fragments, chloroplasts and nuclei were obtained by centrifugation at 4,000*g* for 7 min. of the homogenate from 900 gm. of tomatoes. As a routine procedure, mitochondria were sedimented at 35,000*g* for 15 min. We found (Table 1) that a centrifugal force of 15,700*g* was sufficient to separate this fraction, but 35,000*g* gave additional active particles packed into easily handled pellets. The obvious advantage of isolation of the fraction at 35,000*g* for 15 min. as a

Table 1. COMPARISON OF ETHYLENE PRODUCTION BY SUB-CELLULAR FRACTIONS FROM TOMATOES, SEDIMENTED AT DIFFERENT CENTRIFUGAL FORCES*

(μl. $\times 10^3$/mgm. nitrogen)

Physical state	Time (hr.)	15,710*g* (15 min.)	22,620*g* (15 min.)	35,000*g* (15 min.)	40,210*g* (15 min.)	105,000*g* (10 min.)
		Average centrifugal forces				
Intact	0–4	0	0	—	21·9	—
	4–16	Trace	28·3	Trace	31·4	—
Sonically treated	0–4	47·5	29·6	64·9	71·3	57·4
	4–16	11·2	16·0	48·8	73·8	160·4

Reaction mixture: 0·5 *M* sucrose, 0·125 *M* KH_2PO_4, 10^{-3} *M* $MgSO_4$, 10^{-3} *M* $MnSO_4$, 0·05 *M* L-malic acid, adjusted to *p*H 7·0 with sodium hydroxide. This mixture was sterilized prior to use; $1·98 \times 10^{-3}$ *M* adenosine triphosphate and $3·3 \times 10^{-4}$ *M* diphosphopyridine nucleotide were added after sterilization.

* Cell fragments, nuclei and chloroplasts were removed by a preliminary centrifugation at 4,000*g* for 7 min.

routine preparative procedure was higher ethylene production. The amount of ethylene produced by the sonically treated 35,000*g* fraction during the first 4 hr. (0·174 µl./mgm. nitrogen, Table 6) is comparable with the sum total of ethylene produced by the sonically treated 15,710*g*, 22,620*g* and 35,000*g* fractions (0·142 µl./mgm. nitrogen, Table 1). Another fraction, separated at 40,210*g* for 15 min., undoubtedly consisted of a mixture of fragmented mitochondria and microsomes (fragments of endoplasmic reticulum). (When a 'combined mitochondria-microsomes' fraction was used, it was obtained by direct centrifugation, at 40,210*g* for 45 min., of the supernatant from the separation performed at 4,000*g*). The remainder of the 'microsomes' was sedimented at 105,000*g* for 10 min. The soluble fraction was the supernatant liquid remaining after this centrifugation.

The different cellular fractions (unwashed) were suspended in 25 ml. of buffer-substrate mixture of the composition shown in Table 1. For preparation of 'intact' and 'sonically treated' fractions, the particles were suspended directly in the reaction mixture with the help of a glass rod followed by a magnetic stirrer operated at low speed. Sonically treated particles were subjected to 4 min. in a 250 W., 10 kc./s. Raytheon sonic oscillator turned to 1·1 amp., at 2° C. 'Partially disintegrated' fractions were prepared by suspension of the particulate fractions in the buffer-substrate mixture by the use of a Servall Omnimixer run at a very low speed, at 2° C. These fractions were similar to those reported in our earlier work[4].

Ethylene collection was made from at least 20 ml. of the particle–buffer-substrate suspension maintained at 25° C.; the remainder was used for manometric measurement[9] of carbon dioxide production. Ethylene was accumulated for different time-intervals at 0° C. in mercuric perchlorate (0·25 *M*) = perchloric acid (2 *M* solution). The micro-apparatus for quantitative ethylene absorption was essentially the same as that described previously[10], with precautions being taken to maintain an air flow-rate of 25 ml./min. and a 10–13 cm. column of finely foaming mercuric perchlorate solution. With this collection system it was possible to recover 100 per cent of microlitre quantities (0·01–1·0 µl.) of ethylene.

The ethylene collected in the mercuric perchlorate was quantitatively determined by a gas chromatographic procedure[11]. In the gas chromatographic unit balanced columns of squalane-impregnated firebrick and a hydrogen flame ionization detector[12] were used. We obtained the greatest sensitivity compatible with a working stability by use of a mixture of nitrogen and hydrogen each at a flow-rate of 15 ml. per 2·6 sec. With this flow-rate of carrier gases it was also possible to effect very good separation of ethylene from other low-molecular-weight hydrocarbons and from carbon dioxide and air. Ethylene was quantitatively liberated from the mercuric perchlorate collection solution by addition of one-quarter volume of 4 *M* lithium chloride. The gas was liberated inside serum bottles of known volume, and representative samples were drawn into a gas-tight syringe and injected into the column for analysis. With this method we obtain 100 per cent recoveries of ethylene in concentrations of 0·01–1·0 µl./ml. of gas sample. Values less than 0·01 µl./ml. are reported as 'traces'. Standards for gas chromatography were prepared by serial dilutions of stock solutions of ethylene mercuric perchlorate which had been standardized manometrically[3,10]. Standards were found to be stable for one week at 5° C. and unknowns were analysed within one week of their collection. Nitrogen in the particulate suspension was estimated by the micro-Kjeldahl method[13].

In our earlier work[4] we used a micromanometric method[10] for analysis of ethylene. However, with the amounts of ethylene obtained from cell-free systems, we were working at the limits of accuracy of this procedure, and it was obvious that for quantitative comparisons we would have to turn to a more sensitive procedure such as flame ionization detection. The manometric analysis, which is specific for olefinic hydrocarbons[3], and mass spectrographic analysis have been used in the present experiments to provide qualitative corroborating evidence that we are, in fact, dealing with ethylene. Moreover, negative results for ethylene in mercuric perchlorate solutions, aerated at the rate of 25 ml./min. for 4–16 hr. in our collection assembly, eliminated all possibility of contamination. In none of our gas chromatographic and mass spectrographic analyses have we found evidence for the presence of any olefinic volatile besides ethylene. It should be noted that figures for ethylene reported in this article have been obtained from gas chromatographic analysis of collections made in mercuric perchlorate solution, and liberated by addition of lithium chloride, a procedure specific for olefinic hydrocarbons. Burg and Burg[7], on the other hand, injected the complete mixture of gases evolved by the particles directly into the gas chromatograph. Moreover, they found that the 'ethylene-like' substance of Lieberman and Craft was not absorbed by mercuric perchlorate, although authentic ethylene was. (Neither Meigh *et al.*[5] nor Lieberman and Craft[6] have stated either the method or time they used for ethylene collection.) It is therefore evident that in our analysis we are dealing with a true olefinic hydrocarbon, and not with the "ethylene-like substance" noted by Burg and Burg[7]. Moreover, these authors did detect minute amounts of true ethylene from their acidic particles, although they were prepared under very different conditions from those we had reported. As further indication that we were in fact dealing with ethylene, mass spectrographic analysis showed that the gas collected by our technique exhibited peaks characteristic of ethylene. Further confirmation was obtained by co-chromatography with known ethylene and disappearance of chromatographic peaks when the gas was passed through columns of brominated activated carbon. Huelin and Kennett[14] showed that normal olefines from propylene to hexene were not present in proportions exceeding one part of higher olefine to 1,000 parts of ethylene in apple volatiles collected in mercuric perchlorate. They indicated that branched chain or multi-unsaturated hydrocarbons would not be regenerated with ethylene from the mercuric perchlorate solution on addition of lithium chloride, and they could find no evidence of saturated hydrocarbons.

Experiments on the effect of the physical state of the particles on ethylene evolution were conducted, and results are summarized in Tables 2–6. It will be seen (Table 2) that intact mitochondria do not produce detectable amounts of ethylene during the first 4 hr., but as the reaction time progresses, they presumably disintegrate and ethylene evolution occurs. The partially disintegrated mitochondria (which were prepared similarly to those reported in our earlier work) produce much of the ethylene during the first 4 hr., as do the sonically treated particles. Markedly higher ethylene production is obtained with sonically treated particles than with the partially disintegrated or with the intact particles. It is interesting that, in all cases, ethylene production is continued over extended periods of time, at a slow rate.

Table 2. ETHYLENE PRODUCTION BY MITOCHONDRIA ($\mu l. \times 10^3$/mgm. nitrogen)

Physical state	Time (hr.)	Run No. 1	2	3	4	5	6	7
Intact	0–4	0	0	0	0	0	—	—
	4–8	6 3	14 7	—	—	—	—	—
	8–16	21·1	17 5	5 7	—	13 0	—	—
Partially disintegrated	0–4	—	—	—	—	23 8	29 0	16 6
	4–8	—	—	—	—	Trace	—	—
	8–16	—	—	—	—	3 0	29 0	29 0
Sonically treated	0–4	—	235	—	113	—	—	—
	4–8	—	17·7	—	—	—	—	—
	8–16	—	13 3	—	7 9	—	—	—

Reaction mixture as in Table 1.

The fraction isolated at 40,210*g* for 15 min., when untreated, showed maximum ethylene production during the first 4 hr. (Table 3). Again, partial disintegration or sonic treatment resulted in considerably greater production of ethylene. As with the mitochondria, activity is retained for some time.

Table 3 ETHYLENE PRODUCTION BY MICROSOMES AND FRAGMENTED MITOCHONDRIA ($\mu l. \times 10^3$/mgm. nitrogen)

Physical state	Time (hr.)	Run No. 1	2	3	4	5	6	7
Intact	0–4	56·4	28 0	21·4	0	25·0	—	—
	4–8	13 8	1 0	↓	↓		—	—
	8–16	13 8	3 8	21·4	28 0	2·5	—	—
Partially disintegrated	0–4	—	—	—	—	86·7	80 7	90·0
	4–8	—	—	—	—	Trace	↓	↓
	8–16	—	—	—	—	8·6	80 7	45 0
Sonically treated	0–4	—	87·6	—	93 6	—	—	—
	4–8	—	↓	—	↓	—	—	—
	8–16	—	18·1	—	20·0	—	—	—

Reaction mixture as in Table 1.

The purpose behind isolation of a combined mitochondrial : microsomal fraction was to prepare a cell-free system capable of still higher ethylene production. From the results in Table 4, it will be seen that although this preparation is active, the rate of ethylene production on a nitrogen basis is lower than that of either the mitochondrial or the microsomal fraction. (Sonic treatment does appear to activate the system.) While many possible explanations for the inhibition on the basis of possible uncoupling of oxidative phosphorylation[15,16] or the presence of natural inhibitors, etc., can be advanced, further experiments under a variety of experimental conditions need to be conducted before valid conclusions can be reached. It is conceivable that, rather than a simple inhibition of the ethylene-producing system, the combination of the two particles may provide an alternative, preferred pathway for utilization of intermediates in the ethylene-producing system, or even of ethylene itself.

Table 4. ETHYLENE PRODUCTION BY COMBINED MITOCHONDRIA-MICROSOMES ($\mu l. \times 10^3$/mgm. nitrogen)

Physical state	Time (hr.)	Run No. 8	9	10	11
Intact	0–4	4 5	22 5	11 4	—
	4–16	8 0	0	—	—
Partially disintegrated	0–4	—	—	—	11 2
	4–16	—	—	—	22 6
Sonically treated	0–4	23·7	36·0	52 0	78·4
	4–16	9 0	0	—	7 1

Reaction mixture as in Table 1.

The ethylene-producing activity (trace amounts to 0·01 µl./16 hr.) of the supernatant liquid was found to depend on the stage of ripeness of the tomatoes and on the method of preparation of the particulate fractions. For example, more ethylene was found when 0·25 *M* sucrose was used in the buffer in which the tomatoes were originally ground than when 0·5 *M* sucrose was used. The higher molarity of sucrose is known to be beneficial for preservation of the integrity of mitochondria[17], and ethylene production by the supernatant fraction from preparations where 0·25 *M* sucrose was used was probably due to disrupted mitochondria.

The cell fragments–chloroplasts–nuclei fraction (Table 5) produced ethylene in small amounts, with stimulation of production by either partial disintegration or sonic treatment. Thus, under the experimental conditions used, all the sub-cellular fractions have shown ethylene-producing activity, and maximum production was obtained with sonically treated mitochondria.

Table 5. ETHYLENE PRODUCTION BY CELL FRAGMENTS–CHLOROPLASTS–NUCLEI FRACTION ($\mu l. \times 10^3$/mgm. nitrogen)

Physical state	Time (hr.)	Run No. 8	9	10
Intact	0–4	3 3	—	—
	4–16	Traces	—	—
Partially disintegrated	0–4	—	—	28 1
	4–16	—	—	4 6
Sonically treated	0–4	67·8	12 3	—
	4–16	7·1	0	—

Reaction mixture as in Table 1.

All fractions except the cell fragments–nuclei–chloroplasts produced carbon dioxide (43–58 µl./mgm. nitrogen/0·5 hr.). Our preparations of intact mitochondria showed the typical[17] lag period of 10–15 min. before carbon dioxide production began, but after that continued to produce it at a normal rate during the entire 4-hr. collection period.

In the present series of experiments, sonic treatment has been done on an empirical basis. It is obvious that the efficiency of sonic treatment would depend on such factors as concentration and size of the particles in the medium, viscosity, time and temperature. Another factor to be considered in drawing comparisons is reproducibility. In all our experiments we used the same amount of fruit and followed the outlined procedure closely. However, many steps in the procedure (for example, crushing and pressing the frozen tomatoes to prepare the homogenate) cannot be conducted strictly quantitatively. While nitrogen determinations themselves were reproducible, considerable variation in nitrogen content of the fractions from various runs occurred. Comparisons are thus more valid when

Table 6. ETHYLENE PRODUCTION BY DIFFERENT SUB-CELLULAR FRACTIONS

(μl. × 10^3/mgm. nitrogen, average values)

Physical state	Time (hr.)	Mito-chondria	Micro-somes-frag-mented mito-chondria	Mito-chondria-micro-somes	Cell frag-ments: chloro-plasts nuclei
Intact	0–4	0	32·7	12·8	3·3
	4–8	10·5	7·4	↓	↓
	8–16	14·3	13·9	4·0	Traces
Partially Disintegrated	0–4	23·1	85·8	11·2	28·1
	4–8	Trace	Trace	↓	↓
	8–16	20·3	44·4	22·6	4·6
Sonically treated	0–4	174	90·6	47·5	4·0
	4–8	17·7	↓	↓	↓
	8–16	10·6	19·0	5·4	3·5

Reaction mixture as in Table 1.

made on different fractions from one run, rather than on the same fractions from different runs.

If the results are examined in the light of these remarks, it will be seen that sonic treatment has always activated the system as compared with the intact fractions. The same conclusions emerge if one compares (Table 6) average values of ethylene production from different fractions. It is clear that sonically treated mitochondria produced the greatest amount of ethylene. From the works of Zeigler[17] and others it is known that the biochemical properties of mitochondria vary greatly with their state of morphological and structural integrity. In our preparative procedure when the fruit is initially disintegrated by a Waring blender or similar device, many sub-cellular particles may be fragmented, just as they are in any further mechanical washing and dispersing procedure. As has been pointed out, the fraction isolated at 40,210*g* has to be looked on as containing partially disintegrated sub-cellular particles enriched with fragments of endoplasmic reticulum. The production of ethylene in relatively large amounts by this fraction could be due to the presence of fragmented mitochondria. The fact that the maximum ethylene production occurs during the first 4 hr., and then tapers off, is characteristic of fragmented particles. If this is the case, it could be concluded that the ethylene-producing system is located in the mitochondria.

It is also clear that the ethylene-producing system is activated by disintegration of the mitochondria. This conclusion is of interest in relation to the climacteric rise in ethylene production in fruits, for it has been reported[18] that mitochondria disintegrate as fruits ripen.

From the foregoing discussion, it is clear that the discrepancies in results from different laboratories may well arise from the use of different techniques for preparation of cell fractions and for collection of ethylene.

Preliminary experiments with sonically treated mitochondria have shown that the ethylene-producing system is heat-labile (when subjected to a temperature of 80° C. for 7 min.) and that the major portion of the ethylene is produced within the first hour, after which the rate of production falls off to trace amounts, difficult to analyse quantitatively even at 1-hr. time intervals. With knowledge of the site of ethylene production, and a technique for stimulation of the system involved, progress in solving the problems of the modes of production and action of this gas in biological systems should now be more rapid.

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A TECHNIQUE FOR INVESTIGATING LOCALIZED MICROBIAL DEVELOPMENT IN SOILS

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AN established method for the study of the interrelationships of micro-organisms in soil is the well-known buried-slide technique of Cholodny. Modifications of this technique, using cellulose or deplasticized 'Cellophane', have been devised[1,2] for studying the decomposition of organic matter in microhabitats. In these methods the substrate is, of necessity, an insoluble one capable of removal from the soil, when desired, for isolation, or microscopic examination, of the enriched microflora. No comparable technique is available for soluble substances. Recently, Lehr, Brown and Brown[3] buried pellets of compressed superphosphate in soil, in order to follow the chemical changes which occur during the conversion of superphosphate to insoluble forms. In the technique described here we have adapted the above method for microbiological purposes. Soluble substances (organic and inorganic) are mixed with an inert base and formed into pellets which can be buried in soil. The soluble material is finely ground and mixed in the required proportion (usually 1:1) with the base. Kaolinite has proved to be the most suitable base since it has good binding properties and is inert. The pellets are made in a hardened steel mould similar to that described and illustrated by Ford and Wilkinson[4], but without the outer case,

Table 1. BACTERIA DEVELOPING ON KAOLINITE AND KAOLINITE/GLUCOSE PELLETS BURIED IN A RICH GARDEN SOIL

Incubation (days)	Bacteria (10^6/pellet) Kaolinite	Kaolinite/glucose
0	0·0012	1·14
2	0·080	6·70
4	0·320	14·60
6–7	0·520	20 92
13–15	0·680	15·6
21	0·620	12 52

since evacuation was unnecessary. Further, the base of the mould was recessed to allow the pellets to be pushed out more easily after they had been formed. In the mould 0·2 gm. of the mixture is subjected to a load of 5,000 lb. (in a press). Under these conditions all pellets of a given material are of uniform size. Our mould gives pellets with a diameter of ⅜ in. (9·52 mm.) The thickness of the pellets depends on the nature of the material being used, for example, for 0·2 gm. kaolinite they are 1·35 mm.

In our experiments the pellets are buried in 50 gm. of soil (passing a 3-mm. sieve). Half this soil is placed in a plastic flower pot (2 in. diam.), a pellet is added and then the remainder of the soil. Sterile water is then added carefully to adjust the moisture content of the soil to 26–28 per cent of the dry weight. The moisture content is maintained at this value by check weighing daily and by adding the necessary amount of sterile water. Two series of pots are set up, one containing kaolinite pellets and the other kaolinite/substrate pellets. The pots are incubated under a plastic cover, to minimize evaporation, in a constant temperature room at 25°. At required intervals a pot from each set is removed and the contents are tipped into separate sterile Petri dishes The pellets are removed aseptically with a forceps and placed in known volumes of sterile water in conical flasks. On shaking the flasks in a Microid shaker for 5 min. the pellets break up and dilutions are made from the contents of these flasks in the usual way for plate counts. Table 1 shows the bacterial counts from kaolinite and kaolinite/glucose (1:1) pellets buried over a period of 21 days. The rapid development of organisms on the glucose-containing pellet is clearly seen.

Apart from examining the development of the enriched microflora on the pellets it is also possible to demonstrate the formation of certain metabolic products by the flora. For this purpose the whole contents of the pots are extracted with water at room temperature and the extract evaporated down to a small volume for application to paper chromatograms. Using methods described by Duff and Webley[5] 2-ketogluconic acid has been detected in a rich garden soil during June–October (and also in February) in which kaolinite/glucose pellets had been incubated for 1–4 days. No 2-ketogluconic acid was found in the soil containing the kaolinite pellets. Estimation of 2-ketogluconic acid in the extract was carried out by the method of Lanning and Cohen[6] which uses *o*-phenylenediamine to form the quinoxaline derivative of α-keto acids. In two separate experiments about 1·5 and 3·5 mgm. of the acid was formed per pot after 2 days incubation. Only 0 5 and 2 5 mgm. of the acid remained after 3 days incubation and the glucose substrate was also much diminished or absent at that time. The keto acid estimation is subject to interference by, for example, sugars ; also other keto acids (for example pyruvic) would be estimated together with 2-ketogluconic acid so that the results above represent only rough approximations. There is no doubt, however, that 2-ketogluconate is actually present in the soil since it could be demonstrated on a paper chromatogram of the extract, after concentration at low temperature. No 2-ketogluconic acid has been similarly detected in agricultural soils so far investigated

In addition to soluble organic and inorganic substances, we have also prepared pellets containing finely powdered insoluble organic and inorganic materials, for example, cellulose, chitin, humic acids, calcium phosphates, silicates, etc. It is suggested that the technique may be of value in investigating not only the localized development of micro-organisms in soil microhabitats but also in some cases their physiological activity in these sites.

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AROUSAL, CHANNEL-CAPACITY AND DECISION

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IT is now widely recognized that with increase of arousal or activation many kinds of performance improve up to a maximum but thereafter decline[1]. The improvement is held to be due to an increase of neural activity in the brain which brings the cells of the cortex or some part of it to a state in which they are more readily fired by an incoming signal. Several explanations have been attempted of the fall in performance at high arousal-levels, but none appears to have been much more than an *ad hoc* speculation. Probably the most widely held is the view that, at high levels of arousal, inhibitor centres are activated. The purpose of this article is to indicate what appears to be the simplest possible model which will account for both trends. and to outline a few of its implications.

Let us consider the effects of progressively increasing the level of activity. At very low levels the system will be relatively inert so that all except the strongest incoming signals fail to secure a response. A moderate increase will improve sensitivity and responsiveness, and thus raise the effective strength of incoming signals. At still higher levels, however. cells will tend not only to be rendered sensitive but also actually to be fired. When this happens the system will become noisy, and its channel capacity will be reduced. Once this point has been reached further increases of activity will thus tend to impair performance rather than enhance it. The essential feature of the model proposed here is that it accounts for both the rise and the subsequent fall of performance accompanying progressive increase of activation, without postulating more than one type of process: both follow straightforwardly from the increase of activity in the system.

The great majority of evidence so far gathered does not favour one theory rather than another; but the present model is not, so far as I am aware, contradicted and in at least four cases it either accounts for facts not otherwise covered satisfactorily or provides a more precise lead for further research:

(*a*) If with Lindsley[2] we regard fear and anger as arousing in this sense, it is easy to see why there has been a controversy about whether their effects are 'organizing' or 'disorganizing'. Mild degrees of these emotions could bring the system to an optimum state while stronger ones would impair its ability to deal with tasks requiring a high capacity, and lead perhaps, at very high levels, to a kind of tense passivity and inability to differentiate between alternative, often antagonistic, reactions: the organism would suffer what Darrow termed "relative functional decortication"[3]. If high levels of emotion caused inhibition, performance might become ineffective but not disorganized.

(*b*) The optimum arousal-level would vary with the level of task: for difficult tasks requiring high capacity the optimum level would be relatively low, while for less demanding tasks it would be higher. If incentives can be regarded as activating, as they almost certainly can be, the model thus gives an elegant explanation of the so-called Yerkes–Dodson effect that increase of incentive improves performance up to a point but thereafter disrupts it, and that the point of inflexion becomes lower as the difficulty of the task is raised[4]. There seems to be no reason why a simple increase of inhibition should have effects related in this way to difficulty of task.

(*c*) The model could be tested by examining the details of performance at high activation-levels. If impairment under such conditions was due merely to inhibition, the organism should tend to become inert and performance suffer accordingly. If, on the other hand, the theory outlined here is correct, impairment would result from action being unduly vigorous, impetuous and ill-controlled. Experiments have not hitherto obtained sufficiently detailed results to provide a test. One possible exception is an experiment by Freeman[5] in which times taken to discriminate the stronger of pairs of electric shocks were measured under three conditions of muscular tension. Comparing normal with relaxed conditions the speed and accuracy of discrimination rose, the extent of finger movement in reaction to the shocks increased and its latency shortened. With tension induced in the trunk muscles finger movement increased slightly and latencies shortened still further, but errors of discrimination rose. In short, the increased tension appears to have made performance faster, but at the expense of accuracy. If the errors had been the result of inhibition, latencies would have been expected to rise and finger movement to lessen.

(*d*) Gross increases of arousal from a low level obviously lower sensory thresholds, but the effects on thresholds of smaller changes in activation are conflicting[6]. A fall of thresholds seems likely to result from the facilitatory effects of increased ambient neural activity so long as these are not offset by an increase of neural noise. Various possible effects are made much clearer if activation is considered in relation to the statistical decision model of discrimination proposed by Swets, Tanner and Birdsall[7]. According to this, neural noise can be conceived as varying randomly in intensity at different instants of time so that discrimination is between two distributions of activity in the system, one of signal-plus-noise and one of noise alone. For convenience, the authors cited treat the distributions as normal, although the argument does not depend on their being in any particular form. The subject fixes a cut-off point and treats any level of activity in the system above this as 'signal' and below it as 'noise'. Errors may arise if the distributions overlap, and the cut-off point, can, indeed, be inferred from the relative proportions of two types of error, namely 'omissions', that is, failures to report signals when present, and 'false positives', that is, reports of signals when none in fact is there.

Three possible results of increased activation appear to need consideration in terms of this approach:

(1) A true improvement of discrimination will occur only if there is an increase in the distance, measured in standard-deviation units, between the peaks of the two distributions—in other words, if facilitatory effects raise the signal strength more than the noise-level and thus enhance signal-to-noise ratio. If noise rose more than signal strength, as might happen at very high activation-levels, the distributions would be widened and their peaks moved to higher activation-levels; but both would be moved by the same amount so that the distance between them in standard-deviation units would be reduced and discrimination would fall.

(2) Apparent changes of sensory threshold might nevertheless result either from an increase of noise alone or from a proportionate rise of both signal and noise together. In both cases the distributions would be shifted to higher levels and thus, if the cut-off point remained the same, a higher proportion of signals would be detected. There would, however, also be a rise in the number of false positives—moderate with an increase of both signal and noise together, severe with an increase of noise alone. The same type of result would, of course, be produced if the cut-off point moved to a lower level. This might well occur as an additional effect of increased activation since the cut-off point must represent the threshold of some other mechanism in the brain and this would almost certainly tend to be affected by any general increase of ambient brain activity.

(3) If, however, the subject tried to reduce false positives by moving the cut-off point to a higher level, the proportion of omissions and thus the apparent sensory threshold might either rise or remain unchanged.

It is not easy to distinguish between these various alternatives, but it seems logically possible to do so if psychophysical measurements are made in such a way that omissions and false positives can be counted separately and the results compared with an independent, preferably physiological, measure of activation.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Nature of the Peculiar Galaxy *NGC* 5128

NGC 5128 is a peculiar galaxy which is of great interest, especially because it is at the centre of a very strong and exceedingly large source of radio emission known as Centaurus *A*[1]. The galaxy has been illustrated in the plate published by Baade and Minkowski[2] and also in the *Hubble Atlas*[3]. It consists of a very bright elliptical galaxy classified *EO* with a very wide dust lane crossing it and roughly dividing the bright area of luminosity in half. Baade and Minkowski interpreted the structure as being due to a collision between an elliptical galaxy and a smaller spiral system. At that time the only spectra available were those of Hubble[4] and Humason[2]. Neither gave sufficient information from which the true nature of the object could be deduced from the motions of the gas and stars in it, and the collision hypothesis based on interpretation of the direct plates alone has been a subject of controversy.

In 1958 we carried out a spectroscopic investigation of *NGC* 5128 with the nebular spectrograph at the prime focus of the 82-in. telescope at the McDonald Observatory of the University of Texas[5]. Since the grating that was used at that time is blazed in the region of λ 6300, the features which it records most efficiently are Hα and [NII] λ 6583, the emission lines in the red which commonly appear in spiral galaxies. Since *NGC* 5128 is at a declination of – 43°, it can only be observed for 2–3 hr. at McDonald Observatory. With these environmental limitations the results which were achieved were as follows. Spectra taken within the dark lane and also along its edges showed the emission lines Hα and [NII] λ 6583. Those emission lines in spectra with the slit parallel to the direction of the dark lane showed a tilt which is interpreted as rotation of the gas and dust in the sense that the south-east side is approaching and the north-west side is receding. Superposed on this rotation curve are velocity fluctuations of the order of 50 km./sec. which are attributable to random motions in the gas. The slope of the rotation curve over the central region of the dust lane is of the order of 3 km./sec./second of arc. The distance of *NGC* 5128 is uncertain. In the paper just mentioned we took a distance of 5 megaparsec. The value given by Sersic is 4 megaparsec. With a distance of this order, the mass obtained from the rotation measured in the emission features out to a distance ~ 5 kiloparsec. from the centre, on the assumption that rotation alone is present, is of the order of $1\text{–}2 \times 10^{11} M_{\odot}$.

These results still left in doubt the question of whether this object is two previously separate systems in collision, since we were not able to obtain measures of velocity from absorption lines which come from the stars. More recently, Evans and Harding[6] have obtained some velocity measures from absorption features (they assign greatest weight to Ca II λλ 3934, 3968) with the 74-in. telescope at the Radcliffe Observatory and they find, in central regions lying approximately along the rotation axis derived by us, radial velocities uncorrected for galactic rotation lying in the range + 350 to + 550 km./sec. with a mean value of + 434 km./sec. This is to be compared with our value of + 605 km./sec. from the emission lines. Evans and Harding were doubtful whether the difference between these values was significant, in view of the uncertainties involved.

We have now made further observations with a new grating blazed at λ 4000 in the same nebular spectrograph at McDonald Observatory. In June 1961 spectra were obtained with the slit oriented in position angle 120° immediately to the north-east and south-west of the dark absorbing lane and parallel to it. A third spectrum was obtained in position angle 30° approximately in the direction of the rotation axis determined in the first investigation. The absorption features which could be measured over a total extent of about 2′ parallel to the dust lane were Ca II λ 3968, Hδ, and Fe I λ 4383. In addition, in one of the spectra parallel to the dust lane the emission feature [OII] λ 3727 was measured. Results obtained from these features can be expected to give lower precision than results obtained from Hα and [NII] λ 6583. This is because (*a*) there is more contamination from night sky features; (*b*) the dispersion is lower (~ 390 Å./mm.); (*c*) there may be some effect due to residual twilight in the short June nights; (*d*) it is notoriously difficult to measure the tilt of an absorption line.

Bearing these difficulties in mind we can summarize our results as follows:

(1) The mean radial velocity obtained from the absorption features over the central region is about + 470 km./sec. with an estimated error of ± 50 km./sec.

(2) The mean radial velocity obtained from [OII] λ 3727 which was measured on one spectrum alone is + 630 km./sec.

(3) There is evidence from the absorption lines for rotation in the same sense and the same direction as that obtained from the emission lines in the dust lane. However, it appears to be considerably smaller, the slope in the central region only amounting to about 1 km./sec./second of arc.

Our results confirm the velocities measured by Evans and Harding. In addition, the recession velocity obtained from the emission feature [OII] λ 3727 is in good agreement with that obtained from Hα and [NII] λ 6583. If we correct for galactic rotation, the recession velocities are + 400 km./sec. (Hα, [NII] λ 6583, and [OII] λ 3727) and + 265 km./sec. (CaII λ 3968, Hδ, and FeI λ 4383). Thus there appears to be a significant difference such that the gas and dust are moving inwards with respect to the stars with a velocity of the order of 100 km./sec.

Besides the absorption features which have been measured over an extended region, the *G* band, CaI λ 4227, and a blend of Hγ and FeI λ 4325 were also measured in the strongest part of the spectrum. The appearance of all these absorption features confirms spectroscopically that the galaxy has the stellar population normally expected in a giant elliptical.

The comparatively small difference in velocity between the gas and the stars together with the result that the rotation of both components is in the same sense leads us to the conclusion that this is not a collision between a spiral and an elliptical galaxy. This conclusion was also drawn by Evans and Harding. The simplest model which would explain the observations is that of an elliptical galaxy surrounded by a broad band of dust and gas, which is rotating and also falling inward. Motion of the gas inward can possibly be accounted for if it was first ejected from the galaxy and is now falling back. The apparent rotation is not easy to understand. A nearly spherical system is not expected to have any appreciable amount of angular momentum, so that the observational evidence that in the stellar component any rotation at all is detectable is surprising. If the gas were ejected from the central system the conservation of angular momentum would give a much slower rotation velocity for the gas than for the stars farther in.

We may speculate that the peculiar motion of the gas in this galaxy is a direct consequence of the violent event which may have led to its becoming a powerful radio source[7].

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GEOPHYSICS

Altered Lavas from the Floor of the Eastern North Atlantic

RITTMANN and others have long argued that basic lavas erupted on the ocean floor under great depths of water may suffer immediate alteration and solidify as rocks of the serpentine–spilite association, and recent experimental work by Clark and Fyfe[1] has directed attention to this possibility. The altered basalts, the physical properties and bulk chemistry of which were outlined in a previous communication[2], were almost certainly extruded under deep water and may provide an example of mild alteration under these conditions. The specimens were dredged from a small abyssal hill, named Swallow Bank, standing 4–5 km. above the Mohorovičić discontinuity under 4,960 m. of water at 41° 21′ N., 14° 28′ W. These specimens, and a second collection from a nearby small seamount (the Western Seamount, summit depth 2,600 m.), have been examined petrographically. In thin section the altered lavas are 50 per cent opaque; none retains unaltered groundmass material, and only three specimens out of four hundred preserve unaltered phenocrysts or glass. The present communication outlines the mineralogy of the alteration process which is believed to have affected them.

At Swallow Bank, the lavas first solidified as ordinary basalts. They were vesicular and hypocrystalline, they contained phenocrysts of augite and bytownite but retained little or no early formed olivine, and their textures were those indicative of rapid cooling and partial devitrification. Mineralogically similar lavas were erupted at the Western Seamounts but were accompanied there by pumice containing microphenocrysts of oligoclase. The Swallow Bank lavas probably belong to a tholeiitic series, but those from the Western Seamount could be alkaline. The lavas exposed on major seamounts or oceanic islands could, conceivably, have originated by melting in the oceanic crust; but consideration of the thermal gradients involved shows almost conclusively that the lavas erupted at Swallow Bank must have originated by melting in the mantle. Several authors have recently suggested that oceanic lavas normally originate in the mantle and it is consistent with their view that the Swallow Bank lavas, which did originate there, reveal none of the mineralogical peculiarities customarily attributed to an unusually deep-seated origin, though the ready formation of chlorophæite in them suggests that the magma had a high initial water-content. It has occasionally been asserted that submarine lavas extruded at depths greater than about 2½ km. under a hydrostatic pressure greater than the critical pressure of water should not be vesicular; but this does not appear to be the case since the lavas from Swallow Bank contain numerous small vesicles. The collection includes pieces of pillowy lava with glassy tops, and there are vitric tuffs which suggest that some explosive activity occurred on the sea floor.

The alteration processes which affected the lavas were as follows:

(*a*) *Hydration and oxidation*. The glassy selvage of sideromelane on the pillowy surface of the flow was converted to palagonite. In the interior of the flow the glassy mesostasis was converted to chlorophæite, the mineraloid described by Peacock as "potential chlorite"[3]. Grey pseudo-fragmental rocks from the Western Seamount typify this stage. They consist of residual grains of hypocrystalline lava set in a matrix of chlorophæite.

(*b*) *Felspathization and zeolitization*. Calcic plagioclase in the cores of the zoned phenocrysts was replaced by a regular arrangement of water-clear plates, each about 0·03 mm. thick. These were first thought to be harmotome; but X-ray powder photographs show that the new mineral is a felspar and not a zeolite, and the remarkably low refractive index of 1·514 ± 0·003 (approximately n_β) indicates that it is probably orthoclase. A very similar example, in which potash felspar replaces the plagioclase of submarine lava fragments entombed in red clay, has been described by Mellis[4]. In the (alkaline) lavas on the Western Seamounts zeolites (natrolite and chabazite) plus calcite formed within the chlorophæite matrix and in cavities.

(*c*) *Argilization*. Yellow palagonite (an amorphous gel) was replaced by a coloured cryptocrystalline aggregate, fibro-palagonite, consisting of montmorillonoids (probably iron-bearing) plus chalcedony. Chlorophæite was replaced by a similar cryptocrystalline aggregate, fibrochlorophæite, consisting of coloured chlorites and colourless montmorillonite, together with abundant limonite and some leucoxene. Montmorillonite also replaces plagioclase in the sodic rims of the phenocrysts and in laths in the groundmass. Thin sections of the most altered rocks show that fibrochlorophæite is capable of replacing all the surviving pyrogenic minerals. Pink pseudo-fragmental rocks dredged from the Western Seamount, where alteration was more wholeheartedly destructive than at Swallow Bank, provide extreme examples of this process. Hand specimens resemble lithomarge.

(*d*) *Weathering*. Yellow fibro-palagonite was converted to grey montmorillonite (probably iron-free). Radioactive pelagite ("manganese") was deposited in cracks and on all surfaces exposed to the sea.

The internal evidence does not indicate the temperature of stages (*a*)–(*c*), but comparison with other known cases suggests that stage (*a*) occurred at high temperature during the initial cooling, stage (*b*) probably occurred at moderate temperature (less than 200° C.) during initial cooling or a subsequent hydrothermal cycle, and (*c*) was probably deuteric (cold) though it might have been hydrothermal. Stage (*d*) certainly occurred at about 0° C. It seems arguable that the type of alteration described here represents an early stage of the process which under more vigorous or more prolonged hydrothermal conditions results in the formation of spilites, poenites and weilburgites, and of the beds of jasper manganese ore and limestone that often accompany them.

The alteration scheme found at Swallow Bank is consistent with what is known of the distribution of clay minerals in deep-sea sediments. Montmorillonite, but not chlorite, is found in the sediments near the sites of basic volcanic activity[5]. Detrital montmorillonite could easily become available for sedimentation since it forms, by diagenesis of the palagonite skin on the surface of the flow, and this skin has been shown to exfoliate readily. By contrast, although abundant chlorite is formed by diagenesis of chlorophæite within the rock, it is difficult to imagine a mechanism by which it could be released to the sediments from the surfaces of the angular fragments of lava which litter the slopes of submarine volcanoes.

I thank Dr. R. M. Perrin, Dr. P. Gay and Mr. K. Rixon for X-ray powder photographs, and Dr. S. O. Agrell and Dr. I. D. Muir for discussion and advice. The work was supported by grants from the British Petroleum Co., and the U.S. Office of Naval Research.

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PHYSICS

Determination of Poisson's Ratio Dynamically

This communication describes a novel and practically useful experimental method for determining Poisson's ratio dynamically. The basis of the method is as follows.

When an elastic wave strikes a free surface obliquely, the energy of the wave will be redistributed, appearing partly in the form of a shear wave, the relative amounts of energy in the newly created waves being dependent on Poisson's ratio of the elastic material[1]. During the interaction of the transient stress disturbance with the surface, the surface is set in motion, the direction and magnitude of its movement being governed by Poisson's ratio and the angle of incidence α of the original incident wave. In general, the instantaneous surface particle motion which would be associated with the spherical wave being considered here is illustrated in Fig. 1, where due consideration has been given to the $1/r$ decay of the wave due to divergence. The particle velocity, except for the special point on the axis, is not, in general, perpendicular to the incident wave-front, deviating more and more with increasing angle of incidence. The surface particle velocity decreases with increasing angle of incidence in a complex manner which is not easily expressed analytically, but can be fairly readily computed point-by-point by combining vectorially the normal and tangential particle velocity components of the incident and the two reflected waves.

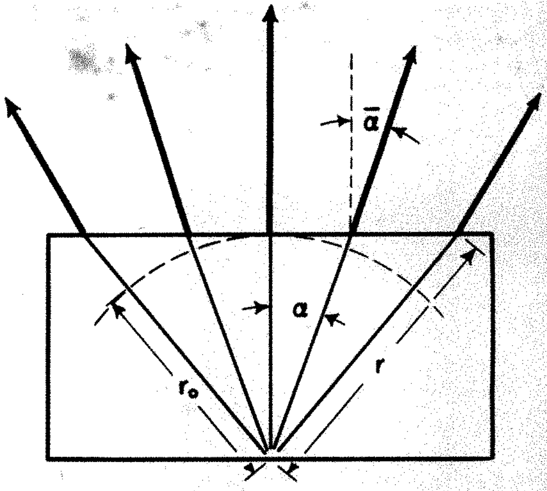

Fig. 1. Diagram illustrating direction in which a small rigidly affixed pellet would fly off when shock wave strikes free boundary obliquely

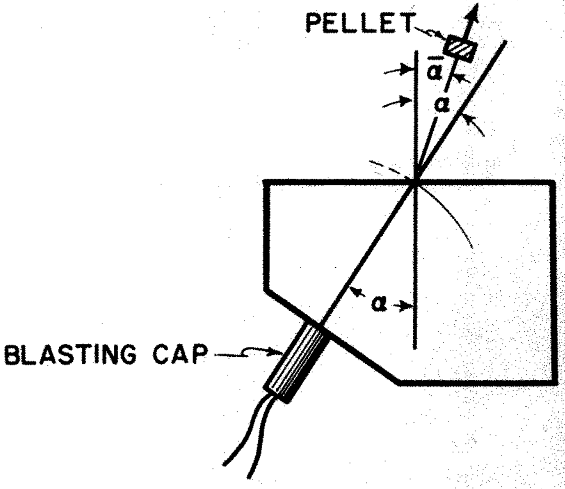

Fig. 2. Angle of emergence plotted against angle of incidence (α, Fig. 1). Solid lines are theoretical curves for three different Poisson's ratios

A small pellet, rigidly affixed to a surface as illustrated in Fig. 2, would be expected to undergo substantially the same movement as a point on the surface. By making the bond between the pellet and the surface of the plate considerably weaker than the 'Plexiglas', the bond can be broken by the tension developed by the reflected longitudinal disturbance, permitting the pellet to detach itself from the plate and fly off. The trajectory which the pellet assumes ought to be directed the same as the movement of a point on the surface of the plate. A number of observations have been made on pellets affixed in

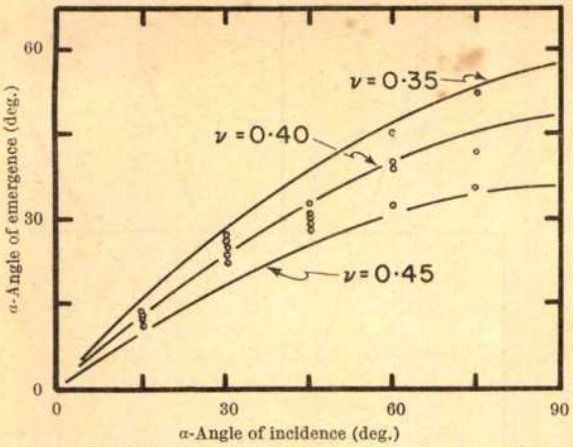

Fig. 3. Experimental arrangement for study of loosely affixed pellets

this way with the results being in excellent agreement with theory. The analytical expression for the direction of motion involves Poisson's ratio through the equation:

$$\nu = \frac{\sin^2\alpha - 2\sin^2(\bar{\alpha}/2)}{2[\sin^2\alpha - \sin^2(\bar{\alpha}/2)]} \qquad (1)$$

where α is the angle of incidence and α is the angle between the trajectory of the pellet and the normal to the surface.

This scheme provides a method for making experimental determinations of the dynamic Poisson's ratio of materials. The results obtained with 'Plexiglas' specimens are illustrated in Fig. 3 in which the angle of emergence is plotted against angle of incidence for a number of tests, the solid lines being the theoretically computed curves for Poisson's ratio of 0·35, 0·40 and 0·45. The experimental points are seen to cluster around the 0·40 curve, indicating a Poisson's ratio for 'Plexiglas' of about this value, a value identical with that computed from measurements made on the same 'Plexiglas' of longitudinal and transverse wave velocities.

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Source of the Light recorded in Photographs of Shocked Granular Pressings

ABOUT ten years ago Stewart Paterson[1] illustrated by means of several wave-speed photographs the previously discovered[2] fact that a large part of the light from a detonating charge was due to the system of shock waves produced in the surrounding air. Further, he showed that the light still reaching the camera when light from these shock waves was eliminated did not arise from chemical reaction of the explosive. He accomplished this by observing the light from a water-surrounded high-explosive charge which included in its length short sections of granular sodium chloride kept dry by a thin plastic tube. The explosive parts of the charge gave very faint wave-traces, but the salt layers gave very intense bursts of light as the disturbance passed through them. Additional photographs showed that a similar brilliant light was produced by explosives when in a granular loose-pressed form.

We have recently performed a simple extension of Paterson's experiments. The explosive part of the charge was 'Composition *B*–3' (60/40: RDX/TNT) at a density of 1·72 gm. cm.$^{-3}$ made up in cylinders 2 in. in diameter. After a suitable length of charge to ensure a full-strength detonation, two separate short layers of granular salt were alternated with explosive sections, followed by two open sections filled only with gas, similarly alternated with explosive. The arrangement is sketched in Fig. 1. The new aspect of the experiment consisted in filling the first salt layer and the first open space with methane while air remained in the interstices of the second salt layer and in the second open space.

The resulting rotating-mirror wave-speed photograph is shown in Fig. 1. First, the contrasting intensities of the light from the shocks in the open spaces are to be noted for methane and for air. On the negative the methane shock is only faintly visible, while the trace from the air shock is quite dense. This difference is then to be compared with the relative intensities emitted by the salt layers, the first permeated by methane and the second by air. An examination made on the negative of the distance over which the intense light was scattered ahead in the salt pressings showed that the light from the air-permeated pressing was slightly more intense than from the methane-permeated pressing. However, the difference was not nearly as large as one might expect if the light were coming entirely from com-

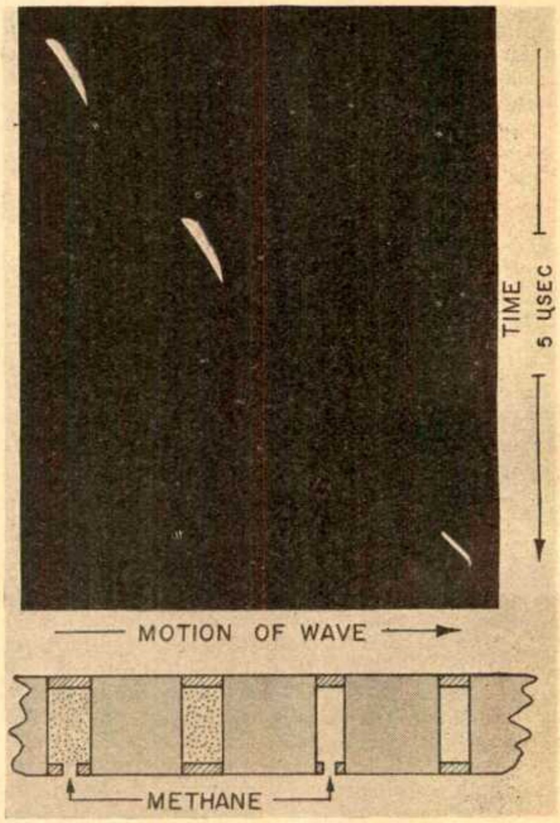

Fig. 1. Side-on photograph of composite charge fired under water. Dotted portions, sodium chloride; shaded portions, 'Composition *B*'; lined portions, polymethylmethacrylate tubing

pressed pockets of the two gases within the pressings. The luminosities given by the traces in the open spaces give a qualitative indication of the temperatures achieved in these two bases by free-running shocks driven by 'Composition *B*'. This temperature differential will not be greatly changed within the range of shock strengths to be expected from possible interactions within the pressing.

From these results we conclude that in a shocked pressing of granular sodium chloride there is an intense source of light which is independent of the nature of the interstitial gas. For a given shock strength this independent component represents a minimum amount of light that will be present for any gas or even when the pressing has been evacuated to 10μ. Experiments which confirm this have been conducted for a different geometry in a number of gases and in vacuum. Since this light does not arise from the gas, we have concluded that it must instead be emitted by hot sodium chloride.

The mechanism by which a part of the sodium chloride can reach a sufficiently high temperature to emit the observed intensities cannot be inferred from the photograph in Fig. 1. Other experiments indicate that the high temperature is produced by stagnation of rapidly moving material. In the present case, the sodium chloride is broken up and accelerated across the open spaces by the action of the shock, locally intensified by the interactions that are expected in a random assemblage of crystals.

A more complete report on this light is being prepared which presents evidence verifying this mechanism. As indicated by Paterson the same type of light exists in detonations of granular explosives pressings, and we would propose that it is produced by the same mechanism. We believe that stagnation hot-spots are important in determining the shock sensitivity of granular explosives pressings.

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ENGINEERING

Spark Track in Air at Very High Voltages

In an investigation[1] now in progress into the mechanism of triggered spark breakdown in air at voltages in the region of 1 MV., observations were made on the trigatron spark track. The track taken by the spark was found to bend sharply at a distance from the anode equal to a few per cent of the gap spacing. Between the anode and the point of bending the spark track was straight, and directed along the axis of the gap, whereas between this point and the cathode the spark path was of random shape. The length of the straight part remained approximately constant for given voltage and gap spacing. Observations showed that filamentary streamers occurred from the point of bending, directed towards the cathode. These appear to represent alternative spark tracks, growth of which ceases when any one streamer bridges the gap and expands to form the main channel.

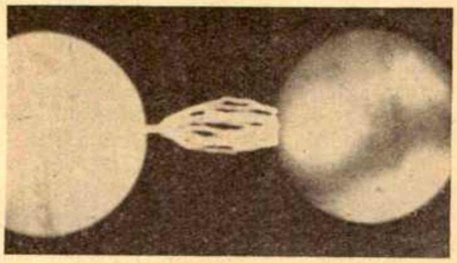

Fig. 1. Photograph of 15 superimposed spark channels. Voltage, 800 kV.; gap spacing, 50 cm.; electrode diameter, 75 cm. Left-hand electrode (anode) contains trigger gap

Similar effects have been observed for untriggered spark gaps[2,3] and for a triggered spark gap[4] at a voltage of the order of 100 kV. By superimposing photographs of several sparks, taken under the same experimental conditions, it is found that the possible spark tracks lie within a well-defined envelope of characteristic shape. A typical photograph, showing this envelope and the straight part of the spark adjacent to the anode, is given in Fig. 1.

In the experiments now in progress[1] an image converter tube is being used as a high-speed optical shutter of adjustable exposure time to measure the temporal progression of luminosity across the spark gap during the breakdown initiation process. Measurements of the current in the gap during the time-interval between the application of a trigger pulse to the system and complete breakdown between the spherical electrodes are also being made. Until these investigations are completed any theory to explain the bend in the spark track can only be speculative. It would appear, however, from the regularity in track shape for a large number of successive shots and the relatively constant length of the straight part of the spark that the bend in the spark track at voltages of about 1 MV. is an inherent consequence of the breakdown mechanism and is not due to the presence of dust or other foreign particles in the gap.

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CHEMISTRY

Evidence of the Influence of Defects on the Szilard–Chalmers Process

A variety of observations made in recent years lead to the conclusion that the fate of the fragments generated in crystalline solids by radiative thermal neutron capture is determined, in part, by the density, and possibly the nature, of the defects in the crystals[1]. It has also been suggested that the annealing reactions which can be carried out after the neutron irradiation by heating or photon irradiation also involve these defects[2].

A well-known method of introducing vacancies into simple ionic crystals is by the addition of small concentrations of ions bearing a different charge to the corresponding ions in the host lattice. Thus each calcium ion incorporated into a potassium chloride lattice leads to the simultaneous inclusion of a cation vacancy[3]. We have prepared homogeneous crystals of potassium chromate containing calcium ions from melts. After neutron irradiation we found the retention of chromium-51 as chromate in the contaminated crystals was not very different from the value for pure chromate crystals, but the rate of thermal annealing in the calcium-contaminated crystals is much greater than for the pure crystals.

Table 1

Calcium (per cent)	Initial retention	Retention after 20 hr. at 190° C.
0	75·0	82·0
0·022	76·5	91·5

Thus the behaviour of the calcium-contaminated crystals, with their cation vacancies, is very similar to potassium chromate crystals into which defects have been introduced by crushing or irradiation with γ-rays or energetic electrons[2].

Previous investigations have shown that if a neutron-irradiated salt is thermally annealed at constant temperature until further annealing only takes place very slowly (that is, more than 6 hr. in the case of potassium chromate), and if the thermal annealing is now interrupted by an irradiation of the crystals by ionizing radiation, on continuing the thermal annealing at the same temperature a further stage of rapid annealing takes place[4,5]. We have now shown that an essentially similar result can be produced by crushing the crystals instead of irradiation with ionizing radiation. In Fig. 1 the full line records the retention as a function of time on heating to 230° and the broken line the retention of a portion of the same material that was crushed after 16 hr. heating.

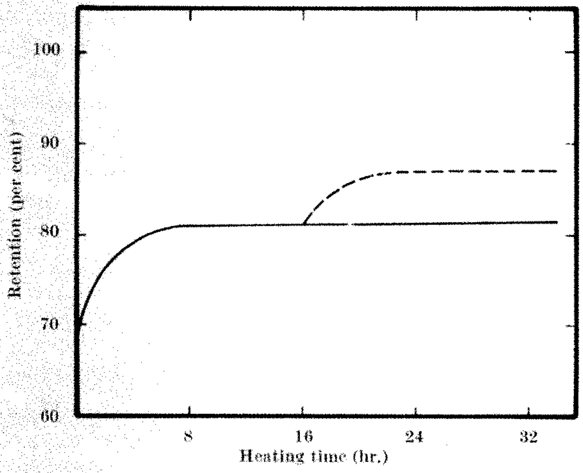

Fig. 1

These observations reinforce the interpretation of such phenomena outlined in our previous work[2].

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Determination of Molecular Weights of Gelatin by Scattered Light Measurements

THE clarification of aqueous solutions of polyampholytes, as a preliminary to determination of molecular weight by scattered light, is particularly difficult and is in fact the main trouble with gelatin solutions. Stainsby's enzymatic digestion technique[1] offered an elegant solution to this problem. This method determines the scattering due to the gelatin by the difference between the light scattered by a moderately clarified solution before and after tryptic digestion. Though Stainsby had confined his use of the technique to gelatin fractions, he nevertheless suggested that it should be applicable to a wide range of systems.

The results of the present work on a large number of gelatins have shown that the Stainsby technique is not universally applicable to commercial whole gelatins or to all their fractions. Unsatisfactory behaviour was indicated by impossibly high values for the dissymmetry ratio, Z, and high values of the weight average molecular weight $\overline{M}_w$ with respect 0·5 M saline, used by Stainsby, with 1 M potassium thiocyanate as the dispersing solvent, in potassium thiocyanate as the dispersing solvent, in many cases reduced the excessive values for Z and gave a much better correlation between $\overline{M}_w$ and viscosity.

The anomalous high results occurred with gelatins in which there was indication of the presence of larger amounts of suspended extraneous matter. The residual scattering after tryptic digestion was used as a means of estimating the presence of non-gelatin suspended material. The possibility of rapid settling out of the larger extraneous particles as a result of the removal, by the digestion, of the colloidal protection had been recognized by Stainsby. These would not then be taken into account in the final readings. This, however, is not applicable in the present work as the gelatin solutions were, immediately before the experiment, passed through a membrane filter with an upper pore diameter of 1μ. Calculation of the rate of descent of the largest particles which could be encountered in the digest showed that even the densest mineral material would not have begun to clear the incident beam during the period of the experiment.

It was found that gelatins which normally had low residual scattering and gave reasonable results for both Z and $\overline{M}_w$ could be made to give anomalous results, in spite of the use of Stainsby's technique, by the introduction of small quantities of very fine suspensions of barium sulphate (prepared by sedimentation and membrane filtration). This produced an increase in apparent dissymmetry and 90° scatter with both acid- and alkali-processed gelatins (Fig. 1). This increase in 90° scatter, for the example shown, corresponded to an increase in molecular weight of 20 per cent and 60 per cent for the acid- and alkali-processed gelatins respectively. The large differences in the effect of the added suspensions are probably due to the differences in the molecular weight rather than differences to be expected between gelatin types. Similar results were obtained by the introduction of small amounts of an anionic surface active agent (mixed olefine sulphates commercially known as 'Teepol'). These effects are attributed to the adsorption of gelatin to the particles introduced in one case and to the formation of complexes with the gelatin

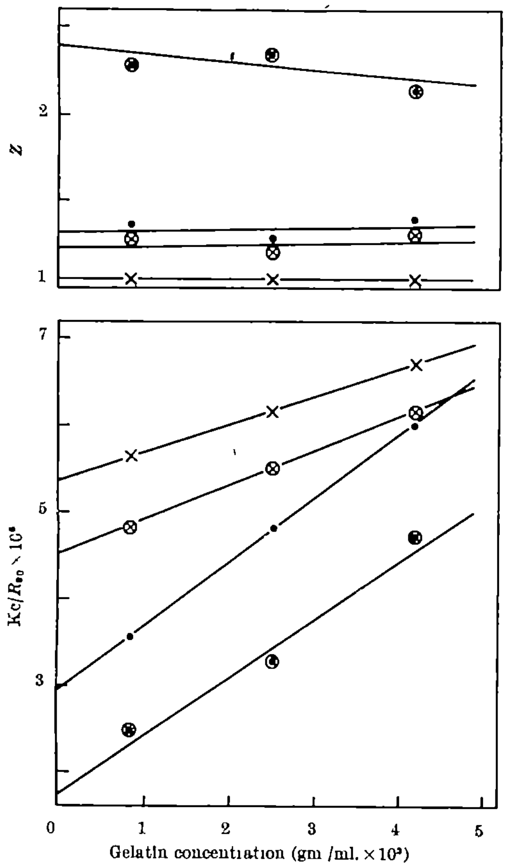

Fig. 1. Effect of addition of small quantities of very fine suspensions of barium sulphate on the dissymmetry and 90° scatter of gelatin solutions, measured by Stainsby's technique. Acid-processed gelatin. ⊗, with suspension, ×, without suspension; alkali-processed gelatin ○, with suspension, ●, without suspension

by the surface active agent in the other. Since both adsorbed layer and complexes are destroyed by enzyme action, their scattering contribution is not fully accounted for by the scattering of the tryptic digest. This general type of behaviour is to be expected if the digestion attacks any component of the initial system other than the simple dispersed gelatin molecules.

It is suggested that this type of mechanism applies in gelatins and fractions having a high residual scatter. This idea is supported by the increase in the number of reasonable results obtained by the use of the disaggregating and desorbing solvent molar potassium thiocyanate. It is also supported by calculations of the number and size of particles required and the amount of gelatin they would have to absorb to produce these effects. In a typical case, the particles had a radius of 0·07μ, were 0·003 per cent of the original material and adsorbed an amount which, expressed as a dry layer, would be 80 Å. thick. The calculations involved simplifying assumptions, but are sufficiently accurate to indicate the reasonableness of the hypothesis.

Lipid and mucopolysaccharide complexes are known to occur in gelatin[2]. Gelatins having appreciable quantities of these materials have a high residual scatter and give a high value for Z. The converse does not necessarily apply. The possibility of small particles of partly denatured collagen was discounted as the residual scatter was not reduced by the enzyme collagenase. Fractionation by alcohol coacervation tends to concentrate extraneous material in the first high molecular-weight fraction and sometimes the last low molecular-weight fraction. This was confirmed by the high residual scatter of these fractions. The middle fractions usually have a low residual scatter and give reasonable results for $\overline{M}_w$ and Z.

It must be concluded that errors can result from the use of Stainsby's technique. They are thought to arise from adsorption on impurities and by complex formation. These errors can be much reduced by the use of molar potassium thiocyanate as the dispersing solvent. Results for gelatins which have a high value for Z and the residual scatter should be rejected in the absence of other evidence. Further work is required to establish the limits of suspended extraneous material which are important.

I thank Dr. E. Pitts of this laboratory for help with the calculations.

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Nitro- and Amino-phenylosotriazoles

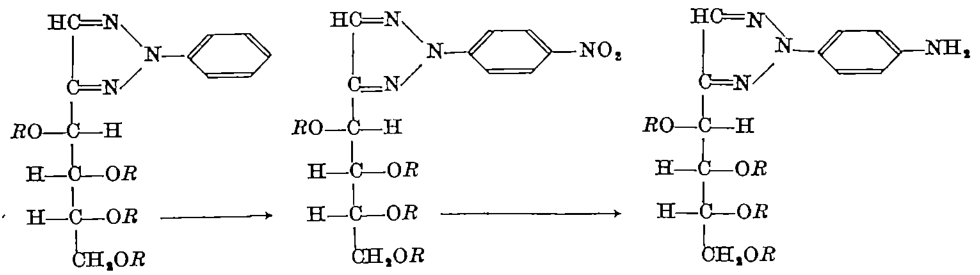
UNLIKE the glycosides, carbohydrate aryl osotriazoles have the sugar residue firmly bound and are unaffected by acids or alkalis. It was therefore interesting to prepare the aminophenylosotriazoles with the view of converting them into sulpha drugs which would acquire added specificity from their stable sugar residues. Alternatively, they could be converted into azo-dyes having affinity towards cellulose fibres.

Aminophenylosazones necessary for the preparation of the aminophenylosotriazoles are difficult to prepare since their hydrazines quickly decompose during osazone formation. Similarly, conversion of nitrophenylosazones into osotriazoles were found to be difficult to achieve[1] and only a small yield of glucose p-nitrophenylosotriazole (II, R = H), m.p. 253°, was obtained when the p-nitrophenylosazone was treated with bromine water.

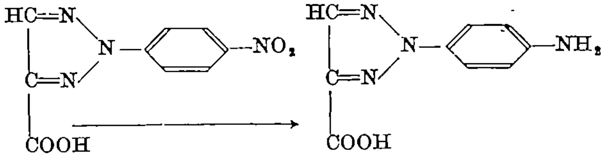
Higher yields of glucose p-nitrophenylosotriazole (II, R = H) were obtained by nitrating glucose phenylosotriazole tetraacetate (I, R = Ac) with fuming nitric acid and sulphuric acid and hydrolysing the resulting p-nitrophenylosotriazole tetraacetate (II, R = Ac) with alkali.

The position of the nitro group was determined by comparison with the p-nitrophenylosotriazole obtained by bromine water and also by oxidizing the osotriazole with potassium permanganate into the known 2-p-nitrophenyl-1,2,3-triazole-4-carboxylic acid (IV), m.p. 237° (refs. 2 and 3). This acid, on catalytic hydrogenation at room temperature using palladium–barium sulphate catalyst, yielded 2-p-aminophenyl-1,2,3-triazole-4-carboxylic acid (V), m.p. 265° (ref. 2), an amino-acid which preliminary experiments showed to possess bacteriostatic activity *in vitro*.

Catalytic hydrogenation of glucose p-nitrophenylosotriazole tetraacetate afforded glucose p-aminophenylosotriazole tetraacetate (III, R = Ac), m.p. 120°, a compound which was converted to a sulphon-



(I) (II) (III)



(IV) (V)

amide and gave a stable azo-dye. Similarly, nitration of glucose *m*-tolyl- and *m*-chloro-phenylosotriazole tetraacetates afforded the 4-nitro derivatives, whereas nitration of *p*-substituted phenylosotriazole tetraacetates such as glucose *p*-tolylosotriazole occurred in the 3-position.

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Rhenium Hexachloride

THE preparation of technetium hexachloride by the direct action of chlorine on the metal has been described in a recent communication[1]. As the pentachloride is the highest chloride known for rhenium[2] the unusual situation arose of a second row transition metal showing a higher co-valency than the corresponding third row metal and it was suggested that rhenium hexachloride should exist[1]. This communication describes the preparation and characterization of rhenium hexachloride.

There are reports in the early literature of a volatile higher green chloride of rhenium which was thought to be the heptachloride or the hexachloride, but it was never characterized[3,4]. No more was heard of this supposed green chloride after Geilmann *et al.* announced the preparation of the brown pentachloride in 1933 (ref. 5). Workers who saw the green chloride made their own rhenium by reduction methods and those who obtained the pentachloride used commercial rhenium sponge or powder.

Accordingly, rhenium metal was prepared by the reduction of ammonium perrhenate with hydrogen at 600° C. After flushing the apparatus with purified nitrogen, chlorine was passed over the heated metal at about 600° C. No pentachloride was observed, but a dark solid, melting very readily and giving a green vapour, was obtained. It should be easily distilled in the chlorine stream to a U-tube cooled in an ice–water mixture. Analysis showed the substance to be rhenium hexachloride. Found: Re, 46·0; Cl, 53 4. Required for $ReCl_6$: Re, 46·6; Cl, 53·4. Hydrolysis of the compound in water gave a precipitate of rhenium dioxide and soluble perrhenate ion in the ratio 1 : 2, agreeing with the usual hydrolysis scheme for Re VI compounds:

$$3 \text{ Re (VI)} \rightarrow \text{Re (IV)} + 2 \text{ Re (VII)}$$

Rhenium hexachloride forms very long needles which are dichroic, appearing red-brown by transmitted light and dark green by reflected light. The crystals melt very readily at a few degrees above room temperature to give a dark brownish liquid which volatilizes on very gentle heating to give a green vapour. In contrast to technetium hexachloride[1], the rhenium compound is thermally stable in both chlorine and nitrogen and there is no sign of decomposition to the tetrachloride.

The reaction between chlorine and a rhenium film is thus similar to the action of chlorine on technetium and is in marked contrast to the action of chlorine on rhenium powder. We have also shown, however, that a small amount of the hexachloride is formed from the powder, which had been previously heated in hydrogen to reduce oxide films, particularly in the early stages of the reaction. The hexachloride had been noted before in these circumstances, but it was never characterized[6]. A rough estimate of the yields is 5 per cent hexachloride, 95 per cent pentachloride. It appears that some hexachloride has been present in all preparations of the pentachloride just as it has recently been shown that some rhenium heptafluoride was always present in samples of the hexafluoride[7]. This explains an observation made by me and others (Lock, C. J. L., and Wilkinson, G., private communication) that when the pentachloride is heated in nitrogen to give the trichloride a small proportion of the material melted very readily and could not be decomposed to the trichloride.

As mentioned above, rhenium hexachloride is thermally stable in nitrogen but in some early experiments a black solid was formed on heating the hexachloride in nitrogen. Analysis gave rhenium : chlorine ratio of 1 : 4, but the compound was not the tetrachloride (cf. technetium[1]) as hydrolysis showed it to contain Re VI. It was, in fact, the oxochloride, $ReOCl_4$, formed by traces of oxygen in the nitrogen. When more care was taken to exclude oxygen it was found that the hexachloride was unchanged on heating.

When rhenium hexachloride is warmed in an atmosphere of oxygen, the only product is perrhenyl chloride ReO_3Cl. In the fluoride system all three possible heptavalent oxofluorides are known[8,9], but the only Re (VII) oxochloride is perrhenyl chloride. It was hoped that by gently oxidizing rhenium hexachloride an oxochloride of Re (VII) containing more chlorine could be made, but this was not achieved.

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BIOCHEMISTRY

New Fermentation Reactions by *Streptococcus viridans* stimulated by Transformation with Pneumococcal Deoxyribonucleic Acid

TRANSFERENCE of genetic material between related bacterial species was described by Bracco *et al.*, who reported reciprocal transformation reactions to streptomycin resistance involving *Pneumococcus* and two strains of *viridans Streptococci*[1]. The present investigation was undertaken to determine if the fermentative capacities of *Pneumococcus* were similarly transferable to *Streptococcus viridans*.

In this work a type II pneumococcus, II*D*39*S*-*Er*^{r}, served as the source of deoxyribonucleic acid (DNA). This organism is resistant to 150 μgm./ml. of erythromycin on blood agar plates (selected for erythromycin resistance by Dr. Robert Austrian). The receptor strains were the *viridans Streptococci D* and *NBSI*, which had been shown previously to be competent in heterologous transformation to streptomycin resistance[1]. DNA was prepared from *Pneumococcus* by methods previously described[2]. Transformation reactions were carried out by adding pneumococcal DNA extract to the streptococcal cells at the start of their incubation in charcoal-absorbed broth plus preheated pleural fluid[1]. In controls DNA was omitted or crystalline pancreatic deoxyribonuclease was added along with DNA at the start of incubation. Tests for transformation were performed following incubation of the cultures for 24 hr.

The fermentation reactions of *Pneumococcus* II*D*39*S*-*Er*^{r}, *Streptococcus D*, and *Streptococcus NBSI* toward thirteen carbohydrates were determined by inoculating loopfuls of these organisms into undefined semi-solid media of standard composition which contained acid-base indicators and the carbohydrates to be tested in concentrations of 1 per cent[3]. All three strains fermented sucrose, maltose, lactose, salicin and arabinose while they were inert towards glycerol, sorbitol, dulcitol, mannitol and xylose. The strains differed, however, in their reaction to trehalose, raffinose and inulin; these substrates were fermented by the pneumococcus but not by the two streptococcal strains.

Aliquots of cultures of *Streptococcus D* and *Streptococcus NBSI* were tested for their capacity to attack trehalose, raffinose and inulin following exposure of the cultures to DNA from II*D*39*S*-*Er*^{r}. The ability to ferment these three carbohydrates developed each time that transformation of the two streptococcal strains was attempted while controls gave consistently negative results.

Quantitation of the fermentation transformants was based on the observation that the transformed streptococcal cells which had gained the capacity to ferment the chief carbohydrate of the semi-solid medium grew into visible colonies while the untransformed cells grew scantily on secondary energy sources and did not form visible units. This observation was confirmed by the demonstration that colony counts in semi-solid media made from a uniform population of fermenters agreed with those made by the classical pour-plate method in blood agar. The numbers of streptococcal cells with new fermentation properties in fully grown cultures of *D* and *NBSI* which had been incubated with pneumococcal DNA, as determined by colony counts in the fermentation media, are given in Table 1.

Table 1. DEVELOPMENT OF NEW FERMENTATION REACTIONS IN *Streptococcus viridans* FOLLOWING EXPOSURE TO PNEUMOCOCCAL DNA

Carbohydrate	No. of fermenting *Streptococci*/ml. culture			
	Streptococcus D		*Streptococcus NBSI*	
	Exposed to DNA	Control	Exposed to DNA	Control
Trehalose	2,500	0	1,000	0
Raffinose	120	0	10	0
Inulin	15	0	20	0

Population of cultures used in this experiment. *Streptococcus D*, 6 × 10^6/ml. *Streptococcus NSBI*, 3 × 10^6/ml.

The two streptococcal strains were also tested for erythromycin resistance following exposure to DNA from the resistant *Pneumococcus* II*D*39*S*-*Er*^{r}. A proportion of the cells in the treated cultures invariably showed increased resistance to erythromycin while controls retained their original susceptibility.

The number of transformants to higher levels of erythromycin resistance was determined by pour-plating aliquots of a 24-hr. culture exposed to DNA with varying concentrations of erythromycin. Table 2 shows that approximately 1 per cent of a fully grown population of *Streptococcus D* which had been incubated with DNA from II*D*39*S*-*Er*^{r} possessed increased resistance to erythromycin. In contrast, new fermentative capacities were noted in a much smaller proportion of a *Streptococcus D* population treated with DNA, the percentages ranging from $2 \cdot 5 \times 10^{-6}$ to 4×10^{-4} for inulin and trehalose respectively (Table 1). These results suggest that cells exposed to transforming DNA may show great differences in their readiness to accept different genetic markers. The possibility that the fermentation transformants have a large replication lag relative to the transformants to erythromycin resistance or that these cells are at a selective disadvantage has not been ruled out, however.

Pneumococci developing new metabolic properties through transformation have been shown, in certain instances, to have acquired the ability to produce specific enzymes, for example, mannitol-6-phosphate

Table 2. TRANSFORMATION OF *Streptococcus D* TO ERYTHROMYCIN RESISTANCE BY EXPOSURE TO PNEUMOCOCCAL DNA

Erythromycin (μgm./ml.)	No. of surviving organisms/ml. culture		
	Culture exposed to:		
	DNA	No DNA	DNA + DNase
0	3 × 10^8	4 × 10^8	4 × 10^8
0 01	1 8 × 10^8		2 3 × 10^8
0·02	3 × 10^6	0	0
10	4 × 10^1	0	0

dehydrogenase[5] and amylomaltase[6]. In the present work the new metabolic properties of the transformed *Streptococci* have not been thoroughly defined, but one can speculate that they involve the elaboration of enzymes required to split the carbohydrates trehalose, raffinose and inulin into smaller units vulnerable to these organisms.

The work reported here was done in the Department of Microbiology of the New York University College of Medicine in the laboratory of Dr. C. M. MacLeod.

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Non-enzymatic Reaction of Fluoro-oxalacetic Acid with Pyridine Nucleotides

β-FLUORO-OXALACETIC acid is a powerful inhibitor of malic dehydrogenase[1] and glutamic-aspartic transaminase[2]. We have recently observed that relatively high concentrations (10 m*M*) of fluoro-oxalacetate also inhibit the reduction of triphosphopyridine nucleotide by L-malate at *p*H 7·4 catalysed by the 'malic enzyme'[3] of chicken liver. The triphosphopyridine nucleotide-dependent decarboxylation of oxalacetate at *p*H 4·5 by this enzyme is inhibited to a greater extent by fluoro-oxalacetate. In the course of this work we found that fluoro-oxalacetate reacts non-enzymatically with di- and tri-phosphopyridine nucleotides, and with some analogues[4] of these coenzymes, to form fluorescent addition compounds. These reactions occur rapidly at 25° in the *p*H-range of 9–10. Oxalacetic acid does not react with diphosphopyridine nucleotide under these conditions. Our experiments suggest that fluoro-oxalacetate reacts at carbon-4 of the pyridine ring of the nucleotides in a manner similar to carbonyl compounds such as acetone and dihydroxyacetone[5]. Fluoro-oxalacetate, unlike oxalacetate, is not appreciably enolized in solution, even at high *p*H[1,6]. The presence of a fluorine atom on the β-carbon of fluoro-oxalacetic acid probably favours the formation of a carbanion in alkaline solution and thus facilitates a nucleophilic attack on carbon-4 of the pyridine ring.

In *tris*(hydroxymethyl)aminomethane buffer (50 m*M*) at *p*H 9·0 and 25°, the reaction of di- and tri-phosphopyridine nucleotides (30 μ*M*) with fluoro-oxalacetate (50 m*M*) is of first order with respect to the nucleotides ($k = 0{\cdot}034$ min.$^{-1}$). Under the same conditions, considerably faster reaction was found with the 3-acetylpyridine and thionicotinamide analogues of diphosphopyridine nucleotide, whereas the corresponding 3-pyridinealdehyde analogue reacted very slowly. Crude preparations of the adducts were prepared by dissolving the nucleotides (30 μmoles) and fluoro-oxalacetic acid (70 μmoles) in 2 ml. of water and then adding 1 *N* potassium hydroxide, with stirring at 25°, in order to keep the *p*H at 10·5–11·5 for 10 min. Ice-cold ethanol (8 ml.) and then diethyl ether (8 ml.) were added. After cooling on ice, the mixtures were centrifuged, and the precipitates washed with ether and desiccated. On paper electrophoresis in ammonium acetate at *p*H 7·5 for 1 hr. at 25° (19 V./cm.; 35 m.amp.), the addition compound of diphosphopyridine nucleotide and fluoro-oxalacetate migrated faster than dihydrodiphosphopyridine nucleotide, and to the same position as dihydrotriphosphopyridine nucleotide. This suggests that at *p*H 7·5 the fluorinated adduct possesses a net charge of -4. The addition compound of fluoro-oxalacetate and triphosphopyridine nucleotide migrated faster than the corresponding diphosphopyridine nucleotide adduct. The addition compounds of the various nucleotides exhibited an absorption maximum at 260 mμ at *p*H 2–9 due to the adenosine moiety. At *p*H 7·4 and 9·0, the adducts of di- and tri-phosphopyridine nucleotide with fluoro-oxalacetate exhibited a second peak at 340 mμ ($\varepsilon = 7{,}900$), while the corresponding addition compound with the 3-acetylpyridine analogue of diphosphopyridine nucleotide possesses a second absorption maximum at 343 mμ ($\varepsilon = 6{,}140$). The absorption maxima of the adducts in the range of 340–350 mμ were abolished by acidification to *p*H 2.

At a concentration of 40 μ*M*, the addition compounds between fluoro-oxalacetic acid and di- and tri-phosphopyridine nucleotides are without influence, at *p*H 7·5, on the action of malic and glucose-6-phosphate dehydrogenases, and of the 'malic enzyme'. Further biochemical properties of these adducts are under investigation. This work was supported by grants C–4681 and C–3211 from the United States Public Health Service, and by a grant of the American Heart Association.

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Isolation of Soluble Glucose-6-Phosphatase from Microsomes of Bovine Livers

IN a recent report[1], we described the isolation of soluble glucose-6-phosphatase from bovine liver by treating the microsomes with both sodium deoxycholate and digitonin. The treatment with sodium deoxycholate attacks the microsomes in such a manner that part of the inactive proteins is solubilized. The main part of active proteins is concentrated in the fluffy layer by centrifugation[2]. Using this method, we obtained better results in rendering digitonin soluble.

The present communication describes some improvements regarding the procedure of ultracentrifugation. The homogenization of the bovine liver and the separation of undisrupted cells, the nuclei

Table 1. INCREASE OF ACTIVITY OF GLUCOSE-6-PHOSPHATASE AFTER TREATMENT OF MICROSOMES WITH DIGITONIN

Time of incubation (min.)	Initial activity present in microsomes (μmoles PO_4/ml./hr.)	Activity in solution			Particle-bound activity		
		Absolute activity (μmoles PO_4/ml./hr.)	Total absolute activity of supernatant (μmoles PO_4/hr.)	Initial activity (per cent)	Absolute activity (μmoles PO_4/ml./hr.)	Total absolute activity of residue (μmoles PO_4/hr.)	Initial activity (per cent)
30	109·0	99·0	748·0	682·0	22·0	61·5	56·4
30	218·0	97·0	660·0	302·0	106·0	350·0	160·5
60	109·0	94·0	686·0	630·0	26·0	72·8	66·8
60	218·0	88·0	607·0	279·0	110·0	341·0	156·5
120	109·0	97·0	707·0	648·0	20·0	56·0	51·4
120	218·0	79·0	545·0	250·0	95·0	294·0	135·0

and mitochondria were the same as in our experiments previously reported[1,2]. Determination of the activity of glucose-6-phosphatase was also carried out in the same manner using the method of Freedland and Harper[4]. The samples were incubated with glucose-6-phosphate at 37·5° C. The liberated phosphate was measured by the method of Fiske and Subbarow[5]. The absolute activity was defined as µmoles phosphate/hr. in 1 ml. samples.

The ultracentrifugation of the suspension of microsomes treated with sodium deoxycholate requires 60 min. During this period sodium deoxycholate still affected the microsomes. Therefore, we attempted to reduce the time of its influence. The agglutination of the microsomes at *p*H 5·4 gives particles of such a size that centrifugation at 18,000*g* for 20 min. was sufficient for separation. This agglutination was reached by mixing the suspension of microsomes and an equal volume of 0·1 *M* citrate buffer, *p*H 5·4 (ref. 6). This method was also used for the separation of the microsomes from cytoplasm and for the sedimentation of undissolved residue after treatment with digitonin.

The use of citrate buffer after the treatment of the microsomes with sodium deoxycholate has the disadvantage that a part of the dissolved proteins became insoluble again. A series of experiments with suspensions of microsomes of different contents of protein (25–150 mgm. protein/ml. suspension) showed that always only 6–12 per cent of the protein initially present was dissolved. It is not possible to increase the soluble part of protein using a suspension of microsomes with lower content of proteins. These results were reached only during agglutination with citrate buffer. Under these conditions the dilution of the suspension of microsomes is of no advantage.

The use of citrate buffer has no influence on a possible disintegration of the structure of microsomes. The result of the following treatment of the microsomes with digitonin is the same in applying the method of agglutination or ultracentrifugation.

Treatment with digitonin was always carried out in a way that a final concentration of 1 per cent of digitonin was reached. Even after standing for a few days the digitonin-treated microsomes do not lose their activity of glucose-6-phosphatase. Relatively smaller amounts of enzyme were dissolved by concentrations lower than 1 per cent. For this reason we recommend the concentration of 1 per cent. The complete removal of digitonin from the microsomal preparations necessary for biochemical experiments is extremely difficult. Therefore we did not investigate its influence at concentrations higher than 1 per cent. A time of incubation, longer than 60 min., has no essential advantage. The results are presented in Table 1.

From Table 1 it can also be seen that the total activity of the supernatant of the suspension of microsomes treated with digitonin was much higher than the total activity of the suspended residue of the sodium deoxycholate treatment. This effect is remarkable because the inhibition of the substrate (glucose-6-phosphate) on more dilute enzymatic solutions would be expected to be stronger in consequence of feed-back mechanism. We explain this effect as a result of a 'demascation' of the enzyme in consequence of a possible protein splitting by treatment with digitonin. A suggested regulation of the enzymatic activity by mascating substances is described by Langdon and Weakley[7]. The authors suppose a partial mascation of the glucose-6-phosphatase in livers of normal animals and total activation in livers of diabetic animals.

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Formation of Pyrroline and Propane Diamine from Spermidine by Extracts of *Serratia marcescens*

THE oxidation of the natural polyamines, spermine and spermidine, by washed cell suspensions of various bacteria has already been described[1–4]. Propane-1 : 3-diamine is formed from spermidine by extracts of *Neisseria perflava*[5] and by washed cells of *Pseudomonas aeruginosa*[4], *Serratia marcescens*[4] and *Mycobacterium smegmatis*[6]. It has been assumed that during this oxidation the four carbon moiety of spermidine is converted to γ-aminobutyraldehyde[4,5] or to pyrroline[5]; however, these compounds have never been isolated. This communication deals with a partially purified enzyme from *Serratia marcescens* which oxidizes spermidine. The oxidation products, which are formed in stoichiometric amounts, have been shown to be propane-1 : 3-diamine and pyrroline.

S. marcescens was grown in a yeast extract medium[4] containing 0·01 per cent spermidine for 20 hr. at 30° C. An enzyme preparation purified 20–30-fold was obtained by protamine precipitation, ammonium sulphate fractionation, hydroxyl-apatite adsorption and DEAE cellulose chromatography.

This partially purified preparation, as well as crude bacterial extracts or an acetone powder suspension, oxidized spermidine to yield a compound which gave a yellow complex on incubation with *o*-aminobenzaldehyde. This reaction is characteristic for pyrroline or piperideine[7]. The yellow complex migrated on paper (R_F 0·79 in butanol/acetic acid/water 2 : 1 : 1) like 2 : 3-trimethylene-1 : 2-dihydroquinazolinium obtained by incubating synthetic pyrroline[8] with *o*-amino-benzaldehyde. The yellow complex had a maximal absorption at 430 mμ as did the complex obtained with authentic pyrroline[9]. The compound was catalytically reduced by platinum oxide and oxidized to γ-aminobutyric acid in the presence of diphosphopyridine nucleotide by a specific enzyme[8]. It was eluted from a 'Dowex 50' column exactly like synthetic pyrroline.

The other product of the oxidation was characterized as propane-1 : 3-diamine by ion exchange chromatography[10] and by paper chromatography[4].

Spermidine, labelled with carbon-14 (in the C_4 moiety[11]), gave rise to labelled pyrroline whereas the propane-1 : 3-diamine formed was not radioactive. The specific activity of the pyrroline formed was similar to that of the added spermidine. Spermidine labelled in the C_3 moiety yielded labelled propane-1 : 3-diamine; the pyrroline formed was not radioactive.

Table 1 shows that 1 μmole each of propane-1 : 3-diamine and pyrroline were formed per μmole of spermidine oxidized. In the presence of catalase 0·5 μmole of oxygen was taken up during this reaction. If no catalase was added to the preparation, the oxygen uptake was about 50 per cent higher. No ammonia was formed during this oxidation. The same preparation did not produce pyrroline from putrescine or spermine.

Table 1. OXIDATION OF SPERMIDINE BY *S. marcescens* ENZYME

Spermidine oxidized*	4·00 μmoles
Propane-1 . 3-diamine formed*	4·03
Pyrroline-formed†	3·90
Oxygen consumed‡	4 01 μatoms
Ammonia formed§	0

Reaction mixture contained 100 μmoles sodium phosphate buffer *p*H 7·0; 0·3 μmole mercaptoethanol, 4·0 μmoles spermidine, 15,000 units catalase; enzyme (equivalent of 1 1 mgm. protein). Total volume, 3 0 ml. Incubated at 30° C. for 120 min.

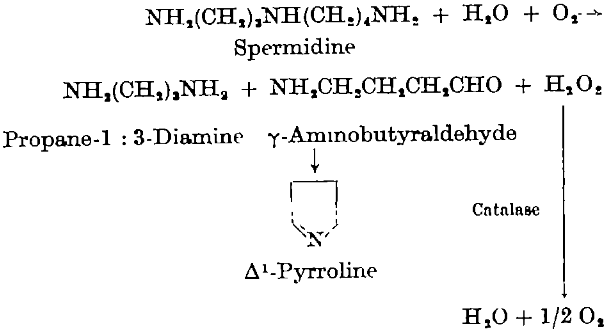
* Estimated by the dinitrofluorobenzene method after separation on 'Dowex 50' column (ref. 10).

† Estimated by the *o*-amino-benzaldehyde method (ref 9).

‡ Estimated by the Warburg manometric technique.

§ Estimated by micro-diffusion and nesslerization (ref. 17) after trapping the pyrroline with *o*-amino-benzaldehyde.

These results indicate that the initial steps in the oxidation of spermidine by *S. marcescens* are as follows:


$NH_2(CH_2)_3NH(CH_2)_4NH_2 + H_2O + O_2 \rightarrow$
Spermidine
$NH_2(CH_2)_3NH_2 + NH_2CH_2CH_2CH_2CHO + H_2O_2$
Propane-1 : 3-Diamine
γ-Aminobutyraldehyde
N
Δ¹-Pyrroline
Catalase
$H_2O + 1/2\ O_2$

γ-Aminobutyraldehyde formed during this oxidation cyclizes to pyrroline spontaneously[7]. A similar process occurs during the oxidation of putrescine by various enzymes[8,12,13]. Spermidine oxidase obtained from *S. marcescens* thus differs from the plasma amine oxidase studied by Tabor *et al.*[14] in that the latter oxidizes spermine and spermidine[14,15], and does not produce pyrroline or propane-1 : 3-diamine[16].

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Removal of Calcium and Relaxation in Actomyosin Systems

EXPERIMENTS described in previous papers[1-4] show that the removal of the essential calcium from actomyosin accounts for the mechanism of relaxing action of the natural relaxing factor[5] as well as that of ethylenediamine tetraacetic acid (EDTA)[6,7]. The difficulty in accepting this view, however, might be that such a large particle as the relaxing factor, about 0·1μ or more in diameter, would produce an action by the same mechanism as that of a small soluble molecule, EDTA.

Recently we found that a chelating resin 'Dowex *A*-I' (Dow Chemical Co.), of which the active site is iminodiacetic acid attached to the styrene-divinylbenzene co-polymer matrix, was capable of relaxing actomyosin systems. As noted in the preceding papers[1,2], the relaxing capacity of a chelating substance is determined by its actual calcium-binding activity under the experimental conditions; the relaxation experiments being performed in the presence of excess magnesium, the effective calcium-binding depends not only on the stability constant for calcium but also on the difference between those for calcium and magnesium. Since the stability constant of the resin for calcium is rather small, that is, 3 × 10^3 at *p*H 6·8, and is about three times that for magnesium, the relaxing action of the resin had been expected to be not so strong; rough calculations based on these figures indicated that, if the relaxation experiments with glycerinated muscle fibres were carried out following the method of Ebashi *et al.*[8], the relaxing activity of the resin would be one-fiftieth to one-hundredth that of EDTA on the basis of molar concentration of chelating sites. Indeed, it was revealed that the relaxing activity of resin suspensions containing 20 m*M* chelating sites approximately corresponded to that of 0·2 ± 0·06 m*M* EDTA in good agreement with the results expected. The super-precipitation of extracted actomyosin was also inhibited by the resin suspension; the activity of the latter was shown to be about one-sixtieth that of EDTA. The size of the resin particle did not significantly influence its relaxing activity in the range of diameter 1–50μ. No relaxing activity of the resin was lost by repeated washings and no soluble substance capable of relaxing came out of the resin, indicating that the resin particle itself was the effective substance.

As has already been noted[1-3], the calcium-binding activity of the physiological relaxing factor is not less than that of EDTA, but exceeds the latter under physiological conditions. It is shown in this communication that a much larger particle than the relaxing factor has a relaxing activity to the extent expected from its calcium-binding capacity. Now it is very probable that the relaxing factor produces the relaxation of the actomyosin systems by a common mechanism with EDTA.

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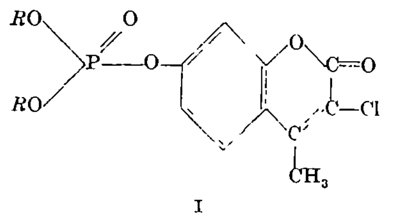
A New Class of Compounds showing Anthelmintic Properties

We wish to report anthelmintic activity of a novel class of chemical compounds (patent pending) for which compound I, (R = ClC_2H_4–), will serve as an example. This compound, bearing our code number 96*H*60, is *O*, *O* di-(2-chlorethyl) *O*-(3-chloro-4-methylcoumarin-7-yl) phosphate, for which the common name 'Haloxon' is suggested.



I

Oral doses of 'Haloxon' given to sheep and cattle have a very high efficiency against adult worms of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Strongyloides* and a high level of efficiency against *Nematodirus* and *Bunostomum*. Immature stages of some of these parasites are destroyed. The recommended dose for sheep and cattle is in the range 35–50 mgm./kgm.; but much lower doses are effective against *Haemonchus* spp. and *Cooperia* spp. 'Haloxon' has a marked anthelmintic effect against *Capillaria obsignata* in poultry, oxyurids, *Nematospiroides dubius* and *Strongyloides ratti* in rodents and against ancylostomes and ascarids in carnivores.

'Haloxon' is much less toxic than the related compound 'Coroxon', (I, R = C_2H_5–), the substitution of a chlorine atom in the ethyl groups of 'Coroxon' having a very profound effect on anticholinesterase activity. 'Coroxon' in glycerol formal shows an acute oral LD_{50} to rats of 12 mgm./kgm., but for 'Haloxon' in a similar formulation the figure is increased to 900 mgm./kgm. Lambs and yearling sheep have tolerated four to six times the anthelmintic dose. Others have received twenty consecutive weekly doses of 100 mgm./kgm. of 'Haloxon' and have shown no ill-effects. A single oral dose of 250 mgm./kgm. 'Haloxon' to sheep results in a slight depression of red cell cholinesterase activity (which returns to normal within one week of dosing), whereas an oral dose of 10 mgm./kgm. 'Coroxon' results in a 95 per cent depression in red cell cholinesterase activity.

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Changes in Absorption during Photosynthesis in the Ultra-violet Spectrum

Seven different types of changes in absorption between 400 and 800 mμ and with lifetimes between 10^{-5} and 10^{-2} sec. have been found in connexion with photosynthesis of green plants. These changes are caused by the formation of the π-π*— triplet states of chlorophyll a_0, resp. b_0 (ref. 1), by probably metastable states of chlorophyll a_1 (ref. 2), by the photo-oxidation of chlorophyll *a* (refs. 3 and 4), by the oxidation of cytochrome[4,5] and by the reduction of added Hill reagents[6]. Changes in absorption at 475 and 515 mμ[7,8] are caused by the reduction of a substance *X* (ref. 6). Changes at 420 mμ are caused by the reaction of an unknown compound. The relations between chlorophyll *a*, cytochrome, *X* and Hill reagents lead to a reaction pattern of photosynthesis as presented in refs. 4 and 5. This report presents evidence for an eighth type of change in absorption.

Bishop[9] has shown that plastoquinone is somewhere involved in transport of electrons during photosynthesis because after extraction of plastoquinone the

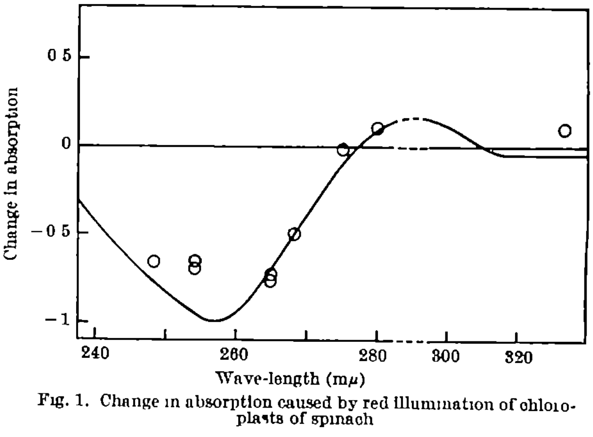

Fig. 1. Change in absorption caused by red illumination of chloroplasts of spinach

photolysis of water stops but is fully restored by recondensation of plastoquinone.

To obtain more information about the function of plastoquinone we looked for changes in absorption in the ultra-violet spectrum. Preliminary measurements are shown in Fig. 1. Changes in absorption were measured with reference to 280 mμ. The magnitude of the observed changes is about 1 per cent. They take place in the region of the absorption bands of plastoquinone and the short wave band of triphosphopyridine nucleotide (TPN). Changes in absorption due to TPN could be eliminated because chloroplasts free of mitochondria show practically no changes at the long wave-band of TPN at 340 mμ. Therefore, we tend to assume that the changes are caused by the reduction of plastoquinone. For comparison we have depicted in Fig. 1 as a solid line the changes in absorption produced by plastoquinone reduction *in vitro* (derived from measurements of Crane[10] and Bishop[9]). The curve is standardized with the measurement at 268 mμ. The light-induced plastoquinone reduction is fast, compared with the slow re-oxidation (several seconds). Addition of ferricyanide has strong influence on the re-oxidation. Probably plastoquinone is at least engaged in a reaction with the reductant Z⁻ (see Fig. 2 in ref. 4) and can be re-oxidized by ferricyanide. We hope that the kinetic analysis of these new changes in absorption lead to an understanding of the role of plastoquinone in the mechanism of photosynthesis.

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PHYSIOLOGY

Activation of Histidine Decarboxylase in Tumour Cells in Mice

IT has been shown in this laboratory that activation of histidine decarboxylase occurs as part of various types of normal and malignant rapid tissue growth, and that such growth can be arrested by inhibition of histamine formation. This applies to the embryo[1] and granulation tissue of healing wounds[2] in rats where histidine decarboxylase activity reaches exceedingly high levels. Elevation of the enzyme activity by artificial means will accelerate the rate of wound healing[2] and increase the collagen content of granulation tissue[3], whereas inhibition of histamine formation has the reverse effect. Tumour tissue obtained from female albino rats bearing subcutaneous transplants of the Walker rat mammary carcinoma produced histamine at considerable rates *in vitro* whereas in normal rat mammary tissue the rate of formation was too low to be measured[4]. A similar finding was made in rats bearing an implanted hepatoma[5]. In these previous investigations it was not possible to define the phase of growth as regards frequency of mitosis.

The results reported here were obtained with the Landschütz I tumour growing in the mouse peritoneal cavity as single free cells where it was possible to determine: (*a*) the total number of cells contained in the tumour; (*b*) the phase of growth expressed as the frequency of tumour cells in mitosis at different intervals after inoculation of the tumour. In the following *T*-cells will be used for tumour cells, mitoses for *T*-cells in mitosis and c.p.m. for radioactivity of histamine labelled with carbon-14 formed during a 3-hr. incubation of the tumour cells with ^{14}C-histidine.

In each set of experiments tumour cells from four mice were pooled. The mice were anæsthetized with ether and bled to death through the carotid arteries. A small incision was made through the abdominal wall; the peritoneal cavity rinsed six times with 2-ml. portions of saline to which a few drops of a 20 per cent potassium oxalate solution were added to prevent coagulation. The rinsings from the four mice were mixed, centrifuged for 15 min., the clear cell-free supernatant discarded and the cells re-suspended in saline. This cell suspension was used for the actual investigation.

The rate of ^{14}C-histamine formation from ^{14}C-histidine was determined *in vitro* in one aliquot by Schayer's isotope dilution method as described by White[6], and the total number of cells estimated

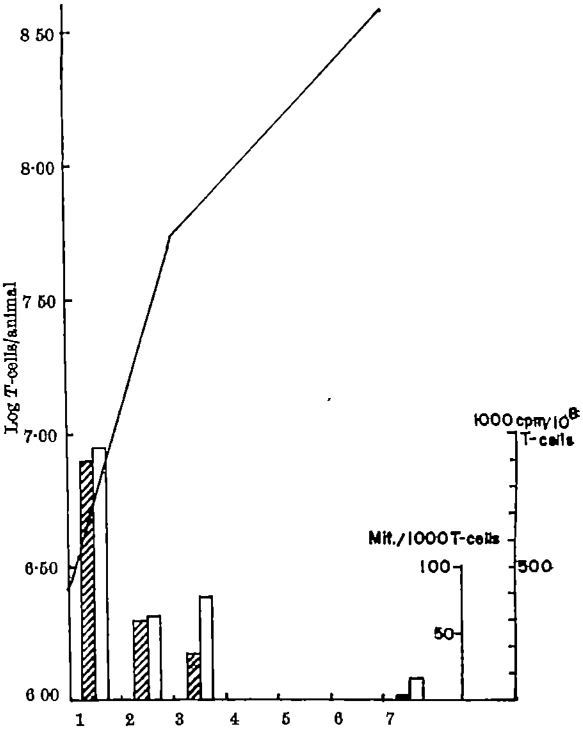

Fig. 1. Measurements in 16 mice simultaneously inoculated with Landschutz I ascites tumour cells. The continuous line represents growth in terms of total number of tumour cells per mouse. The hatched columns stand for amount of ^{14}C-histamine formed and the unhatched for frequency of tumour cell mitosis at 1, 2, 3 and 7 days after inoculation. Hatched, c.p.m./10^8 *T*-cells; white, mitoses/1,000 *T*-cells

according to Klein and Révész[7] in another aliquot. A differential cell count was made on a sample of the suspension by staining with a 2 per cent 'Orcein' solution, placing a drop on a slide and examining the cells under the microscope. It was hereby possible to count differentially the number of T-cells, mitoses and non-T-cells.

The results from one typical experiment are given in Fig. 1. This shows first that at the onset of tumour growth the histidine decarboxylase activity is correlated with the frequency of tumour cell mitosis and, further, the lack of correlation between enzyme-level and total number of tumour cells. These results display striking resemblance to the situation in healing wound tissue where histidine decarboxylase is activated to peak-level on the first day after wounding[2].

These experiments strongly support the idea of histidine decarboxylase activation as part of certain types of rapid tissue growth[8]. This concept is now further strengthened by the clear correlation between mitosis frequency and enzyme activity in the initial stages of tumour growth.

Attempts at arresting tumour growth by enzyme inhibition are in progress.

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Orientation of Spermatozoa in Mucus of the Cervix Uteri

Bovine cervical mucus has been shown to contain a mucoid which behaves as though its molecules were long, flexible and thread-like[1]. The elastic properties and the Weissenberg effect shown by native mucus (at œstrus) are traceable to this molecular form[2]. Native mucus may be drawn out into thick threads in which the mucus is anisotropic due to the uncoiling and alignment of the molecules along the lines of strain. It is probable that mucus secreted by the cervix uteri and expressed into the vagina is in this condition, the lines of strain running along the path the spermatozoa should follow.

It has been suggested that spermatozoa in cervical mucus would swim preferentially in the direction in which molecules were aligned. To test this possibility, threads of bovine œstrous mucus were drawn out with platinum hooks on to the central part of slides with parallel grooves of the type described by Harvey and Jackson[3]. A drop of bull semen was pipetted on to one end of each strip of mucus. The slides were then incubated for approximately 15 min. at room temperature in Petri dishes lined with filter paper damped with saline. For control purposes semen was also added to one end of a comparable streak of 0·9 per cent sodium chloride solution (saline) on a similar slide.

After incubation the mucus (or saline) was examined under a microscope. In fields very close to the semen drop, the sperm were so numerous that scoring would have been impossible. A number of fields were therefore chosen at a distance of about 2 or 3 cm. from the semen source. All the sperm in a given field were scored according to the direction in which they were travelling, as judged by eye in relation to the grooves in the slide (parallel to the direction in which the mucus had been stretched) and the cross-markings at right-angles to the grooves.

Table 1 shows the results of nine experiments in which two different samples of mucus and three samples of semen were used.

Table 1. ORIENTATION OF SPERMATOZOA IN A THREAD OF MUCUS. MEAN PERCENTAGES WITH STANDARD ERRORS (RANGE IN BRACKETS)

Direction of sperm movement	Mucus (9 exp.)	Saline (3 exp.)
Along the axis of orientation	70 ± 4 (59–91)	33 ± 1 (31–36)
At right-angles to the axis	20 ± 3 (4–35)	37 ± 1 (35–39)
Diagonally	8 ± 2 (0–18)	17 ± 1 (15–19)
Pivoting and circling	3 ± 1 (0–7)	12 ± 1 (10–14)
Proportion of those orientated along the axis going away from the semen source	65 ± 7%	65 ± 6%
Total No. of sperm counted	708	701

Orientation could not have been induced by flowing of the mucus along the slide because, in the first place, epithelial cells in the mucus were stationary, and, in the second place, there was little difference between the swimming-rates of sperm in the two directions, away from and towards the semen source.

Since the technique allows for unconscious selection of sperms for scoring, it was arranged for a further two experiments to be carried out by another observer who did not know the 'expected' result. Both sets of results from these experiments showed the same trend as those in Table 1, but to an even greater extent; in the first 84 per cent and in the second 95 per cent of the sperm were orientated along the strip of mucus. Of these spermatozoa 85 per cent and 97 per cent respectively were orientated away from the semen source. In the first none, and in the second only 1 per cent, of the sperm was circling.

Similar experiments were carried out with preparations of purified mucopolysaccharides dissolved in saline. These solutions did not have a prolonged relaxation time and the molecules, though similar to those in native mucus, were therefore randomly orientated. The results for two experiments with each of two such preparations were very similar to those obtained with pure saline. Of 1,092 sperm scored, 30 per cent were orientated along the streak, 34 per cent at right-angles.

It appears, therefore, that spermatozoa travelling in a thread of mucus are preferentially orientated in the direction in which the thread has been drawn out.

This work forms part of a programme of research on cervical mucus supported by the Population Council, Inc., Rockefeller Institute, New York.

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Sodium Transport and Phosphoproteins

In previous work[1] we have shown that liver slices suspended in a medium free of sodium ions show a depressed turn-over of phosphoprotein phosphorus. This effect is reversed by addition of sodium. In these and the experiments shown here, the phosphoproteins have been identified by isolation of radioactive phosphorylserine from acid hydrolysates of the crude proteins. We have found that the phosphoproteins sensitive to Na^+ are non-mitochondrial[1], the mitochondrial phosphoproteins appearing to have different properties[2].

We now find that the effect of Na^+ on phosphoprotein turn-over is strongly influenced by the concentration of externally added Ca^{2+} ions. In Table 1, we show that Ca^{2+} ions tend to inhibit phosphoprotein turn-over as well as that of adenosine triphosphate. Na^+ reverses this effect, but it is clear that as the Ca^{2+} level is reduced the inhibition of uptake of phosphorus-32 falls away, while the system still requires Na^+ for full activity.

Table 1. Effect of Na^+ and Li^+ Ions at Two Ca^{2+} Concentrations

Ca^{2+} level m*M*	2·8			0·93		
Addition	Choline	Na^+	Li^+	Choline	Na^+	Li^+
ATP, sp.a.	97,000	147,000	150,000	155,000	228,000	172,000
Phosphoprotein P, sp.a.	11,700	18,000	12,000	18,400	39,000	17,200

Slices of about 200 mgm. wet weight were suspended in Ringer solution containing choline-chloride instead of sodium chloride. *p*H 7·2 was maintained with sodium bicarbonate, gas phase was 95 per cent oxygen – 5 per cent carbon dioxide. Temperature, 38°. After 10 min. incubation in the medium, which contained phosphorus-32 of specific activity 3×10^6 c.p.m./μmole, Na^+ choline or Li^+ was added to a final concentration of 0·039 *M*. 15 min. later, trichloroacetic acid (final concentration, 5 per cent) was run into the flasks and the contents homogenized rapidly and chilled.

The effect of Li^+ ions is also shown in Table 1. At high levels of Ca^{2+}, Li^+ causes a marked stimulation of ATP, but has no such effect on phosphoproteins. This experiment is further evidence that the action of Na^+ on the latter compounds is specific.

Cosmos and Harris[3] have shown that calcium ions are gained by frog skeletal muscle on reduction of the external Na^+, and expelled on further addition of Na^+; and have postulated that Na^+ and Ca^{2+} ions compete for a common site (see also Niedergerke and Harris[4]).

The present results strongly support the idea that phosphoproteins are involved in Na^+ transport, and are in keeping with previous suggestions to that effect[5,7].

The evidence for this can be summarized as follows:

(1) Systems deficient in Na^+ show reduced turn-over of phosphoprotein phosphorus;

(2) The addition of Na^+, but not of Li^+, K^+ or of choline reverses this effect;

(3) The level of Ca^{2+} greatly influences the rate of turn-over of phosphoprotein phosphorus and supports the idea of a competition between Na^+ and Ca^{2+} for a common site.

(4) Li^+ ions can be shown to stimulate adenosine triphosphate turn-over while having no effect on phosphoprotein turn-over.

(5) Drugs such as strophanthin which inhibit Na^+ transport prevent the effect of Na^+ on phosphoprotein turn-over while having no action on adenosine triphosphate (Table 2).

Table 2. Effect of Strophanthin on Phosphoprotein Turn-over

		Specific activity, c.p.m./μmole phosphorus of			
Addition	3×10^{-4} *M* strophanthin	Phosphoprotein as phosphoserine-P	Increase (per cent)	ATP	Increase (per cent)
Choline	–	479	—	306×10^3	—
Na^+	–	700	46	483×10^3	58
Na^+	+	520	9	460×10^3	51

The conditions were as in Table 1 with 2·8 m*M* Ca^{2+}. The phosphoproteins were subjected to acid hydrolysis and chromatography to give the activity of phosphorylserine derived from them. Carrier phosphorylserine, 10 μmoles, was added before acid hydrolysis.

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Gamma-Aminobutyric Acid and Other Blocking Substances extracted from Crab Muscle

As part of an attempt to identify the transmitter agent released by inhibitory nerves in crustacean muscle we have extracted and purified heat-stable 'inhibitory' substances of a crab muscle and its associated nerve supply. The fibres of the extensor muscle (opener) of the dactyl of the walking leg of *Cancer borealis* are densely innervated by two inhibitory nerve fibres, and by extracting the whole muscle, although adding non-neural elements, we were assured of obtaining the materials stored within the nerve endings. The extracts were assayed on the extensor muscle of the dactyl in the crayfish, *Orconectes virilis*. Only those extracts which imitated the effect produced by neural inhibitory activity (reduction of the excitatory junctional potentials without shifting the resting potential by more than 5 mV.) were considered 'inhibitory' and were further purified.

Fifty-five extensor muscles were dissected free from hypodermis and from the larger bundles of sensory nerves. The muscles were frozen on dry ice, ground to a powder and lyophilized. The resulting powder (1·4 gm.) was extracted three times with 30 ml. of boiling distilled water. The pooled extracts were concentrated by freeze-drying and dialysed against three changes of 100 ml. of distilled water at 4° C. for 32 hr. The physiological activity was confined to the dialysate, which was taken to dryness. 215 mgm. (a half) of this material was dissolved in 0·5 *M* acetic acid (*p*H 2·5) and fractionated on an Elphor continuous electrophoresis apparatus at 4° C. The extract was applied to the paper at the rate of 1 mgm. of starting material per hour, and the voltage was adjusted so that potassium appeared at one border of the paper and inorganic phosphate near the other. All the fractions were lyophilized, and a portion of each was then dissolved in van Harreveld's solution for assay.

Each active fraction was further purified by ascending paper chromatography as follows. A large amount of the fraction was applied in a long line, near the bottom of a sheet of washed Whatman No. 1 filter paper. After developing with a suitable solvent the paper was dried for a day. Narrow vertical strips were stained with ninhydrin or other colour reagents, and, using these strips as guides, the entire paper was

Table 1

Compound	Method of identification*	Approximate sensitivity of crab muscle to the pure substances (on a molar basis, GABA taken as 1)
γ-Amino-butyric acid	*E*, 1′, 2, 4′, 5′	1
Taurine	*E*, 1′, 2′, 3′, 4′, 5′	0·0012
Alanine	*E*, 1′, 2, 3′, 4, 6	0·00027
β-Alanine	*E*, 1′, 4′, 2	0·0086
Proline	*E*, 1′	0·0022†
Unidentified compound		

* Column 2, identical behaviour with authentic sample in: *E*, electrophoresis; 1, butyl alcohol–acetic acid–water (60 : 15 : 25); 2, butyl alcohol–pyridine–water (1 : 1 : 1); 3, ethyl alcohol–ammonia–water (90 : 5 : 5); 4, methyl alcohol–pyridine–water (80 : 4 : 20); 5, 80 per cent phenol–water (80 : 20); 6, ethyl alcohol–2 *N* acetic acid (80 : 20). Prime after number indicates that physiological activity ran at same rate as authentic sample. Otherwise, the R_F values of the unknown and authentic sample were compared by ninhydrin staining.

† Proline was tested only on crayfish muscle.

divided into horizontal sections which were separately eluted with water. A fraction of each of these eluates was dried, dissolved in saline and assayed. The remaining portions of the active eluates were chromatographed again in a series of solvents to determine their purity and to establish their identity.

Five blocking substances (Table 1) were identified by comparing their chromatographic and electrophoretic behaviour with that of authentic samples. Gamma-aminobutyric acid (GABA) was further identified by a micro-adaptation of the highly specific enzymatic assay of Jakoby and Scott[1]. The amounts of GABA determined by the enzymatic assay and by the biological assay were in good agreement. The 'unidentified compound' of Table 1 was present in very large amounts, and must have a very low physiological activity relative to GABA. Taurine and alanine were present in such large amounts that they accounted for about one-half and one-tenth respectively of the total blocking activity of the original hot-water extract in spite of their low physiological activities compared to GABA. GABA contributed about 20 per cent of the total blocking activity and β-alanine and proline together less than 5 per cent. Blocking activity was found in fractions which contained large amounts of inorganic phosphate, calcium or magnesium. The purification and assay procedures were such that a compound which contributed less than 3 per cent of the total blocking activity of the extract might have been overlooked. The unidentified compound shared with citrulline the following properties: (*a*) very similar R_F values in solvents 1, 2, 4 and 6 (but not in solvent 5); (*b*) white fluorescence under ultra-violet illumination (2537 Å.); (*c*) similar colour reactions to ninhydrin, the Sakaguchi reagent and Ehrlich's reagent. Presumably, it is a carbamyl compound. It was accompanied by very large amounts of an unidentified and inactive guanido compound which also stains with ninhydrin and is not arginine or lombricine.

Using similar techniques, GABA has also been found in peripheral nerves of crabs (10–12 μgm. GABA per 100 mgm. dry weight of nerve) and in the lobster central nervous system (about 60 μgm. GABA per 100 mgm. dry weight of ganglia (to be published)). GABA is of considerable interest because it is the most active of the stable blocking compounds found by these methods in the crustacean nervous system or muscle, and because it closely imitates the actions of the natural transmitter at inhibitory junctions in crayfish[2-6] and lobster[7]. D. Hamilton and R. S. Eisenberg (unpublished results) have found in preliminary experiments that it reduced the size of the excitatory junctional potential and increased the membrane conductance in extensor muscles of *Cancer borealis*. Crab and crayfish muscles have similar sensitivities to GABA; strong blocking effects are seen with concentrations of 1–3 μgm./ml. On the other hand, our chemical extraction methods would have destroyed active heat-labile compounds. Florey and Chapman[8] reported that they found in methanol extracts of crab nerve a compound which they believe is more active than GABA.

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HÆMATOLOGY

Starch-Gel Electrophoresis of Mouse Hæmoglobins

The hæmoglobins of mice were first shown to exist in two different inherited forms by Ranney and Gluecksohn-Waelsch[1,2] using paper electrophoresis. Their work was confirmed and extended by Russell and Gerald[3], who found only two hæmoglobin types, diffuse and single, among twenty inbred lines of mice, using the starch-block technique. Starch-gel electrophoresis gives a better resolution of proteins than either of these techniques, and there have been three accounts[4-6] of the detection of slow-moving bands in the diffuse hæmoglobin using starch gel with continuous borate buffer. Popp and St. Amand[4] noted these bands and also two peaks in the ultracentrifuge analysis of diffuse hæmoglobin. Ranney, Marlowe-Smith and Gluecksohn-Waelsch[5] observed that both the slowest band of the electrophoretic pattern and the faster peak of the ultracentrifuge pattern increased when the hæmoglobin solution was stored for 4 weeks at 4° C. Rosa *et al.*[6] tested six inbred lines, five of which were among those used by Russell and Gerald[3], and claimed that they could be further sub-divided into at least four hæmoglobin types, when these bands were taken into account.

In the present investigation, in order to type with certainty the slower bands, very strong solutions of carbon-monoxy-hæmoglobin (10–15 per cent) were used within 48 hr. of the bleeding of the mice, and a variety of buffers tried. The discontinuous buffers of Poulik[7] and Aronsson and Grönwall[8] gave good results, but the best were obtained with a system using *tris*(2-amino-2(hydroxy-methyl)-1 : 3-propanediol), EDTA (disodium ethylene-diamine-tetra-acetate dihydrate) and citric acid in the gram-molecular concentrations 0·0385, 0·0009 and 0·0035 respectively in the gels, and veronal buffer of *p*H 8·6 and ionic strength 0·05 in the troughs.

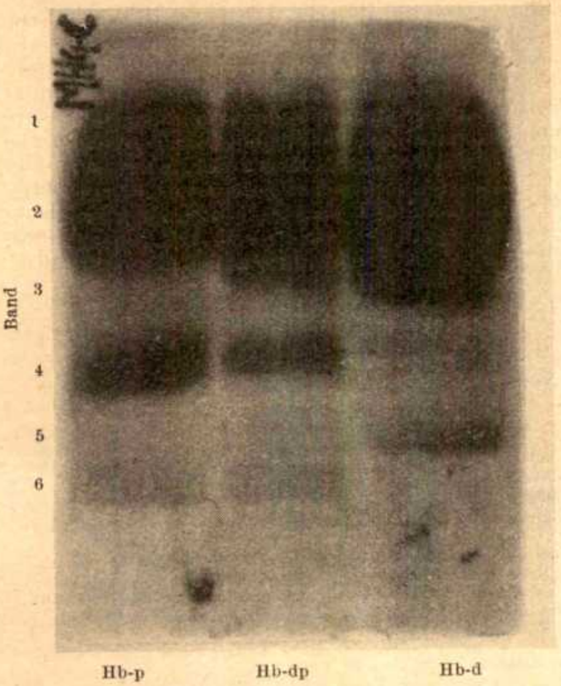

Fig. 1. The pattern after starch gel electrophoresis of three hæmoglobin types, all from the polydactylous line

With this technique the diffuse hæmoglobins consistently gave five bands (Fig. 1, Hb-d). Numbered in order of their mobilities, the strongest is 2 and coincides with the single hæmoglobin type, 5 is weak and 4 very weak. Standard strains tested were *C57BL/6* and *C57BR/cd* of the single type, and *CBA/Fa*, *A/He*, *DBA/2*, C_3H and *AKR* of the diffuse type. No differences within these types were observed, supporting the classification of Russell and Gerald[3], as opposed to that of Rosa *et al.*[6]. (B. L. Cohen (personal communication) classified these lines similarly, using Poulik discontinuous buffer in starch gel.[7])

However, in the non-inbred strain developed by Fisher and Holt for the expression of polydactyly[9], two variants of the normal diffuse pattern were observed in low frequency (Fig. 1). In one form, of which only three examples were found, band 3 is absent and 4 greatly increased, and 5 is replaced by a new slower band 6. The other form shows all six bands simultaneously. Both forms give the same ultracentrifuge pattern as diffuse hæmoglobin. The polydactylous strain was nearly extinct, so that few breeding data exist for these variants. What there is suggests that they are the heterozygous and homozygous products of a gene allelic with those of single and diffuse. They are therefore provisionally called Hb-p and Hb-dp respectively. The results of matings of mice of types Hb-p and Hb-dp with those of type Hb-d (diffuse) are summarized in Table 1. An outcross of an Hb-dp female to an Hb-s male of the *C57BL/6* line produced 2 offspring classifiable as Hb-ds and 4 undistinguishable from Hb-p, but presumably of the fifth genotype Hb^p/Hb^s. A mating between two such mice has yielded 6 apparent Hb-p to 1 Hb-s young.

Table 1

Mating type		No. of matings	Offspring		
♂	♀		Hb-d	Hb-dp	Hb-p
Hb-d	Hb-dp	3	8	9	0
Hb-dp	Hb-d	1	4	3	0
Hb-p	Hb-d	2	0	10	0

Mice from line C_3H were supplied by the Department of Radiotherapeutics, Cambridge, and from lines *C57BL/6*, *C57BR/cd* and *DBA/2* by the Medical Research Council Laboratory Animals Centre, Carshalton.

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Interspecific Hybridization of Hæmoglobin

Formation of hybrids between hæmoglobin of different species (human and canine) have been described[1]. It is the result of a complete dissociation of the two hæmoglobins into chains with subsequent re-association in equal proportion; the two new molecules of hybrids become apparent; 50 per cent of the canine hæmoglobin participates in the formation of hybrids.

In other cases hybridization between hæmoglobins of different species has been impossible[2]. We present here: (1) an example of incomplete hybridization in which less than 50 per cent of animal hæmoglobin is involved; (2) conditions under which 100 per cent of animal hæmoglobin is involved.

The hæmoglobins were prepared as described in ref. 3. The dissociation occurred after a dialysis against acetate buffer 0·2 *M*, *p*H 4, 7 (ref. 4). Control was obtained by starch-block electrophoresis accord-

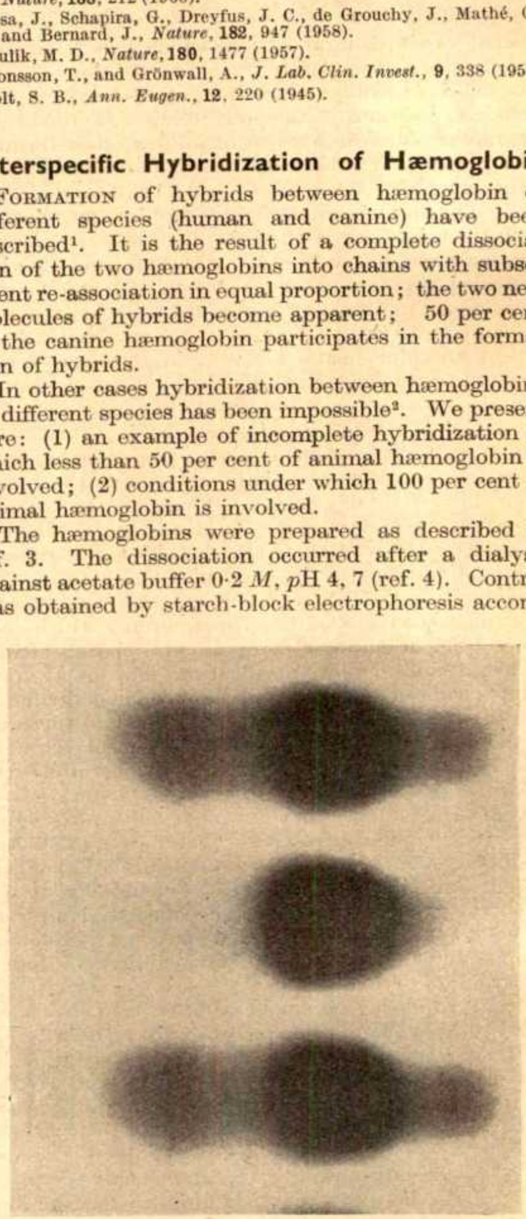

Fig. 1. Incomplete hybridization between human and rabbit hæmoglobins. Top, hybrid; middle, extemporaneous mixture; bottom, hybrid

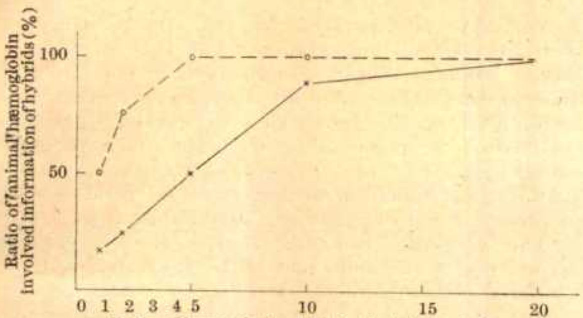

Fig. 2. Variation of the fraction of animal hæmoglobin involved in hybrids with the ratio of the initial mixture. ×, Rabbit; ○, dog

ing to the method described in ref. 5. The spots were eluted and hæmoglobin estimated[6].

Hybridization of human and rabbit hæmoglobins in equal proportions gave rise to two hybrids; but the ratios of each never reached 25 per cent of the initial mixture. Several conditions as temperature, concentration, *p*H, time, gave the same results (Fig. 1). The interpretation of this incomplete hybridization has to be discussed.

(1) An ultracentrifugation control (a single component of $S_1 = 2{\cdot}65$ of acidic *p*H instead of 3·6 at *p*H 7) allows the exclusion of incomplete dissociation of rabbit hæmoglobin.

(2) We can exclude the hypothesis of a hybridization of a minor fraction of rabbit hæmoglobin because after a first hybridization, the hybrids can be eliminated, and the hybridization on the remaining human and rabbit fractions repeated; the same quantities of hybrids reappear.

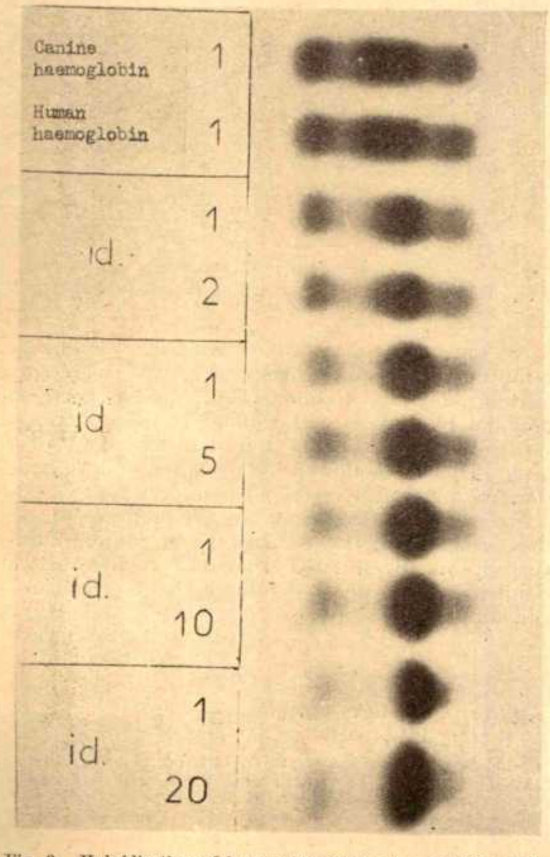

Fig. 3. Hybridization of human and canine hæmoglobin with change in the ratio of the two hæmoglobins

(3) The final hypothesis is that the affinity of one rabbit chain toward the other is stronger than that of a rabbit chain toward the corresponding human chain.

To test these hypotheses we changed the ratio of the two hæmoglobins in order to displace the equilibrium. Fig. 2 shows that the proportion of rabbit hæmoglobin which is involved in hybrids is 16 per cent when the initial mixture of human and rabbit hæmoglobin is 1 : 1 and more than 50 per cent when the ratio is 5 : 1.

The same experiment was performed with human and canine hæmoglobin (Fig. 3). When the ratio is 1 : 1, 50 per cent of canine hæmoglobin is involved in formation of hybrids; when the ratio is 5 : 1, 100 per cent of canine hæmoglobin is involved (Fig. 2).

Perhaps the application of systematic variations of ratio during hybridization of normal and abnormal hæmoglobin will allow the detection of abnormalities otherwise undetectable.

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Absorption-Elution Grouping of Modifications and Development of Bloodstains

THE ABO grouping of dried blood smears by the absorption-elution method has already been described[1]. That a similar technique can be applied to bloodstains on fabric is also known[2]. This communication is intended to describe the modifications and development which have followed subsequent to the introduction of the absorption-elution method to this laboratory as a routine test and how the procedure has been made even more simple and its application even more effective.

In the grouping of smears, one of the difficulties encountered was to ensure that the cavities produced by the application of gum–dammar–beeswax mixture remained intact. Not infrequently the wax was found to have become lifted from the slide while in the elution chamber and inadvertent mixture of the cell suspensions to have taken place. This can be avoided by the use of three-cavity 3 in. × 1 in. glass slides (12 mm. diam. wells). For practical purposes, only anti-A, anti-B and anti-H antisera are generally used, and the three cavities are adequate for routine work.

In the grouping of bloodstains on fabrics, group specific substances present in some fabrics are known to interfere with bloodstain grouping. Furthermore, the cutting of a garment may not be desirable. Extracts of the bloodstain can be made either by soaking a piece of bloodstained fabric in water or by the application of water to the uncut garment and scraping with a scalpel. Minimum quantities of water should be used (6–12 drops). Similar treatment to a control area as near as possible to the stain is essential. Saline extracts are not satisfactory due to the formation of salt crystals on drying. The procedure is as follows: (1) Prepare the slides by placing one or two drops of extract in each of the three cavities. With a glass rod, cover the whole of the cavity with extract. Control A, B and O stains may be made from 1/10 solutions of A, B and O cells in water. Extracts from control areas of fabrics will not adhere to the slide unless protein is added. For this purpose, to fabric controls, add one drop of 1/10 O serum in water to each cavity. (2) Dry in air or on a hot plate at 40–45° C. (3) Fix in McIlvaine buffer at 99° C. for 1 min. (For large-scale grouping the use of stainless steel racks is recommended). (4) Blot dry. (5) Dispense one or two drops of anti-A, anti-B and anti-H antisera to the three cavities, ensuring that all the stain is covered. (6) Place in a moist chamber and leave for 3 hr. to absorb. (A plastic refrigerator box approx. 9 in. × 4 in. is a suitable chamber. Three or four layers of filter paper should be moistened with water and forced into the bottom of the box. Invert the box, and use the lid as a slide tray.) (7) Place in refrigerator at 4° C. for ½ hr. to increase agglutination intensities. (8) Wash off excess antibody with cold saline. Place slides in stainless steel rack and wash by alternate raising and lowering in each of two beakers of cold saline, ten or fifteen times in each beaker. (9) Blot dry. (10) Dispense one or two drops of A, B and O cells 2 per cent v/v to the relevant cavities. (11) Elute at 50°–55° C. for 10 min., in a moist chamber. (12) Remove from oven, rotate, leave for 15 min., read.

All bloodstain grouping tests in this laboratory are performed in duplicate. Known A, B and O controls are run at the same time, together with a 1/10 O serum blank. Slides may be marked for purposes of identification with a lead pencil if one end of the slide is finely ground into an opaque surface. Sensitivity tests have shown that the minimum quantities of dried whole blood necessary to produce good macroscopic results are approximately 10 μgm. when testing for A or B antigens and 200 μgm. when testing for H antigen.

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PHARMACOLOGY

Hæmatological Effect of a Derivative of the Monocarboxylic Acid of Vitamin B_{12} in Primary Polycythæmia

AN increasing number of derivatives of the monocarboxylic acid of vitamin B_{12} is known to antagonize the growth-promoting effect of vitamin B_{12} in various microbiological cultures (*Escherichia coli, Euglena, Ochromonas*, etc.) in the chick embryo and the rat[1–3].

Very few papers have, however, appeared dealing with the clinical indications of the B_{12} antagonists. Beard[4] reports that if administered in combination with 5,000γ of anti-vitamin B_{12}, 5γ of vitamin B_{12} will not be followed by a reticulocyte crisis in patients with pernicious anæmia. Smith[5] found that in patients suffering from chronic myeloid leukæmia, anti-vitamin B_{12} failed to improve general condition or depress the high level of serum vitamin B_{12}.

The present communication describes clinical experience gained with one of the B_{12} antagonists in primary polycythæmia. It is true that the number of patients involved is very low, and 6 months only have passed since the trials were begun, yet the results already observable are thought remarkable enough to merit preliminary communication.

The B_{12} antagonist used was the homogeneous monocarboxylic acid of vitamin B_{12} prepared by Kelemen[6]. It was administered to four patients in intramuscular daily doses of 1–3 mgm. Fig. 1 demonstrates the substantial reduction in the number of red cells in circulation after a total dosage of 20–30 mgm. in three cases, and of 90 mgm. in the fourth. The white cell count and the qualitative blood picture remained in all patients practically unchanged, nor was there any deterioration in the platelet count. Marrow puncture showed the erythroid–myeloid ratio to have come within the normal range in one case, and to have improved markedly in two, and moderately in one, patient. The hæmatocrit decreased and the sedimentation-rate increased. The spleen remained unchanged in size.

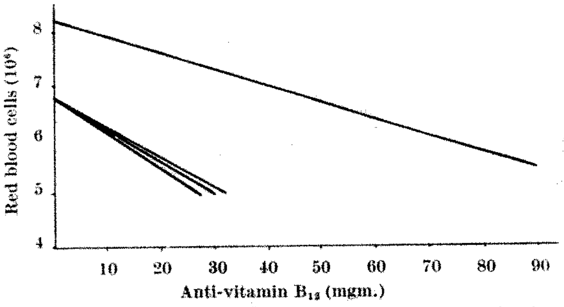

Fig. 1. Effect of anti-vitamin B_{12} on red blood cells of polycythæmic patients

All four patients showed alleviation in subjective symptoms. In three cases this was followed by lasting remission. In one patient who had been given previous treatments with radioactive phosphorus, the slow remissive response required 93 mgm. of the drug. This was followed by a relapse, and renewal of the treatment proved of no avail.

Extended investigations are in progress to see if the reduction in the red blood cell count following anti-vitamin B_{12} in polycythæmia vera is statistically significant, and to understand the mechanism involved.

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Myocardial Catechol-O-Methyl Transferase Activity after Chronic Cardiac Denervation

In a recent study of the denervated dog heart[1], we noted a marked increase in cardiac sensitivity to norepinephrine, a finding anticipated from previous investigations of denervated organs[2]. Catechol-*O*-methyl transferase activity was measured in these denervated hearts to determine whether this pharmacological supersensitivity might be accompanied by a decreased ability of the myocardium to *O*-methylate norepinephrine. The results showed that cardiac denervation produces a decrease in the catechol-*O*-methyl transferase activity of the myocardium; whether this decrease is related to the phenomenon of supersensitivity, however, is open to considerable doubt.

Six dogs were subjected to cardiac denervation by regional neural ablation[1]. After 7–84 days the response of the cardiac contractile force[3] to several sympathomimetic amines and to cervical sympathetic and vagal nerve stimulation was measured. Portions of the left ventricular myocardium were then removed and frozen for subsequent determination of catechol-*O*-methyl transferase activity[4] and catecholamine content[5]. Control tissue was obtained in a similar manner from normal dogs. Nerve stimulation failed to produce a cardiac response in the denervated dogs, as did the injection of tyramine; in addition, catecholamines could not be detected in the denervated myocardium (Table 1). When compared with normal dogs, the cardiac-denervated animals showed nearly a ten-fold decrease in the threshold dose of L-norepinephrine required to give a detectable response in cardiac contractile force; there was also a three-fold increase in the cardiac response to a standard dose of 0·25 μgm./kgm. L-norepinephrine. These findings confirmed the adequacy of the denervation and the presence of marked supersensitivity to injected L-norepinephrine. Catechol-*O*-methyl transferase activity in the denervated myocardium was significantly less than in normal dogs, the mean value after denervation being 58 per cent of control (Table 1).

Table 1. EFFECT OF CHRONIC CARDIAC DENERVATION ON THE CATECHOL-*O*-METHYL TRANSFERASE ACTIVITY AND CATECHOL AMINE CONTENT OF THE LEFT VENTRICLE OF THE DOG

	Innervated	Denervated
No. of dogs	7	6
Catecholamine content:		
Mean ± *S.D.*	0·77 ± 0·148 μgm./gm.	< 0·03 μgm./gm. †
Range	0·57–0·98 μgm./gm.	< 0·03 μgm./gm.
Catechol-*O*-methyl transferase activity:		
Mean ± *S.D.*	0·12 ± 0·024 units *	0·07 ± 0·021 units †
Range	0·07–0·14 units	0·03–0·09 units

* 1 unit = 1 μmole L-norepinephrine metabolized/gm. tissue/hr.
† Significantly different from innervated ($P < 0·01$).

In other studies we have shown that the complete inhibition of catechol-*O*-methyl transferase by pyrogallol produces no potentiation and only moderate prolongation of the cardiac response to injected norepinephrine in the dog[6]. It therefore seems unlikely that a 42 per cent decrease in myocardial catechol-*O*-methyl transferase activity could account for the several-fold increase in cardiac sensitivity produced by denervation. Norepinephrine may also be metabolized by monoamine oxidase, but Burn *et al.*[7] have shown that this enzyme remains intact in the denervated foreleg arteries and iris of the cat. These investigations taken together, suggest that the supersensitivity of denervated organs to norepinephrine cannot be attributed to a marked loss of catecholamine-metabolizing enzymes from denervated tissue.

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Interference of Chlorpromazine with the Metabolism of Aromatic Amino-acids in Rat Brain

CHLORPROMAZINE (2-chloro-10-(3-dimethylamino-propyl)-phenothiazine, 'Largactil^R') and chlorprothixene (2-chloro-9-(ω-dimethylamino-propyliden)-thioxanthene, 'Taractan^R') counteract the changes in the monoamine content of rat brain induced by monoamine oxidase inhibitors, reserpine and 5-hydroxytryptophan[1]. It has been suggested that this might be due to reduction of the permeability of storage organelles for aromatic monoamines[1]. However, investigations have not yet been undertaken to determine whether chlorpromazine interferes also with the penetration of monoamine precursors (for example, aromatic amino-acids) into the brain. In this communication we will show that chlorpromazine interferes with the metabolism of 3,4-dihydroxyphenylalanine (dopa) and of 5-hydroxytryptophan in rat brain.

After fasting for 14–16 hr. Wistar rats weighing 70–130 gm. received chlorpromazine intraperitoneally and 30 min. later either 20 mgm./kgm. D,L-dopa-2-^{14}C or 75 mgm./kgm. unlabelled D,L-5-hydroxytryptophan subcutaneously. In the experiments with dopa, whole blood and brain were assayed for dopa-^{14}C, for catecholamine-^{14}C, as well as for carbon-14 in the remaining fraction, containing presumably phenylcarbonic acids, phenylglycols, glycine conjugates. This procedure (to be described elsewhere in detail) included hydrolysis of $HClO_4$-extracts by 'glusulase' (enzyme mixture containing glucuronidase, sulphatase; Endo Laboratories), fractionation on 2 micro-columns of 'Dowex' 50 × -4 ($pH = 6·5$ and H$^+$-form, respectively) and use of a Packard liquid scintillation counter. With this fractionation procedure the recovery of dopa-^{14}C and norepinephrine-2-^{14}C added to $HClO_4$-extracts was 95–100 per cent. The radioactivity found in 1 gm. (fresh weight) of brain or 1 ml. of blood was calculated for each fraction in per cent of the radioactivity administered per gm. of body-weight. After injection of 5-hydroxytryptophan the following procedures were carried out simultaneously: (*a*) immediate homogenization of the brains and spectrophotofluorometric assay of 5-hydroxytryptamine[2] (5-hydroxytryptamine rise *in vivo*); (*b*) anaerobic incubation of the intact isolated brains in wet chambers for 2 hr. at 37° C. before homogenization and determination of the 5-hydroxytryptamine rise occurring post mortem[3].

Experiments with dopa-2-^{14}C: In rats treated with 20 mgm./kgm. chlorpromazine 30 min. before administration of dopa-^{14}C the blood concentration of dopa-^{14}C was found to be remarkably higher than in control animals. Thus, in total blood the quotient dopa-^{14}C content of chlorpromazine-treated animals/dopa-^{14}C content of controls showed the following increase: 30 min. after injection of the amino-acid, 1·09; 60 min., 1·43; 90 min., 1·79; 120 min., 1·96. The effect of chlorpromazine was dependent on the dose of the drug (Fig. 1, above). In brain no significant change of dopa-^{14}C occurred after chlorpromazine within the intervals mentioned above and at different doses (Fig. 1, above). In consequence, there was a fall in the brain/blood ratio of dopa-^{14}C, indicating that dopa-^{14}C within the brain cells was relatively decreased by chlorpromazine. Even in doses as low as 2 mgm./kgm. chlorpromazine had a significant effect ($P < 0{\cdot}05$; *t*-test) (Fig. 1, below). Chlorpromazine did not decrease the brain/blood ratio of other carbon-14 labelled fractions significantly. The results with dopa-^{14}C suggest that chlorpromazine either reduces the uptake of this amino-acid by brain or increases its cerebral turn-over.

Preliminary experiments with 5-hydroxytryptophan-2-^{14}C gave results similar to those obtained with dopa-2-^{14}C.

Experiments with unlabelled 5-hydroxytryptophan: In rats treated with 5-hydroxytryptophan the brain 5-hydroxytryptamine increases *in vivo* as well as for several hours after death[3], probably due to continuing decarboxylation of 5-hydroxytryptophan. Chlorpromazine in doses of 5–20 mgm./kgm. injected prior to 5-hydroxytryptophan caused an inhibition of the 5-hydroxytryptamine rise in the brain *in vivo* as well as post mortem (Fig. 2). Since 5-hydroxytryptophan decarboxylase was found unaffected[4] in the brain of chlorpromazine-treated rats, this action of chlorpromazine might be explained by decreased penetration of 5-hydroxytryptophan to the cellular site where decarboxylation into 5-hydroxytryptamine occurs.

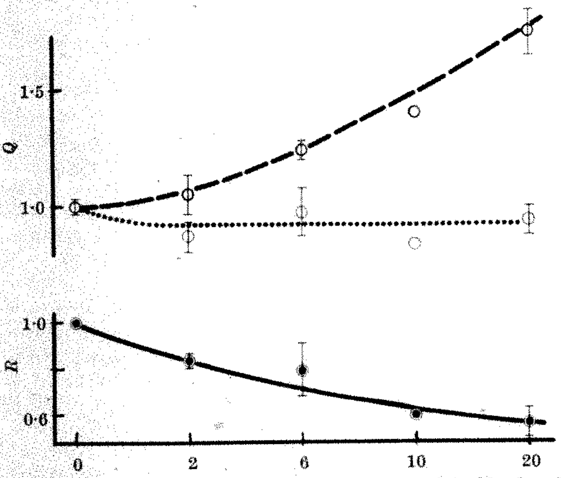

Fig. 1. Influence of chlorpromazine on dopa-^{14}C in blood and brain of rats. Abscissa: doses of chlorpromazine in mgm./kgm. Ordinate, above, Q indicates the following quotient:

$$\frac{\text{Dopa-}^{14}\text{C-content in chlorpromazine-treated rats}}{\text{Dopa-}^{14}\text{C-content in controls}}$$

– – – ○ – – –, blood; · · · ○ · · ·, brain

below: R means the ratio $Q_{\text{brain}}/Q_{\text{blood}}$. Chlorpromazine was injected intraperitoneally 30 min. prior to 20 mgm./kgm. D,L-dopa-2-^{14}C (specific activity 0·67–3·33 μc./μmol.) The animals were decapitated 1 hr. after subcutaneous administration of dopa-^{14}C. For the quotient Q chlorpromazine-treated animals were related to controls killed simultaneously. The ratio R was calculated for each individual experiment. The points (except that for 10 mgm./kgm.) represent means ± standard errors of three experiments, each experiment was carried out with pools of brain and blood respectively from three animals. Control values in percentage of dopa-^{14}C administered per gm. of body-weight; brain, 3·06 ± 0·24/gm. fresh tissue; blood, 8·00 ± 0·63/ml.

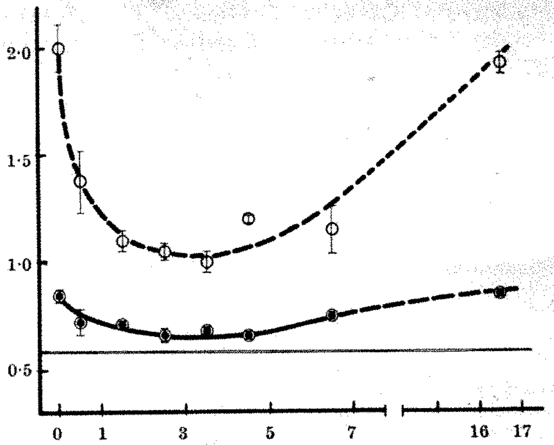

Fig. 2. Inhibition by chlorpromazine of the 5-hydroxytryptophan-induced rise of 5-hydroxytryptamine in rat brain *in vivo* and post mortem. Abscissa: Interval in hr. between intraperitoneal injection of 20 mgm./kgm. chlorpromazine and decapitation. Ordinate: 5-Hydroxytryptamine in μgm./gm. fresh brain. 75 mgm./kgm. of D,L-5-hydroxytryptophan were given subcutaneously 30 min. prior to decapitation. Time 0 represents animals receiving 5-hydroxytryptophan only. The points represent mean values ± standard error of 2–8 estimations of 5-hydroxytryptamine (each with pools of 2 brains). —⊙—, Brain homogenized immediately after decapitation; - - - ○ - - -, intact brains incubated at 37° C. for 2 hr. after death; =, untreated controls, brain homogenized immediately after decapitation

Inhibition of dopa- and 5-hydroxytryptophan-uptake by brain could partly explain earlier findings that chlorpromazine reduces the *in vivo* accumulation of cerebral monoamines due to administration of monoamine oxidase inhibitors or of 5-hydroxytryptophan[1]. There remain, however, other effects of chlorpromazine on monoamine metabolism which are probably due to interference with the permeation of monoamines, for example, inhibition *in vivo* of the reserpine-induced monoamine depletion of the brain[1], decrease in the uptake of epinephrine-^{14}C by several tissues[5,6], acceleration of the epinephrine-^{14}C-turnover in intact animals[7]. Thus, it may be concluded that chlorpromazine reduces *in vivo* the permeability of membranes responsible for the uptake of aromatic monoamines and possibly also that of aromatic amino-acids.

Interference of chlorpromazine with biological membranes has already been described for other substances *in vitro* and *in vivo*. Thus, *in vitro* the drug diminishes the uptake of 5-hydroxytryptamine by blood platelets[8], the water imbibition of mitochondria by thyroxine and other swelling agents[9], the uptake of glycine by brain slices[10], the binding of *N*-ethyl-3-piperidyl benzylate to mitochondrial granules[11] and the release of acetylcholine from storage organelles[12]. Chlorpromazine reduces *in vivo* the diffusion of trypan blue[13], the intestinal absorption of lipids[14,15] and sugars[16], dextran-induced œdema[17,18], the increase of permeability due to hyaluronidase[19], etc. Recent[1] and present findings support the idea that chlorpromazine might primarily affect the permeability of the membrane[9]. They show, furthermore, that this action of the psychotropic drug affects *in vivo* the metabolism of aromatic amino compounds that have been considered of special importance for brain functions.

It is unlikely that the interference of chlorpromazine with the penetration of aromatic amines and amino-acids is a consequence of hypothermia produced by the drug. The finding that chlorpromazine acts in the brain (kept at constant temperature) post mortem as well as *in vivo*, and the experiments *in vitro* mentioned here, do not support such an assumption.

We thank Mr. E. Bühler for skilful technical assistance and valuable contributions to the analytical procedure.

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HISTOCHEMISTRY

Starch-Gel Electrophoresis of Alkaline Phosphatases using a Histochemical Method

We have recently reported a technique for the demonstration by a histochemical method of phosphatase bands following starch-gel electrophoresis[1,2].

Examination of phosphatases of various organs have demonstrated the following: (1) That rat liver cells contain six separate glycerophosphatases. (2) That there are phosphatase bands which hydrolyse glycerophosphate, adenosine triphosphate and adenosine monophosphate, and there are other bands which are specific for either adenosine monophosphate or adenosine triphosphate. (3) Specific phosphatase bands were also found for diphosphopyridine nucleotide, triphosphopyridine nucleotide, glucose-6-phosphate, fructose-6-phosphate and hexose-diphosphate with, however, some overlapping of some of the bands.

In order to determine the spectrum of activity of a single glycerophosphatase a commercial preparation of calf intestinal alkaline glycerophosphatase (obtained from California Biochemicals) was subjected to starch-gel electrophoresis according to the foregoing technique[1,2]; and the preparation was given the opportunity to hydrolyse each of eleven separate phosphate esters (adenosine monophosphate, adenosine diphosphate, adenosine triphosphate, diphosphopyridine nucleotide, triphosphopyridine nucleotide, creatine phosphate, sodium β-glycerophosphate, glucose-6-phosphate, fructose-6-phosphate, glucose-1-phosphate and acetyl phosphate). The electrophoresis of this purified phosphatase demonstrated only one band of phosphatase activity which was active in hydrolysing all the substrates studied. At first we believed that this contradicted our findings on specific phosphatases or at least that intestinal phosphatase was different from other phosphatases. This was a distinct possibility, since Brandes *et al.*[3,4] had demonstrated that the brush border phosphatase of the intestinal cells lay between the microvilli outside the cell membrane. It was thus an extracellular enzyme and also, in view of its site, might be expected to be non-specific in its activity and to differ from the intra-cellular enzymes which are concerned with metabolic processes. However, Ahmed and King[5] in their discussion of placental phosphatases make the point that all the purified alkaline phosphatases studied to date (from kidney, intestine and placenta) hydrolyse all monophosphate esters non-specifically, but that polyphosphates and metaphosphates are not hydrolysed. On the other hand, Schmidt *et al.*[6] state that purified intestinal and kidney alkaline phosphatases hydrolyse all the phosphate esters studied (including poly- and meta-phosphates). Our results have shown that the single band of calf intestinal phosphatase in our starch-gel preparations actively dephosphorylates adenosine diphosphate and adenosine triphosphate as effectively within the limitations of the technique as it does adenosine monophosphate or glycerophosphate, or indeed as effectively as it hydrolyses any of the other phosphate esters.

Although we have not carried out investigations on homogenates of intestine to date, work on kidney homogenates has demonstrated specific phosphatase bands after starch-gel electrophoresis for certain phosphate esters. We did not find a single band which would hydrolyse all the esters presented to it. It seems possible, therefore, that the act of extraction and purification of a phosphatase may have altered the active centre of the enzyme in such a way as to

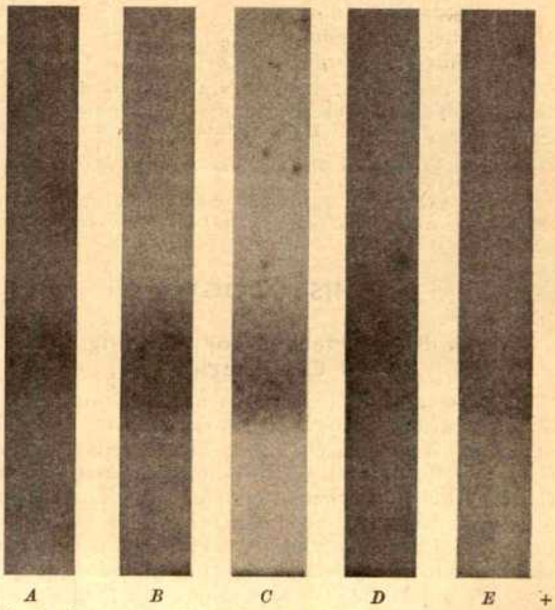

Fig. 1. Zymograms obtained with a purified calf alkaline phosphatase preparation. Note the same enzyme hydrolyses all the substrates. *A*, Incubated with fructose-6-phosphate as a substrate; *B*, incubated with glucose-6-phosphate as a substrate; *C*, incubated with creatine phosphate as a substrate; *D*, incubated with adenosine triphosphate as a substrate; *E*, incubated with adenosine diphosphate as a substrate

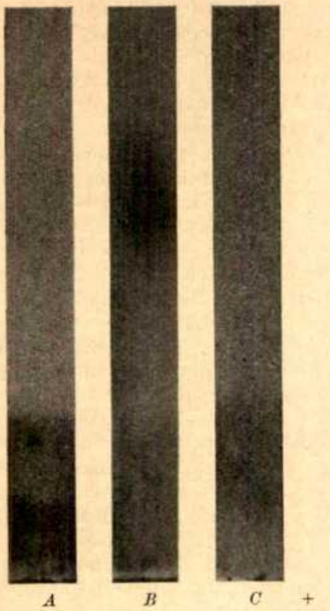

Fig. 2. Zymograms obtained with a rat kidney homogenate. Note the bands of enzymatic activity are different for each substrate. *A*, Incubated with sodium β-glycerophosphate as a substrate; *B*, incubated with adenosine triphosphate as a substrate; *C*, incubated with adenosine monophosphate as a substrate

render it non-specific. We believe this possibility should be allowed for in future investigation with purified enzyme preparations and underlines the fact that experiments conducted in test tubes on starch gel or any *in vitro* method should not necessarily be assumed to bear any relation to what goes on *in vivo*.

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HISTOLOGY

Sudan Black Method for Staining Sites of Calcification

IN 1958[1] a method was described for staining sites of calcification at the 'calcification front'—the edge of the preosseous matrix in bone, the cartilage around the hypertrophic cells of the epiphyseal cartilage and the junction of predentin and dentin. The method consisted, after fixation, in extracting the tissue with hot pyridine, decalcifying, embedding in gelatin, and staining with an alcoholic solution of sudan black *B* after sectioning on a freezing microtome. The sections were mounted in glycerine jelly. A fuller account of the method was published later[2].

This method has been used with success by Ponlot[3] and by Johnson (private communication). However, I have since found that the results after pyridine extraction may be somewhat erratic. At present the reason is not known; it may be due to differences in the quality of the various batches of pyridine used. Recent work[4] suggests that the sudanophil material may be lipid in nature, and that prolonged extraction with pyridine may remove it completely, partial extraction unmasking it and making it sudanophil.

A reliable method is to place the tissue, after fixation, in absolute alcohol for one day and then into benzene at 60° C. for 24 hr., after which it is again transferred to absolute alcohol. Then, after washing, decalcification and embedding are carried out. The sudanophil material is apparently not removed by this procedure.

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PATHOLOGY

Seromucoid-Levels in Osteolathyrism in the Rat

SCHWARTZ[1] has recently reported significant increases in mean levels of serum mucoproteins in lathyric rabbits, and has interpreted these as reflecting an increased depolymerization of ground substance. Grant, Hathorn and Gillman[2] have reported increased levels of serum mucoproteins in rats made lathyric by feeding sweet pea diets. They have suggested the occurrence in these animals of an increased synthesis and discharge of mucoproteins by the liver, associated with a decreased rate of utilization of mucopolysaccharide by the aorta and other connective tissues during synthesis of new fibrillar components. Our own unpublished observations of seromucoid-levels made over a period of years in a number of series of rats in which osteolathyrism had been produced by feeding β-aminopropionitrile are not in agreement with these reports.

Our findings have indicated that changes in seromucoid-level do not uniformly occur in osteolathyrism and, when present, are transitory and seem to result from a re-enforcement by the lathyrogenic agent of a stressful situation to which the animals were subjected—such as might result from a change in living quarters—rather than being a specific effect of the disease itself. It has previously been suggested that elevated serum glycoproteins may represent a response to non-specific stress[3].

All rats in our work were males of the Holtzman Sprague–Dawley strain and varied in age from 21 days in some experiments to 3 months in others. Experimental diets consisted of finely ground Rockland rat stock diet with additions of β-aminopropionitrile fumarate varying in amounts corresponding to 0·175–0·8 per cent of the diet, levels which are markedly to severely lathyrogenic when fed to rats. Control animals were fed unsupplemented stock diet. In the earlier experiments seromucoid was determined by the original method of Winzler *et al.*[4]. In recent determinations the modification of Weimer and Moshin was used[5]. Tyrosine content was used as a measure of seromucoid in all determinations.

Results obtained with older rats paralleled those from weanling rats. When the rats were caged in

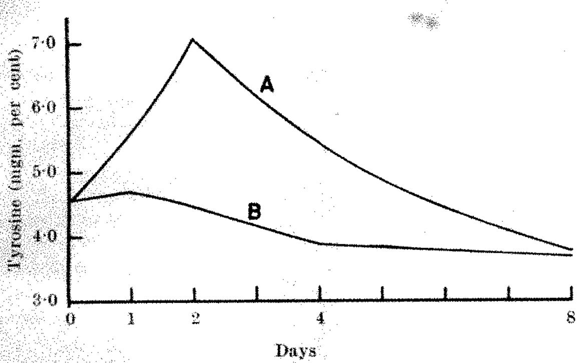

Fig. 1. Seromucoid-levels. Rats, 30 days of age on arrival, were placed six in a cage and fed stock diet. After 3 days they were placed into different individual cages (day 0, on chart). Curve *A*, rats receiving 0·4 per cent β-aminopropionitrile fumarate from day 0. Curve *B*, rats receiving normal diet. Curves were plotted from means of values obtained from individual sera of 5 or 6 rats per group (blood obtained by heart puncture under ether anæsthesia, each rat used only once)

groups of 3–6 after their arrival from the supplier, then distributed to different individual cages at the beginning of the experimental period, transitory rises in seromucoid-levels occurred in the groups receiving the diets containing β-aminopropionitrile, reaching a maximum after 2 days and declining gradually to coincide with control-levels at 8–15 days after the beginning of the experiment (Fig. 1). Individually caged rats which were accustomed to their individual quarters before being changed to the experimental diets showed no such elevations in seromucoid-levels. In fact, when young rats were placed in their experimental cages in pairs immediately on arrival from the supplier, then started on experiment on the following day without a change in quarters, the added 'security' afforded by the cage-mate apparently prevented even a transitory rise in seromucoid-level even though the period of acclimatization was minimal (see Table 1). Acclimatized rats showed no significant differences from normals for periods up to 28 days, our longest experimental period.

The explanation for the sustained elevation in the serum mucoprotein fractions in experimental lathyrism reported by others[1,2] is not apparent. Their 'mucoprotein' fractions appear to represent the same perchloric acid-soluble and phosphotungstic acid-insoluble fraction as our 'seromucoid'. Species or strain differences might conceivably be at least a partial explanation for the differences in results. It may also be pertinent to point out that the experimental diet used by Grant *et al.*[2] differed appreciably from their control diet in other aspects besides lathyrogenicity. It has been shown that the levels of certain blood constituents may be more dependent on other variables in the diet than they are on lathyrogenic activity[6]. Even the symptomatology of osteolathyrism can be affected by dietary changes not involving changes in the level of the lathyrogen[7–10]. Whatever the explanation for the differences between our results and those of others may be, it seems apparent that elevated seromucoid-levels are not a necessary concomitant of osteo-lathyrism in the rat. This finding does not necessarily imply, however, that mucopolysaccharide metabolism is not affected in osteolathyrism.

Table 1. Seromucoid-Levels in 30-day-old Rats placed Two in a Cage on Arrival and started on Experiment the Following Day (Day 0) without changing Cages

Day	Seromucoid in mgm. per cent tyrosine*	
	Experimental†	Normal
0	—	4·66
1	4·98	5·49
2	4·07	3·38
4	3·46	3·33
7	10·55‡	9·20‡
14	4·72	4·78

* Each value was determined in quadruplicate on the pooled sera from 6 rats (blood obtained by decapitation).

† The experimental diet contained 0·25 β-aminopropionitrile fumarate.

‡ The reason for the high values in both groups on day 7 is not known. The same factors, whether analytical or environmental, apparently affected both results. The rats were housed in a room that was used by several groups of investigators and contained also other species of small animals.

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Antibrain Antibodies of the 19*S* γ-Globulin Type in Rats with Allergic Encephalomyelitis

It is known that complement-fixing antibrain antibodies can be detected in rats and other animals with allergic encephalomyelitis following sensitization to nervous tissue plus Freund's adjuvant[1]. Although these complement-fixing antibrain antibodies fix complement with autologous brain antigen[2], failure to transfer passively allergic encephalomyelitis with immune serum and the lack of correlation between antibody titre and occurrence of disease in individual animals have left the role of such antibodies undefined[1,3]. This communication summarizes additional studies which indicate that complement-fixing antibrain antibodies in rats appear to belong to 19*S*-type γ-globulins and are incapable of giving passive cutaneous anaphylaxis.

Attempts to elicit passive cutaneous anaphylaxis were made using the technique of Ovary[4]. Rat sera tested for passive cutaneous anaphylaxis were from animals sensitized to guinea pig spinal cord-adjuvant and had been assayed previously for antibrain antibody by the quantitative complement fixation technique described in detail elsewhere[5]. Test sera (undiluted or diluted) were injected intracutaneously in 0·1-ml. amounts over the back of normal albino rats each weighing approximately 200 gm. These test rats were challenged intravenously 1–3 hr. later with rat brain antigen and Evans blue dye. The type of brain antigen used for challenge consisted of either 'detergent solubilized' lyophilized rat brain in physiological saline or a 20 per cent rat brain homogenate in saline (clarified by low-speed centrifugation and heated at 80° C. for 30 min. to eliminate thrombo-

Table 1. ATTEMPTS TO ELICIT PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN RATS WITH ANTIBRAIN SERA

Rat immune serum tested*	Serum antibrain antibody content	PCA results (sera tested in dilutions indicated)		
		undiluted	1:10	1:100
*C*537	29†	0	NT	0
*D*596	44	0	NT	0
*D*614	41	0	NT	0
*D*774	35	0	NT	0
*D*789	36	0	NT	0
*D*791	26	0	NT	0
*C*528	24	0	0	NT
*C*529	16	0	0	NT
*C*535	16	0	0	NT
*E*704	41	0	0	0

* Sera obtained from rats 11–29 days after sensitization to guinea pig spinal cord-adjuvant.

† Maximum number of 50 per cent hæmolytic units of complement ($C'H_{50}$) fixed, of exactly 100 $C'H_{50}$ available, by 0·25 ml. serum and rat brain antigen diluted 1 : 250.

NT, Not tested.

plastin activity). Each test rat received 6–113 mgm. of brain (dry weight) for challenge. Serum test sites were inspected for blue staining (positive passive cutaneous anaphylaxis reaction) 1–2 hr. after intravenous injection of brain antigen and dye. Several passive cutaneous anaphylaxis experiments are shown in Table 1. Each of the 10 sera tested contained antibrain antibody, fixing between 16 and 44 units of complement *in vitro* with brain antigen. All 10 sera gave negative passive cutaneous anaphylaxis reactions when tested either undiluted or diluted 1 : 10; or 1 : 100. Similar attempts to elicit passive cutaneous anaphylaxis in guinea pigs, a more sensitive test animal, were carried out using 14 test sera from guinea pigs or rats sensitized to homologous spinal cord-adjuvant, respectively. None of these 14 sera gave positive passive cutaneous anaphylaxis reaction.

From the work of Ovary *et al.*[6] it seemed likely that our inability to elicit passive cutaneous anaphylaxis with rat immune sera might be explained, at least in part, if the antibrain antibodies in question were of the 19*S* γ-globulin variety. In order to clarify this point, immune rat sera known to contain antibrain antibody were treated with 2-mercaptoethanol, a procedure reported to inactivate 19*S*-type antibodies[7–9]. Immune sera were mixed with an equal volume of 0·2 *M* 2-mercaptoethanol, or dialysed against 0·1 *M* 2-mercaptoethanol, at 4° C. for 1–4 days. The treated sera were then dialysed against physiological saline for 3 days and reassayed for their capacity to fix complement with brain antigen. Representative results are shown in Table 2. Before 2-mercaptoethanol treatment, the 11 rat serum samples and the immune serum pool fixed from 14 to more than 67 units of complement with rat brain antigen. The capacity of all sera to fix complement with rat brain was regularly abolished by 2-mercaptoethanol treatment. This striking effect of 2-mercaptoethanol was noted with 6 additional rat immune serum pools as well as with 13 other individual rat sera. Since 19*S*-type antibody has been reported by Ovary *et al.*[6] to prevent detection of 7*S*-type antibody in passive cutaneous anaphylaxis, 6 immune rat sera were tested for their capacity to elicit passive cutaneous anaphylaxis after mercaptoethanol treatment. All 6 sera failed to give passive cutaneous anaphylaxis reactions, suggesting that they contained little, if any, 7*S*-type antibrain antibodies.

Table 2. EFFECT OF 2-MERCAPTOETHANOL (2-*M*) TREATMENT ON ANTIBRAIN IMMUNE RAT SERA

Rat serum tested*	Units of complement fixed by serum and brain antigen	
	Before 2-*M*	After 2-*M*
*E*210	14†	1
*E*563	> 67	2
*E*565	65	1
*E*566	18	0
*E*567	> 57	0
*F*310	38	0
*F*311	44	0
*F*312	> 63	0
*F*206	25	1
*F*207	33	4
*F*208	36	8
Pooled sera (> 50 rats)	55	5

* Individual serum samples obtained from rats 17–21 days after sensitization to guinea pig spinal cord plus Freund's adjuvant; serum pool prepared from sera obtained from rats 7–41 days after sensitization.

† Maximum number 50 per cent hæmolytic units of complement ($C'H_{50}$) fixed, of exactly 100 $C'H_{50}$ available, by 0·25 ml. serum and rat brain antigen diluted 1 : 250.

Direct evidence for the high molecular weight nature of the complement-fixing antibrain antibodies under study was obtained by density gradient ultracentrifugation of one immune rat serum known to fix 80 units of complement with rat brain antigen. Following ultracentrifugation the fractions which contained less than 20 per cent of the total protein and all the 19*S*-type molecules fixed 84 units of complement. No significant fixation of complement could be detected by the fractions containing the remaining slower sedimenting molecules of the 7*S* variety.

Our data indicate that complement-fixing antibrain antibodies, produced by rats sensitized to guinea pig spinal cord and adjuvant, are high molecular weight γ-globulins. This finding accounts, at least in part, for failure of such antibodies to give passive cutaneous anaphylaxis. The observations reported here are of particular interest in view of work by others showing that positive passive cutaneous anaphylaxis reactions can be obtained with sera from animals with auto-immune thyroiditis or with human thyroiditis sera containing antithyroid antibodies of the 7*S*-type[6,10]. Our interest in the nature of the complement-fixing antibrain antibodies in rats, described in this communication, has been heightened by our recent finding that passively administered rat immune serum containing high-titre complement-fixing antibrain antibodies exerts a striking suppressive effect on development of allergic encephalomyelitis in actively sensitized recipients[11].

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RADIOBIOLOGY

Chromosomes of Two Cases of Human Chronic Myeloid Leukæmia

THREE reports have described an abnormally small chromosome (Ph^1) replacing one of the four smallest, acrocentric autosomes in the chromosome complement of cells from patients with chronic myeloid leukæmia[1-3]. The Ph^1 chromosome has been found in cultured peripheral blood or bone marrow cells from twenty patients with chronic myeloid leukæmia, ranging from untreated cases to those in terminal, acute stages, and a causal relationship between the chromosome abnormality and the disease has been suggested. Tough *et al.*[3] failed to find the Ph^1 chromosome in five cases of this leukæmia. Two of these had entered an acute leukæmia phase where diverse chromosome changes can be expected, whereas the other three cases had in common an unusually long history after diagnosis and followed a relatively benign course. Two of these latter cases had a previous history of radiotherapy for ankylosing spondylitis and there was a high probability that these leukæmias were radiation induced.

Two cases of leukæmia, which add to these findings, have been examined in this laboratory. The chromosome investigations were made on peripheral blood cells which had been cultured for two days by a modified form of the method of Moorhead *et al.*[4]. Case 1 is a 49-year-old woman who was recently diagnosed as chronic myeloid leukæmia, and was untreated at the time of the chromosome studies. The Ph^1 chromosome was found in 16 of the 27 cells which were examined (Table 1). This is a further example of the few cases which show that the presence of Ph^1 is not the result of any therapy measures causing chromosome breakage.

Table 1. CHROMOSOME COUNTS IN TWO CASES OF CHRONIC MYELOID LEUKÆMIA

Case no.	Chromosome counts 40 —	45	46	47	Total	Ph^1 present	Ph^1 absent	Ph^1 ?
1	1	1	24	1	27	16	10	1
2	–	–	53	2	55	0	49	6

Case 2 is a 49-year-old man whose white cell count was first found to be high in 1956, and fluctuated afterwards with an overall tendency to increase. In 1959, sternal bone marrow cells showed a marked granulocytic hyperplasia with a particular increase in myelocytes and metamyelocytes consistent with a diagnosis of asymptomatic chronic myeloid leukæmia. An increased white cell count and splenic enlargement were successfully treated with 'Myleran' in 1960. This patient has a long varied medical history, and since 1944 has received a minimum of 33, and probably more diagnostic irradiations from which it has been estimated he has received a total skin dose of at least 180 r. Although the dose is small compared with those given in radiotherapy, the possibility that this leukæmia was radiation-induced cannot be dismissed. The Ph^1 chromosome was not found in any of the 55 cells, in which the small acrocentric chromosomes were studied (Table 1). This case appears to be of the same nature as the three cases described by Tough *et al.*[3], and supports their view of the existence of a distinct sub-group of chronic myeloid leukæmia characterized by unusually slow progression and absence of the Ph^1 chromosome.

The existence of such a group raises a problem as to the relationship of the Ph^1 chromosome to chronic myeloid leukæmia. If this leukæmia can occur without the Ph^1 abnormality, the latter can then be only one of its causes and, indeed, a different, more fundamental cause common to all cases of chronic myeloid leukæmia might be suggested. Alternatively, the malignant nature of the clinically benign cases without the chromosome abnormality may be doubted; the blood abnormality in these may have the nature of a long-term leukæmoid reaction, or it may be a pre-malignant stage, malignancy being consequent on the occurrence of the Ph^1 chromosome abnormality. The greatest interest of this group of cases lies in the possibility that they represent an elementary stage in the ætiology of the disease and that progression from their present malignant, but benign, course awaits the development of the Ph^1 chromosome abnormality. The Ph^1 chromosome is not therefore a primary event causing the leukæmia, but is a secondary effect of the neoplastic condition which has some other cause. Secondary chromosome stem-lines, which are important in the progression of tumours, usually result from chromosome breakage and reunion, which occur in an essentially random manner, and consequently tumour stem-lines show marked individuality and are rarely if ever repeated. The Ph^1 chromosome is the only chromosome abnormality which has been found consistently in neoplastic tissue, and as such, it differs from known secondary chromosome changes. If it is a secondary effect, and the result of random chromosome breakage, its presence in all cases of chronic myeloid leukæmia would require a rigorous selection for this particular chromosome abnormality.

The specificity of the Ph^1 chromosome to a particular form of leukæmia is striking, whether it is primary and causal or a secondary development.

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Biogenesis of Glucobrassicin, the *in vitro* Precursor of Ascorbigen

RECENT work by Gmelin and Virtanen[1], concerning the isolation and elucidation of the structure of glucobrassicin, and its reaction with ascorbic acid in the presence of myrosinase to give rise to ascorbigen, prompted us to investigate some biochemical interrelations of indole derivatives in *Brassica oleracea* L. by means of labelled (radioactive) compounds. The main question in this connexion was why, in our previous communication[2], we were not able to prove the presence of glucobrassicin in our material. According to Gmelin[3], our method of working up the plant material was not suitable for demonstrating the presence of glucobrassicin in it. The reaction of glucobrassicin with ascorbic acid in the presence of

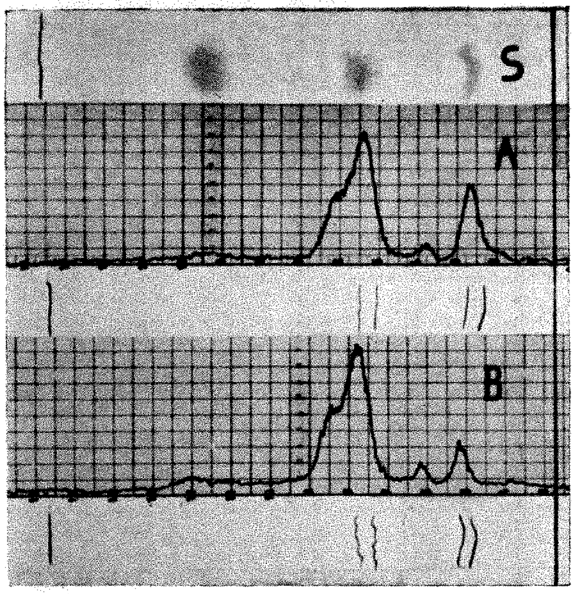

Fig. 1. Distribution of the radioactivity in chromatograms of the methanolic extracts of cabbage leaves given D,L-tryptophan-3-^{14}C. *S*, Chromatogram of standards: ascorbigen, tryptophan, glucobrassicin (in decreasing order of R_F values); *A*, radioactivity on a chromatogram (below) of the extract of leaves held in light; *B*, radioactivity on a chromatogram (below) of the extract of leaves held in darkness. Solvent system: *n*-butanol/acetic acid/water (4 : 1 : 3)

enzymes (myrosinase) set free in the damaged plant tissue is so rapid as to make impossible the proof of the presence of glucobrassicin in the fresh juice.

We have, therefore, tried another method of working up the plant material, suggested by Gmelin[3], namely, we first inactivated the myrosinase by heat, by placing the fresh, intact leaves in boiling methanol for 2 min. The methanol extract contained an appreciable amount of glucobrassicin; but we were not able to demonstrate either ascorbigen or 3-indolylacetonitrile.

When radioactive D,L-tryptophan-3-^{14}C (5·7 mgm. of specific activity 16·9 μc./mgm.) was administered to the plant[2] (50 gm. of fresh cabbage leaves) and the plant treated after 48 hr. with hot methanol, appreciable radioactivity was found in glucobrassicin (Fig. 1). In an experiment in which the plants were kept in the light, the synthesis of glucobrassicin from tryptophan proceeded more actively than in the plants held in darkness. This is in agreement with our earlier experiments[2,4], where more ascorbigen was formed from tryptophan in light than in the darkness. From these experiments at least two conclusions may be drawn: (1) ascorbigen is not present in the intact, living plant, but is an artefact formed in damaged or in stored, ageing tissue; (2) tryptophan is incorporated into glucobrassicin, which in certain circumstances[1] gives rise to a series of indole degradation products, notably ascorbigen, and relatively small amounts of indolyl-3-acetonitrile.

In order to prove whether the transformation of glucobrassicin to ascorbigen and 3-indolylacetonitrile is a purely *in vitro* degradation, or whether these reactions are involved in the metabolism of glucobrassicin, we have tried to ascertain experimentally whether the transformations referred to are reversible *in vivo*. Radioactive ascorbigen-^{14}C (ref. 5) (16·5 mgm. of specific activity 9·1 μc./mgm. per 85 mgm. of fresh Savoy cabbage leaves) and indolyl-3-acetonitrile-1-^{14}C (7·15 mgm. of specific activity 21·8 μc./mgm. per 20 gm. of fresh brussels sprouts leaves) were therefore administered to the plants. Radioactivity of ascorbigen was in the methylene bridge, connecting the indole nucleus with ascorbic acid. In neither case was glucobrassicin found radioactive after 48 hr. of incubation. In view of the physiological inertness of ascorbigen[6,7] and of its absence in the living and undamaged plant, we can conclude that it is physiologically unimportant. Many of our results, for example, those concerning the occurrence of ascorbigen in plants[8–11], the changes of its level during the vegetation period[12], its level in various parts of the plant[8,9,12,13], the influence of various external factors on its level in plants[4,13], must be understood as relating, in fact, to glucobrassicin. The question of the physiological activity of indolyl-3-acetonitrile seems more difficult to answer. We only can state that the reaction glucobrassicin→indolyl-3-acetonitrile is not reversible *in vivo*.

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BIOLOGY

Storage of Fœtal Sheep Skin and its Growth as an Autograft on the Lamb

SKIN has been removed from sheep fœtuses, stored until after the birth of the donor and then replaced as an autograft to a position where its subsequent development could be closely watched. The object of this was to by-pass any systemic factors peculiar to the latter half of gestation which might influence the development of the skin. Also, by having the developing skin on the surface of the post-natal animal, there are many opportunities for experimental work such as altering the rate of expansion and imposing various other conditions.

To remove pieces of skin from the developing fœtus general anæsthesia of pregnant ewes was induced with sodium pentothal and maintained with *cyclo*propane. Through a 6-in. para-median incision of the abdominal wall the uterus was exteriorized and the fœtus held against an avascular portion of the greater curvature of the uterus. A 2-cm. incision was made through the horn of the uterus and the fœtal membranes over an area of the fœtus judged by palpation to be the mid-side. From this region a small piece of fœtal skin, 1–2 cm.^{2} in area, was excised. In most cases the skin wound in the fœtus was left open and a length of braided nylon was inserted at the wound edge to assist in the identification of the operation site in the post-natal animal. Loss of amniotic fluid was negligible. The incision in the amnion was closed by picking up the edges with small artery forceps and then carefully rotating them so as to form a pedicle of the membrane. This pedicle was then ligated with linen thread and the uterine horn repaired with a continuous Lembert suture using No. 0 'Chromic' catgut.

Of seven 2-year old Romney ewes, subjected to surgery between 75 and 90 days gestation time, only two produced living lambs. One ewe died two days after the operation. Three aborted, the first 15 days, the second 28 days and the third 54 days after surgery. The second abortion consisted of twins, the fœtus untouched at surgery apparently living until the time of abortion, while the fœtus, from which the skin had been removed, was distinctly smaller and probably had been dead for some brief but indeterminate time before abortion. The third abortion was a mummified fœtus which had died at or shortly after surgery. The remaining ewe apparently resorbed her fœtus.

Compared with the high proportion of survivors in Schinckel and Ferguson's[1] experiment, the greater loss of fœtuses in our work may be associated with the method of exteriorizing the uterus and the holding of the fœtus against the uterine wall. The breed of sheep might also have some influence.

The piece of skin from each fœtus was divided into three: a small portion for histological examination and two larger pieces, some 50 mm.^{2} in area, for storage. These were frozen according to the technique of Billingham and Medawar[2], being soaked for 1–1½ hr. in a 15 per cent glycerol/Ringer solution before being slowly frozen to – 79° C. in quartz tubes within stoppered 'Pyrex' tubes. One piece was stored continuously at this temperature in solid carbon dioxide, while after 3 days the other piece was transferred to a liquid nitrogen store and kept continuously at – 195° C.

The first lamb was born normally at 148 days and received the fœtal skin grafts 26 days after birth, the skin having been stored for 87 days. The second lamb was produced by Cæsarian operation, the ewe's gestation time apparently being 154 days, 5 more than the normal mean. It may possibly have been born naturally if events had been allowed to follow their normal course. This lamb did not thrive well at first, and transplantation of the fœtal skin was delayed until the lamb was 98 days old and the skin had been stored for 165 days. In both animals the two pieces of skin stored at the different temperatures were grafted to opposite sides of the lamb on to areas prepared by the removal under local anæsthetic of areas of skin about 5 cm. in diameter. The grafts were placed in the centre of these areas and were covered with polythene sheet. Pressure was then applied with a wad of cotton wool under an elastic bandage.

All four grafts survived and expanded rapidly, being kept moist with isotonic saline so long as they were still of a pre-natal age. Wool fibres penetrated the skin and became clearly visible to the naked eye at times equivalent to fœtal ages of 106 and 108 days in the first and second lambs respectively. There was no marked difference between grafts stored at the different temperatures and between grafts stored for the different times. It is very doubtful if the extra work involved in maintaining a liquid nitrogen store can be justified.

These techniques should be of great value in the study of factors controlling the development of fœtal tissues. The results of Schinckel and Ferguson[1] and of Barnard[3] suggest that the main disadvantage encountered, that is, the poor survival of fœtuses, can be overcome.

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Experimental Evidence for Regeneration following Spinal Section in the Minnow (*Phoxinus phoxinus* L.)

Little is known of regenerative processes in the central nervous system of lower vertebrates. In fishes a few experiments have been made on the spinal cord. Koppányi and Weiss[1], Pearcy and Koppányi[2] and Hooker[3] cut the cord of *Carassius carassius* and *C. auratus* and reported the reappearance of locomotory movements after 8–10 weeks and cases of morphological regeneration. Hooker[4] transected the cord in young specimens of *Lebistes reticulatus* and claimed full co-ordination of movement in little over 4 days after the operation. Kirsche[5] carried out spinal section in adult *L. reticulatus* and reported both physiological and morphological regeneration of the cord 15 days after the operation. Like previous investigators he assessed physiological regeneration by observing the locomotory movements. He also reported a posterior twitch following electrical stimulation of the cord anterior to the section.

Two objections may be raised regarding experiments involving electrical stimulation and those involving estimation of co-ordinated motor activity. First, it is difficult to be certain that there is no spread of current when stimulating the spinal cords of such small animals. Secondly, it is difficult and sometimes impossible to be sure by direct observation that sinuous rhythmical movements throughout the length of the body of a fish are truly co-ordinated and not largely passive. In order to avoid these difficulties experiments were made which involved colour changes and the effects of a second spinal cut.

In the minnow the fibres controlling active aggregation of the melanophores pass from the medulla along the spinal cord, leaving the latter at about the level of the 15th vertebra to enter the sympathetic chain. From the chain they, or their post-ganglionic associates, reach the melanophores by way of the spinal nerves and the trigeminal nerve[6]. The existence of nerve fibres controlling active dispersion of the melanophores has not been satisfactorily demonstrated. If there are such fibres in the minnow, the evidence indicates that they follow a similar path to the aggregating fibres[7]. A minnow which is fully adapted to an illuminated black or white background and then subjected to background reversal changes colour very rapidly for the first 5–6 min. Thereafter it changes more and more slowly. After 15 min. the colour change is very advanced, but a total time of about 2 hr. is required for the complete change[8]. In these changes both nervous and hormonal mechanisms are concerned. The former may be eliminated by transecting the spinal cord anterior to the level at which the chromatic fibres leave it. If the operation is not carried out farther forward than about vertebra 8 the fish can generally still maintain an upright posture and so receive normal light stimuli on its retinæ. After the operation such chromatically spinal fish become dark, regardless of the background tint. However, an ability to change colour gradually returns and after about 10 days they are able to change slowly over a somewhat smaller range than before. These slow colour changes require about 3 days for their completion[8] and appear to be entirely under hormonal control[7].

In November 1959 thirty-two chromatically spinal fish were placed in illuminated black-and-white tanks and regular observations were made of their ability to change colour on background reversal. Colour changes were estimated by comparing the fish with slightly yellow-tinted black–grey–white cards based on the Ostwald series. The temperature of the water in the tanks varied from 10° to 18° C. in the course of a year.

For the first 4 months after the operation the fish showed a typical hormonal colour change, but in the fifth and sixth months the majority suddenly showed a rapid partial change and by the seventh month several had a rate and degree of colour change characteristic of the normal minnow with intact nervous system. A few, however, continued to change colour at the hormonal rate. The twenty-four surviving fish were kept until May 1961, that is, 18 months after the operation, to see whether those which had not developed a rapid rate of colour change in the first 7 months would do so after a longer time. However, it appeared that 7 months was the approximate limit and that there was no improvement after this time.

The spinal cord of each minnow was then transected a second time at a level about 2–3 vertebræ anterior to the original cut. Fish with rapid colour change darkened and after some days once more showed a slow colour change, typically hormonally controlled. It thus appears that in those fish which regained an ability to change colour rapidly after the first spinal section there had been some degree of re-establishment of nervous continuity in the cord. This was disrupted by the second cut.

So far as postural and locomotory reactions are concerned, changes following the original spinal section were not nearly so easy to judge in terms of nervous regeneration. Immediately after the operation the region posterior to the cut was usually immobilized and the animal was only able to progress along the bottom by means of a series of wags to the right and left of the anterior region combined with variably co-ordinated movements of the pectoral fins. These anterior wags were passively communicated to some extent to the posterior region. After some weeks the movements of the pectoral fins became better co-ordinated until many fish could use them very efficiently for progression through the water. The initial immobilization of the posterior region gradually gave place to activity. This typically first appeared as rhythmical to-and-fro movements of the tail fin and, later, of the entire tail. In time these became stronger and could, in many cases, raise the animal off the bottom and drive it through the water. Such movements have already been described[9]. Propulsive power came either from the region anterior to the spinal section or from the region posterior to it or from both regions at once, but the movement was obviously not co-ordinated throughout the animal. However, after some months it became, in many cases, increasingly difficult to be certain whether or not there was co-ordination. Finally, several fish swam so well that neither I nor impartial observers could detect any difference between them and normal minnows. It was easy to recognize the recovery of rapid colour change, but difficult to estimate the time necessary for this recovery of good swimming. However, the time appeared to be roughly the same in both cases and there was no very obvious improvement in locomotion after the first 6–8 months. Fish which could not swim well then never did so in the remaining 10–12 months of observation.

When the second section was made in the cord, co-ordination between the anterior and posterior regions was immediately lost. Here it is necessary to distinguish between the development of separate well co-ordinated movements in the anterior and posterior regions and a total co-ordination throughout the entire fish. It was this total co-ordination which was lost when the cord was cut for the second time. The separate well co-ordinated movements of the anterior and posterior regions appeared to be unaffected (Dijkgraaf[9] has reported a somewhat similar state of affairs), and several fish, immediately after recovery from the anæsthetic, were able to carry out locomotor movements which drove them efficiently through the water.

The second cut was made a little anterior to the first in order to allow a histological examination. This has no importance so far as the evidence from colour changes is concerned, but from the point of view of neuromuscular control it may well be significant. The observations on posture and locomotion following the second cut can therefore only be regarded as not contradicting the evidence obtained from colour changes. Definite evidence regarding the re-establishment of physiological continuity of fibres controlling posture and locomotion can only be given by a preparation in which the second cut is in the same position as the first. Series of fish are being prepared for this and other experiments and a full report will be published elsewhere.

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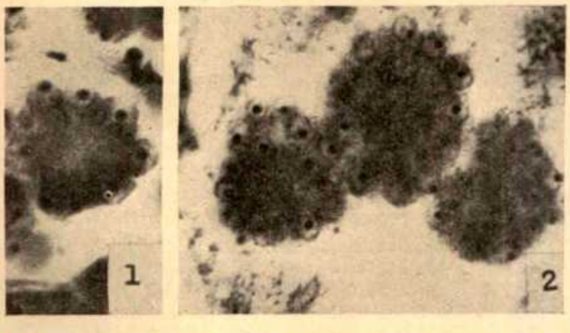
Spermatogenesis of a Cestode

THE interpretation of the structure of the spermatozoa of cestodes and of the process of spermatogenesis is subject to wide divergence of opinion. In 1935 Young[1] directed attention to the lack of knowledge regarding cestode structure and development, and among the main problems awaiting solution discussed the method of cell division and the structure of the sperm. Regarding the latter he wrote that "we have even less information than we have about most cestode tissues and until the spermatogenesis is known this ignorance will continue". His observations[2,3] and those of Child[4] "suggest a very simplified structure correlated with rapidity of development and the production of enormous numbers of spermatozoa"[1]. Watson[5] believed that the spermatozoon of *Gyrocotyle* is provided with a head. He did not, however, make a detailed study of spermatogenesis and described the sperm as "a slender thread tapering at the posterior end, with a well marked head, several times the diameter of the body". In a description of a figure of two spermatozoa he mentioned head, tail and acrosome. Presumably the acrosome is a short thread-like structure figured at the anterior end of the head. Since the publication of Young's paper[1] no further information on the spermatogenesis of cestodes has become available.

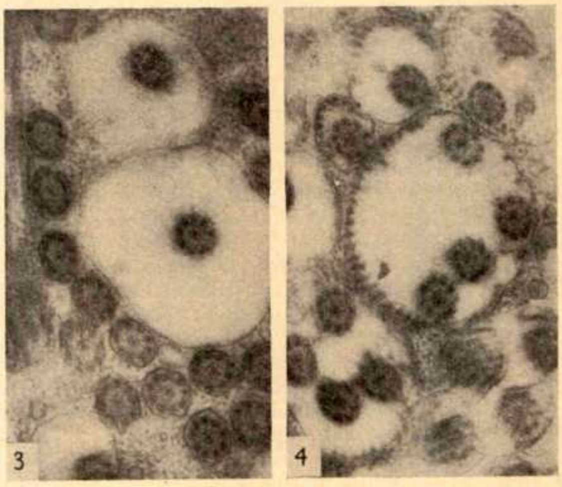
According to Young[2,3], the sperms of three species of cestodes "taper to a point at either end without any visible differentiation". On the other hand, Child[4] considered that the spermatozoa of *Moniezia expansa* and of *M. planissima* possess a head, derived from two granules in the nucleus. Child discussed earlier work, and pointed out that Sommer and Landois[6], for *Bothriocephalus latus*, and Leuckert[7] for *Taenia saginata*, stated that a small sperm-head is present. Other species were examined by Salensky[8], Sommer[9] and Moniez[10], who concluded that the spermatozoon does not possess a nucleus. Later, Roboz[11] described a minute structure which may represent the sperm-head of *Solenophorus megalocephalus*.

My examination of sections of the testes of *Proteocephalus pollanicola* Gresson[12] shows that in general spermatogenesis resembles that of trematodes[13-15] and indicates that 'the developmental processes' of cestodes are not, as Young suggested[1], markedly different from those of other animals. The primary spermatocytes occur in groups of eight cells whose nuclei contain Feulgen-positive material. These cells undergo the first meiotic division to form sixteen secondary spermatocytes (Fig. 1) that in turn divide to form a group of spermatids (Fig. 2). During spermateleosis the single large nucleolus (Feulgen-negative) disappears and the nucleus becomes spindle-shaped. At this stage Feulgen-positive granules are a prominent feature of the nucleus. The ripe spermatozoon is thread-like, possessing a Feulgen-positive nucleus and a long flagellum.



Figs. 1 and 2. 1, Section through a group of secondary spermatocytes (× 1,400). 2, Three groups of spermatids. At this stage the nuclei lie at the periphery of a mass of residual cytoplasm and the nucleolus is deeply stained (hæmatoxylin, × 1,000)

Electron micrographs of sections of the testes of *P. pollanicola* reveal the presence of electron-dense material in the nuclei of the spermatozoa and of a flagellum possessing an axial filament consisting of two central fibrils surrounded by nine double peripheral fibrils (Fig. 3). The two central fibrils lie in an area of moderate density limited by a thin membrane-like structure of higher density. Arms, similar to those first described by Afzelius[16], extend from the membrane to one of the members of each pair of peripheral fibrils. Each arm seems to be associated with the corresponding member of each pair of peripheral fibrils.



Figs. 3 and 4. Electron micrographs of tails of spermatids (× c. 32,500). 3, The axial filament is shown in the broad and terminal regions of flagella. 4, Sections of flagella to show the coiled or twisted condition of the axial filament

In micrographs of late spermatids the flagellum is often seen in transverse section as a relatively broad structure while in other cases it is narrow. Frequently micrographs of the broad flagella show two, three or four sections of the axial filament (Fig. 4). As the tails of ripe spermatozoa possess a single axial filament it is concluded that at a certain stage of spermateleosis the axial filament becomes coiled or twisted within the external sheath of the tail. In many cases fibril-like structures, frequently eleven or more in number, are spread out in contact with the inside of the external sheath (Fig. 4). Sometimes one or more of the peripheral fibrils of an axial filament, cut rather obliquely, appear to be continuous with a fibril or fibrils in contact with the sheath. Appearances suggest that not only is the axial filament coiled as a whole, but also that the individual fibrils are coiled independently of one another. Later, the axial filament uncoils and the sheath now closely surrounds the former throughout the whole or greater part of the length of the flagellum (Fig. 3). The above interpretation of the unusual appearance of the axial filament is put forward tentatively.

I wish to thank Associated Electrical Industries, Ltd., Barton Dock Road, Urmston, Manchester, for cutting ultra-thin sections, and taking the electron micrographs shown in Figs. 3 and 4.

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Post-harvest Ripening of Avocados

It is a common observation that fruits of many species ripen quickly if they are harvested but more slowly or not at all if left on the tree. An extreme case of this behaviour is that of the Fuerte avocado (grown in southern California) which will not soften while attached to the tree by a healthy stem[1]. Similarly, Florida avocados (var. Choquette and others) are stimulated to ripen when they are detached, but they differ from the California fruit in that most varieties eventually will successfully ripen on the tree. Recently, several objections[1] to the theory that ethylene plays an important part in fruit ripening have been eliminated, and it has been established that an active concentration of the ripening hormone accumulates within cantaloupes[2], bananas, mangoes and probably many other fruits[3] before or coincident with the climacteric rise. From the work of Biale[4] it is known that Fuerte avocados are stimulated to ripen by as little as 0·1 p.p.m. ethylene, and this same minimum concentration of gas is effective with most other fruits and plant tissues which have been investigated[5]. Therefore, a very small change in ethylene content induced after separation of the fruit from the tree could lead to an acceleration in ripening, and this possibility is investigated in the present communication.

Late-season Choquette avocados were picked in a local orchard and the internal gas was immediately sampled (within 10 sec. of harvesting) by immersing the fruit in water and withdrawing a few ml. of air from beneath the skin with a hypodermic syringe. The fruit was kept at 24° C. and additional samples were taken at frequent intervals during a 5-day period. Carbon dioxide content was determined by gas chromatography using a silica-gel column and a high-sensitivity katharometer, and ethylene was assayed with a flame ionization instrument following chromatography on an aluminium oxide column. These methods have been discussed elsewhere[3,6]; but it is important here to note that the smallest amount of ethylene which could be measured was approximately 0·01 p.p.m. in a 2·5-ml. sample of air. The production of carbon dioxide by the fruits was determined at frequent intervals with an infra-red gas analyser.

Results are shown in Figs. 1 and 2. Data for the changes in internal concentration of carbon dioxide have been omitted because in general the pattern only reflects the respiration-rate. However, the observation by Ben-Yehoshua *et al.*[7] that outward diffusion of carbon dioxide is restricted in post-climacteric avocados was corroborated by the fact that the ratio between the internal carbon dioxide content and the rate at which the gas was produced increased 2–4-fold during the 5-day period. The average internal concentration of ethylene at the time of harvest was approximately 0·04 p.p.m.; fruit number 4 had the highest value (0·064) and fruit number 3 the lowest (< 0·01). Directly after the avocados were picked there occurred a transient emanation of ethylene and a subsequent decline to a very low value within 4 hr. (Fig. 1). This was followed by a surge of ethylene production, clearly in advance of the pre-climacteric respiratory minimum. In Fig. 2 the time to reach 0·1 p.p.m. ethylene is indicated by an arrow, and this occurs within 9 hr. after harvest and at least 12–24 hr. before the onset of the climacteric rise. By that time the ethylene content exceeds 0·5–1 p.p.m., and it rapidly increases to a maximum value of 300–700 p.p.m. When the fruit is picked the barrier

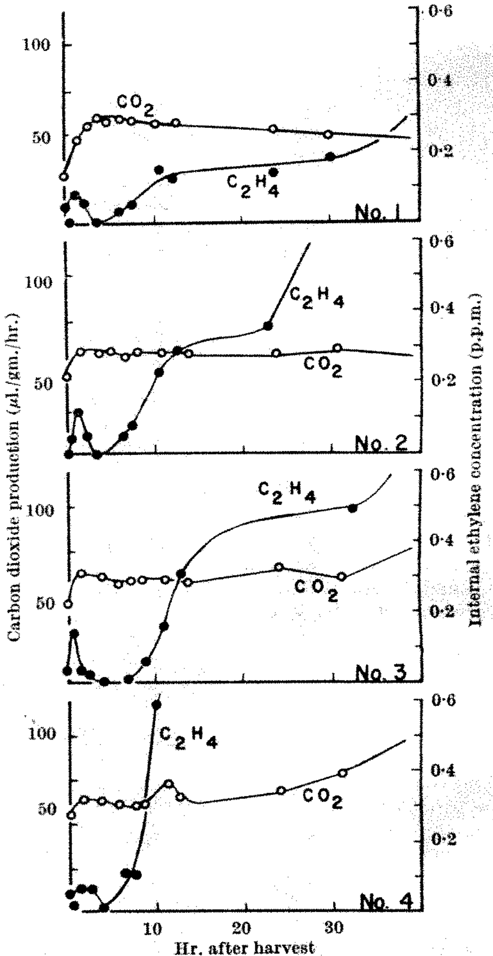

Fig. 1. Post-harvest changes in the respiration-rate and internal ethylene content of Choquette avocados at 24° C. The initial rate of respiration has been inferred from measurements of the internal carbon dioxide concentration taken during a 1-hr. period before the first infra-red gas analyser determination. It has been assumed that the barrier to outward diffusion of carbon dioxide remained constant throughout that interval

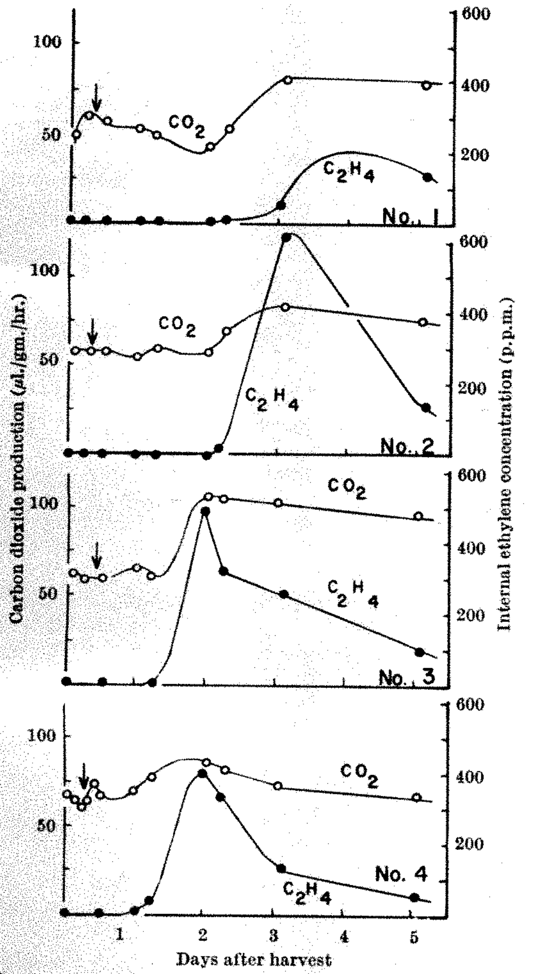

Fig. 2. Climacteric pattern of respiration and the associated changes in internal ethylene content of Choquette avocados at 24° C. The arrow indicates the time at which a 0·1-p.p.m. ethylene concentration has been reached (see Fig. 1)

to outward diffusion of ethylene is several fold lower than when it is ripe, but a rate of ethylene production of only 0·2 µl./kgm./hr. still suffices to create a physiologically active ethylene concentration (0·1 p.p.m.) and this clearly has been overlooked in previous work[1].

It is significant that ethylene could not be detected in early-season, full-sized Choquette avocados, and these fruits did not ripen properly unless they were gassed with ethylene. Since Biale has found that early-season avocados require higher concentrations of applied ethylene for a rapid climacteric rise than do late-season fruit[4], both the low production-rate and low susceptibility may work in unison to prevent fruit ripening on the tree at that time. That the production rate may be the critical factor is suggested by the experiments of Kidd and West[8] in which it was shown that a slight restriction in ventilation hastened the ripening of apples on the tree. Late-season avocados possess a quasi-stimulatory concentration of ethylene while attached to the tree, and tree-ripe fruit has a complete complement of the gas. Since the post-harvest acceleration in ripening is anticipated by a substantial increase in ethylene content, it follows that the Choquette avocado behaves at all times as if ethylene plays a vital hormonal part in its ripening processes.

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Interference by Tungsten with Uptake of Molybdenum by Plants

ALTHOUGH tungsten competitively inhibits the functions of molybdenum in bacteria[1,2], fungi[3], and poultry[4], it has been reported as substituting for molybdenum in legumes[5]. Several workers have reported stimulatory effects on plant growth by low concentrations of tungsten[6–8]; but the element is toxic above 5 p.p.m.[6]. We have investigated the effect of growing tomato plants in sand culture using a wide range of molybdenum/tungsten ratios, supplied as molybdate and tungstate: other elements were supplied from Hewitt's nutrient solution[9].

Chemical nutrients were purified by scavenging with copper sulphide[9], and the sand used was washed successively with nitric acid, ammonia and demineralized rain water. Despite these precautions, molybdenum deficiency symptoms were not observed in plants grown without added molybdenum (treatment 6). Negligible amounts of molybdenum and tungsten were introduced with the seeds, from which plants were grown for 200 days, after which the leaf tissue and seeds were dried, ground in a 'Perspex' mill and analysed for molybdenum and tungsten by neutron activation analysis[10,11]. After activation for 24 hr. the samples were wet ashed with perchloric and nitric acids in the presence of known amounts of molybdenum and tungsten carriers. The tungstic acid precipitate was purified and counted as described previously[11], and the molybdenum was separated from the supernatant liquid by a chemical procedure including two sulphide precipitations, extraction into diethyl ether from 6 *N* hydrochloric acid, scavenging with tungstic acid, ferric hydroxide and magnesium ammonium phosphate, and finally precipitation with α-benzoin oxime.

The p.p.m. of molybdenum and tungsten in the nutrient solutions, dry plant tops and seeds are given in Table 1: all figures are means of three replicate analyses. There was no obvious difference in growth, fruit production, deficiency or toxicity symptoms between the treatments.

Table 1. P.P.M MOLYBDENUM AND TUNGSTEN IN NUTRIENT SOLUTIONS, TOMATO PLANTS AND SEEDS

Treatment	Mo Solution	W Solution	Mo Plant	W Plant	Mo Seed	W Seed
1	0·19	< 0·001	37·8	0·10	17·3	0·011
2	0·19	0·0019	47·2	0·31	17·1	0·038
3	0·19	0·019	43·4	1·38	17·5	0·121
4	0·19	0·19	44·2	19·8	16·7	1·64
5	0·019	0·19	11·2	15·5	6·28	1·46
6	< 0·001	0·19	0·60	15·1	0·42	1·59

The mole fractions of molybdenum (molybdenum/molybdenum + tungsten) in the solutions, plants and seeds are given in Table 2.

Table 2. Mole Fractions of Molybdenum in Nutrient Solution, Tomato Plants and Seeds

Treatment	Solution	Plant	Seed
1	> 0·997	0·998	> 0·999
2	0·995	0·996	0·999
3	0·952	0·985	0·996
4	0·667	0·810	0·951
5	0·167	0·582	0·892
6	< 0·010	0·068	0·336

Summarizing the results in Table 2 we can say that the tomato plant, and especially the seed, discriminates against tungsten at every level tested. This discrimination is especially marked when tungsten exceeds molybdenum in the nutrient solution, in which case the combined uptake of the two elements is considerably depressed. These observations suggest that tungsten is not essential for the growth of the tomato plant, but that it may be able partly to replace molybdenum when the latter element is in short supply.

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Accumulation of a Distinct Macromolecular Component in Rye Embryos as a Result of Vernalization

Many attempts have been made to relate the phenomena of vernalization to chemical entities, particularly macromolecular components, such as nucleic acids[1], proteins[2], and related substances[3]. The results so far obtained have been either negative or inconclusive. The present communication reports a successful demonstration of the accumulation of an electrophoretically homogeneous macromolecular component in the vernalized embryos (and seedlings) of Petkus winter rye (supplied by F. von Lochow, Petkus Co., Bergen, Germany). This component was greatly reduced by devernalization and was absent from non-vernalized embryos.

Grains of Petkus winter rye were germinated in the dark in Petri dishes receiving full moisture supply and subjected to different temperature régimes (Table 1). The parts of the embryo above the base of the first internode were excised. This tissue was ground in the cold in a mortar with one half its volume of 0·05 *M* borate buffer (*p*H 9·2). This was further homogenized by hand in a glass homogenizer. Further extraction and analysis procedures have previously been described[4]. The results are summarized in Fig. 1 and Table 1.

Table 1. Concentration of Component V in Embryos attaining Different Sizes, as affected by Various Temperatures

(Total yield was calculated from the area of the electropherograms. Arrows designate transfer from one temperature to another)

Temperature (° C.)	Duration of treatment (days)	Average embryo weight (mgm.)	Component V as percentage of total yield	Total yield (mgm. per gm. embryos)
Small embryos				
26	7	4·9	0·6	11·3
20	7	4·8	7·0	8·4
18	7	3·7	3·7	11·7
14	9	3·5	8·1	7·0
2	8	3·0	12·1	12·7
2	20	3·6	17·5	9·4
2	40	8·7	43·7	14·4
2° (+30° 7 hr. daily)	9	4·0	4·0	7·6
Medium embryos				
26	7	14·4	5·3	—
20	11	23·2	19·5	12·7
18	7	14·2	11·9	11·5
14	14	14·4	23·2	10·5
2	20	17·1	37·1	12·8
2	40	30·0	49·8	15·2
2°, 20 days → 33°,	5 days	8·5	6·4	9·1
26°, 7 days →	2°, 18 days	14·9	46·5	13·5
Large embryos				
20	11	58·0	6·4	10·5
26°, 10 days →	2°, 18 days	62·0	45·1	6·9

As can be seen from Fig. 1, germination at 2° C. brings about the accumulation of a slow-moving, homogeneous macromolecular component. This component, having approximate mobility of $-2{\cdot}7\mu$ (corresponds to 1·3 mm. migration in Fig. 1), will be referred to as component V. This component is practically absent in the embryos germinating at 26°

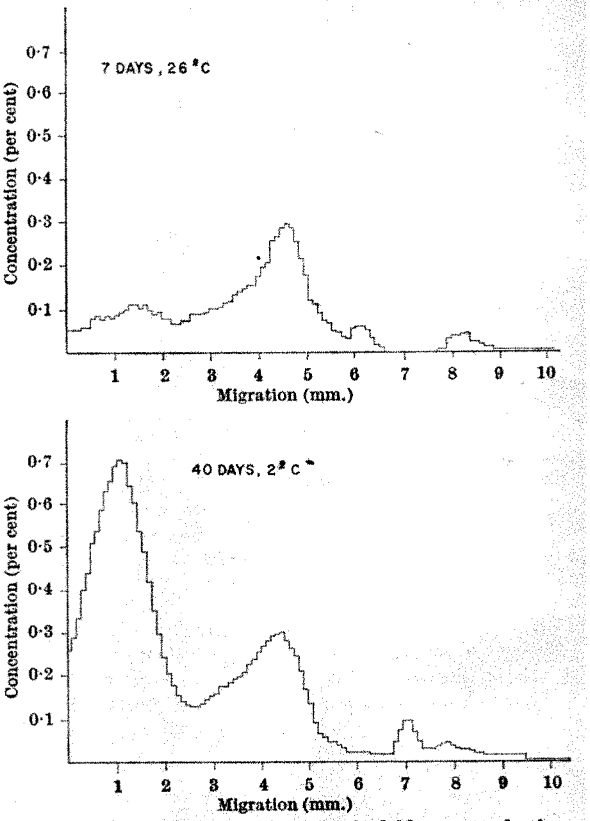

Fig. 1. Free-boundary electrophoresis of soluble macromolecular components from vernalized and unvernalized embryos of Petkus winter rye. Vernalized embryos (8·7 mgm.) were kept 40 days at 2° C. and unvernalized embryos (14·4 mgm.) 7 days at 26° C. Electrophoresis was carried out for 11 min. in 0·05 *M* borax *p*H 9·2 under 1·5 m.amp.

C. It is fairly evident from Table 1 that the accumulation of component V is closely associated with the phenomenon of vernalization. Since the rye embryos germinate and grow at different rates I was able to divide the seedlings, after 20 days at 2° C., into two groups; one with small seedlings having an average weight of 3·6 mgm., the other with larger seedlings having an average weight of 17·1 mgm. The concentration of component V was 3·5 times higher in the larger seedlings. An enhanced growth of the embryo is known to increase its response to vernalizing temperatures[5]. The gradual increase in component V at 2° C. from the 8th to the 40th day is in accordance with the kinetics of vernalization[6–8]. Similarly component V is greatly reduced under devernalizing conditions, that is, at 35° C. (ref. 9) and is absent from seedlings grown under non-vernalizing conditions—alternating high and low temperatures[10]. It is further evident from Table 1 that the accumulation of component V decreases with increasing temperatures and at 26° C. its accumulation is negligible. As the temperature is reduced from 26° to 14–20° C. component V increases. This reflects the increase observed in flowering response of *Hordeum bulbosum* as a function of decreasing growth-temperatures[11]. A re-investigation of the flowering response of Petkus winter rye in this range of temperatures seems warranted.

Parallel experiments under continuous light (100–200 foot-candles) in the low and higher temperatures were performed. Accumulation of component V was not affected significantly in any way by light.

Identical experiments with Petkus spring rye, obtained from the same source, showed that component V is also accumulated in this strain when exposed to low temperatures. It seems therefore reasonable that "the absence of flowering response (in spring varieties) when these are vernalized does not necessarily imply that the vernalization product is not formed in such varieties"[12].

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ENTOMOLOGY

Heterologous Transplantation of Corpora Allata between Lepidoptera Diapausing in Egg and Pupal Stages

WHEN corpora allata from the same species of Lepidoptera are removed and implanted into naturally diapausing pupæ or pupæ diapausing as a result of extirpation of the brain ('Dauer pupæ'), the recipients develop into moths or pupal–adult mixtures (second pupæ)[1–4]. The results obtained when donor and host are not so closely related are also of interest. Therefore, heterologous grafts of corpora allata between insects usually diapausing in the pupal and egg period respectively have been made and the effect on metamorphosis compared to homologous transplants.

Species used were *Hyalophora cecropia*, *Samia cynthia*, *Antheraea polyphemus*, and *Bombyx mori*. Except for *Bombyx mori*, the insects were divided into two groups. Group *A* were the pupæ maintained at 5° C. for more than six months; Group *B* were pupæ maintained at 5° C. for less than one month. The F_1 hybrids between strains *J*. 122 and *C*. 115 of *Bombyx* were deprived of pupal brain immediately after pupation. These operations were facilitated by immobilization at 5° C., and the procedure in other species was carried out under narcosis with carbon dioxide. Post-operatively the wounds were sealed, and the operated animals were maintained at 25° C. Reciprocal experiments were performed. In the first, 'Dauer pupæ' of the *Bombyx* silkworm 55 days of age were the recipient of transplants from the three wild species; in the second, corpora allata from *Bombyx mori* were implanted into diapausing, chilled, brainless pupæ of the wild silkworms.

When corpora allata from wild silkworms were implanted into 'Dauer pupæ' of *Bombyx* silkworms, only 3 moths emerged within 30 days in a total of 25 implanted; and 4 additional eclosions occurred within 60 days with 18 of the 25 undeveloped at the end of 60 days. Therefore prompt emergence only occurred in 20 per cent of the group in which *cecropia A* and *B* and *cynthia B* were donors. Although the number was relatively small, at least one moth emerged in all groups of donor preparations.

In the second experiment, second pupæ appeared when corpora allata from *Bombyx* were implanted into brainless *cecropia*, but they did not appear in any other group. Five similar preparations of *polyphemus* resulted in the emergence of one moth and no second pupæ. The remainder of the preparations showed no imaginal differentiation within 100 days after implantation.

In contrast to these results of heterologous transplantation of corpora allata, previous experiments[4] demonstrated that 73 per cent of the 'Dauer pupæ' of *Bombyx* became imagines within 30 days at 25° C. following homologous implantation of corpora allata derived from 'Dauer pupæ'. Also, Kobayashi and Yamashita[3] found that 17 of 20 'Dauer pupæ' of *Bombyx mori* emerged when corpora allata from 0-hr. pupæ of the same species were implanted, and 45 per cent of 'Dauer pupæ' became moths and no pupal–adult mixtures occurred when the corpora allata were derived either from *Bombyx* pupæ 5 days old or from moths. Williams[2] noted the formation of second pupæ in work on heterologous implants of corpora allata into brainless pupæ of *cecropia*. When corpora allata from *Bombyx mori* moths were implanted by Ichikawa and Takahashi[5] into diapausing

Table 1. EFFECT OF HETEROLOGOUS IMPLANTATION OF CORPORA ALLATA ON THE INDUCTION OF IMAGINAL DIFFERENTIATION OF 'DAUER PUPÆ' OF *Bombyx mori*

Donor		Pairs of corpora allata implanted	No. of 'Dauer pupæ' recipients	No. of moths emerged		No. of undeveloped pupæ 60 days after operation
				1–30 days	31–60 days	
cecropia	(*A*)	3	5	1	2	2
	(*B*)	3	5	1	0	4
cynthia	(*A*)	3	5	0	1	4
	(*B*)	3	5	1	0	4
polyphemus	(*B*)	3	5	0	1	4
Control (mock implantation)		0	5	0	0	5

A, Pupæ maintained at 5° C. for more than 6 months; *B*, pupæ maintained at 5° C. for less than 1 month.

Table 2. Effect of Heterologous Implantation of Corpora Allata derived from *Bombyx* Silkworm into Brainless, Diapausing Pupæ of Other Lepidoptera

Host		Operation	No. of preparations	No. of second pupæ	No. of moths emerged	No. of undeveloped pupæ in 100 days after operation
cecropia	*A*	EB	5	0	0	5
		Control	4	0	4	0
		EB + CA *	2	2	0	0
cecropia	*B*	Control	5	0	3	2
		EB + CA †	2	0	0	2
cynthia	*A*	EB	6	0	0	6
		Control	6	0	5	1
		EB + CA †	2	0	0	2
cynthia	*B*	Control	5	0	0	5
		EB + CA *	5	0	0	5
polyphemus	*B*	Control	5	0	2	3
		EB + CA *	5	0	1	4

EB, Extirpation of brain.
EB + CA, Three pairs of corpora allata implanted into each 'Dauer pupa'.
* Corpora allata from moths originating from 'Dauer pupæ'.
† Corpora allata from 'Dauer pupæ'.
Control, mock operation.

pupæ of *Philosamia cynthia ricini*, 3 emerged as moths and the remaining 17 became pupal–adult mixtures.

The results tabulated in Tables 1 and 2 show less action of heterologous implants than homologous corpora allata implanted into 'Dauer pupæ' of *Bombyx mori* previously reported. However, these F_1 hybrids of *Bombyx* emerge more often than the wild species when the latter are implanted with corpora allata from *Bombyx*. In the recipient group of *cecropia*, there is suggestive evidence that response to heterologous transplants occurs more often when the chilling is longer (Table 2) and that corpora allata derived from pupæ chilled longer may induce differentiation more readily (Table 1). However, the corpora allata which were implanted into wild silkworms chilled longer than 6 months were from *Bombyx* moths, whereas transplants from pupæ were transferred into those chilled for less than one month in this experiment with *cecropia*.

Pupal–adult mixtures are induced by hormones from both the prothoracic gland and from the corpus allatum[2]. The two functions of brain hormone, tropic action on the prothoracic gland and synergistic induction of metamorphosis with ecdysone[6], involve it in the appearance of these mixtures as well. Pupæ of wild moths chilled for a long period may secrete more brain hormone than those chilled for a short time; and the host may become a moth, pupal–adult mixture, or undeveloped pupa depending on the amount of brain hormone secreted in combination with hormone secreted from prothoracic gland and also from the corpus allatum following its implantation.

How much of the differences in results observed are due to failure of the implanted corpora allata to function in a foreign environment, the physiological state of the gland when extirpated, the stage of metamorphosis and sensitivity of the recipient, and possible variations in the hormone secreted by different species cannot be distinguished by preliminary data. However, it is apparent that the action of corpora allata differs when they are functioning in natural and host environments and that the physiological state of the host and donor may affect the result of transplantation.

This work was aided by grants from the U.S. National Institutes of Health and the American Cancer Society.

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Response of *Aedes aegypti* to Moist Convection Currents

The behaviour of *Aedes aegypti* in the vicinity of warm surfaces of various colours has been examined by mounting warmed copper bars (at 30°–45° C.) on the floor of a flight cage, covering them with cloth of different kinds and colours, and counting the number of alightments during a 5-min. interval. The insects were activated by human breath introduced through an opening midway between the test surfaces. The temperature was 20–25° C. and the relative humidity about 30 per cent.

It was found that cloth with high initial attractiveness to mosquitoes lost its attraction within 10–15 min. The attractiveness could be restored by washing the cloth or hanging it in the open air for a few days. The initial attractiveness depended in part on the colour—black was the most and white the least attractive—but even more on the material. Wool was nearly always very attractive initially, cotton moderately so, and synthetics like 'Terylene' almost unattractive. Bare copper was wholly unattractive.

The spectrum of the visible and ultra-violet light reflected from the surfaces was recorded with a Cary model 14 *M* spectrophotometer without revealing any differences sufficient to account for the difference between new and exposed cloth of the same kind, or between different cloths of the same colour.

In an attempt to examine the effect of surface texture, samples of cloth were ironed smooth. This reduced or wholly eliminated the attractiveness of all the samples, and suggested that guidance is brought about by warm, moist convection currents.

The experiments were repeated with samples of cloth which had been ironed, and then pre-dried in a desiccator or moistened in a humidity chamber. The results were striking. A pre-dried sample laid on a warm surface had little or no initial attraction. On the other hand, a humidified piece of black wool measuring 3 × 5 in. was very attractive initially, but after 15 min. it had lost both its attractiveness and about 0·25 gm. in weight. If a piece of wet filter paper was momentarily pressed on to its surface it immediately became attractive again, and if a piece of wet filter paper was placed between it and the warm under-surface it became very attractive indeed, and its attractiveness persisted for as long as the filter paper remained wet. Probably because of the larger specific surface, damp wool was more attractive than wool that was saturated and visibly wet.

A surface that was damp but not warm was as unattractive as one that was warm but not damp.

Water saturated with carbon dioxide but not warmed was also unattractive, but the presence of the carbon dioxide made other warm, wet surfaces in the chamber more attractive.

Thus it appears that *Aedes aegypti* are stimulated to fly in search of a warm-blooded host by an alteration in the ambient carbon dioxide level, and that they are guided to the appropriate surface partly by colour, but mainly by warm, moist convection currents rising from it.

This explains the decline in attractiveness of dark-coloured wool presented to the insects on a warm surface, and the poor initial attractiveness of a hydrophobic synthetic material like 'Terylene'. If the 'Terylene' was underlaid by wet filter paper it was as attractive as wool of the same colour presented in the same way.

There have been many attempts to isolate specific mosquito-attractive substances in blood or sweat or other biological fluids. The reported results have generally gone unconfirmed, and it seems possible that the difference in attractiveness of various solutions may have been related to the rate of evaporation of water from them. This rate of evaporation would be governed in part by the vapour pressure (a function of the concentration) but even more by the obstruction or otherwise of the surface by a surface film of some kind.

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MICROBIOLOGY

Demonstration and Possible Significance of Uricase in Human Pathogenic Fungi

Uricase has been demonstrated in several moulds, bacteria, and yeasts[1-4] and in animal tissues[5,6]. The elimination of uric acid as the chief waste product of nitrogen metabolism from birds and the isolation of certain human pathogenic fungi from soils in poultry pens and yards[7-9] have suggested the possibility that these fungi may be able to utilize uric acid as a source of nitrogen for growth.

Uric acid (Merck) was washed three times with warm distilled water and used for preparing a semi-fluid medium containing 10 gm. glucose, 5 gm. sodium chloride, 0·5 gm. magnesium sulphate, 1·0 gm. uric acid, 25 mgm. calcium chloride, 10 mgm. ferric chloride, 50 μgm. biotin, and 1·0 gm. washed agar (Difco) in 1 litre of 0·03 *M* phosphate buffer. Stock culture and routine media were adjusted to *p*H 7·0. Sodium thioglycolate was made 0·001 *M* in media for *Histoplasma*. The media were dispensed in screw-capped tubes, autoclaved, cooled, and inoculated with 5- to 10-day yeast-phase cells. The inocula were prepared by collecting cells from a solid stock culture medium[10] or from infusion blood agar slants, washing them four times with sterile phosphate-buffered 0·85 per cent saline at *p*H 7·0, and finally suspending them in the above uric acid media. The reactions of the experimental media were varied from *p*H 6·5 to 9·0 by altering the proportions of the constituent phosphates in the buffer solution.

The organisms used were *Histoplasma capsulatum* (A.T.C.C., 11407) the natural occurrence of which has a recognized correlation with avian species and poultry yard soils, and *Blastomyces dermatitidis* (N.I.H., 6046) which until recently, although resembling *H. capsulatum* in many respects, has never been demonstrated in soils from any source. (The improved and successful methods used by Denton *et al.*[11] may explain why previous attempts to isolate the organisms from soils failed[7,8,12].)

Since uric acid was the sole source of nitrogen available for growth in the media, growth was used as the indicator of uricase activity in the fungi, and was determined by measuring the turbidity (per cent transmittance) of cultures at 600 mμ in a Coleman Junior spectrophotometer. Un-inoculated media were incubated and used as controls and blanks.

Uricase activity was not great in any series of cultures (Fig. 1). The rather heavy growth which occurred within 8–14 days in the 27° C. cultures is not represented to advantage in the spectrophotometer because the mycelial phase cells were difficult to suspend evenly throughout the medium. (Twelve-day cultures of *B. dermatitidis* at 27° C. could have been recorded as 4-plus; growth of *H. capsulatum* was slower and somewhat less extensive.)

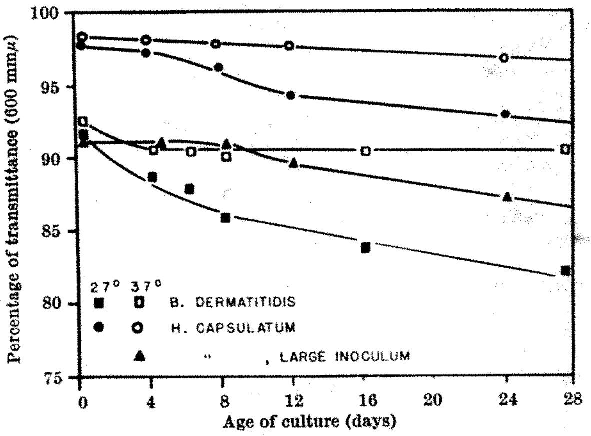

Fig. 1. Effects of incubation temperature on *B. dermatitidis* and *H. capsulatum* cultures in uric acid medium at *p*H 7·0

At 37° C., *B. dermatitidis* exhibited a slight initial decrease in per cent transmittance. This change does not seem significant when compared with the growth at 27° C. and with *H. capsulatum* cultures. If the change actually represents increase in cell numbers, then the increase probably occurred at the expense of traces of nitrogenous materials other than uric acid since such a change was not uniform among all cultures (Fig. 2).

Initially, growth of *H. capsulatum* was very slow at 37° C. (upper curve in Fig. 1). When the inoculum size was increased to that used in *B. dermatitidis* cultures, however, *H. capsulatum* grew out well following an 8-day lag period. This lag, similar to that which occurred initially in 27° C. cultures, is probably directly related to the oxidation-reduction potential of the medium and the thioglycolate added to it as a sulphydryl source. (Uricase activity is reported to be a strictly aerobic process.) Increasing the inoculum size has not as yet altered the slope of the curve representing 37° C. *B. dermatitidis* uric acid cultures.

Although the series of cultures indicated in Fig. 2 was not sufficiently varied to determine the precise

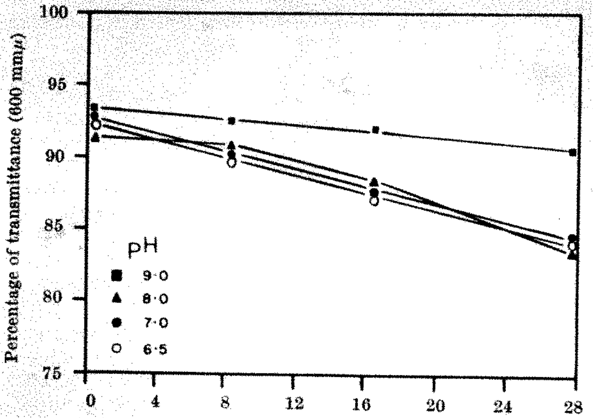

Fig. 2. Effects of *p*H on growth of *B. dermatitidis* in uric acid media at 27° C. (Similar results were obtained using *H. capsulatum*)

reaction for optimum uricase activity, it is apparent that growth was uniform and good from *p*H 6·5 to 8·0, but was strongly inhibited at *p*H 9·0. That the inhibition may have been the result of changes in the surface activity of the cell toward the uric acid molecule is indicated by: (1) the good growth of *B. dermatitidis* on routine laboratory media adjusted to *p*H 9·0; (2) the activity of extracted uricases at that reaction[5,6].

Since the reaction for optimum uricase activity could have differed from the mycelial to the yeast phase, thereby preventing growth in neutral media at 37° C. (a possibility which seemed remote), the reactions of 37° C. culture media were varied in an attempt to demonstrate growth. The results were negative (Fig. 3); no significant change occurred in any of the media.

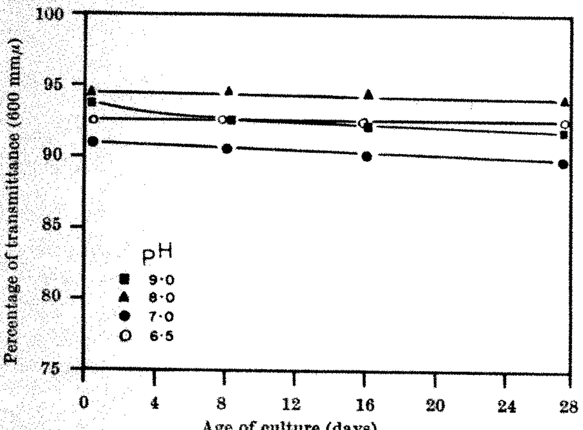

Fig. 3. Effects of *p*H on growth of *B. dermatitidis* in uric acid media at 37° C.

It appears that uricase may aid the survival of some human pathogenic fungi in soils containing uric acid from avian or other sources, and may be in part responsible for the unusual association of *H. capsulatum* with poultry. The fact that yeast-phase *B. dermatitidis* appears to be unable to utilize uric acid, although the mycelial phase utilizes it readily, stimulates some interesting conjectures on the necessity of yeast-phase cells shed from a host to undergo morphological variation in certain soil environments in order to survive. Additional investigations into the survival of pathogenic fungi in natural environments are at present in progress i this laboratory.

This is the first report, to my knowledge, of a apparent qualitative difference between the tw morphological phases of *B. dermatitidis* in activel growing cultures.

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Note added in proof. While this paper was in th press, I have demonstrated uricase activity in both culture phases of *Sporotrichum schenckii.*

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An Immobilization Reaction against *Balantidium coli*

An immunological test, the immobilization reaction, has been found to be positive for a number of protozoa[1]. I have found it useful in differentiating various species of *Entamoeba* and strains of *E. histolytica*[2]. However, it is with *Tetrahymena*[3] and *Paramecium*[4] that the reaction has been studied in greatest detail. Against *Paramecium* it is a highly specific and sensitive test. In view of these positive results with various ciliates, it was thought of interest to determine whether a positive reaction could be obtained with *Balantidium coli.*

The strain of *B. coli* used was isolated from the cæcal contents of a pig slaughtered at the Singapore abattoir. The strain after some initial difficulty grew well in *hsm*+*S* (ref. 5) and Balamuth's medium[6], kept at 37° C.

A rabbit was immunized by subcutaneous injections of a washed suspension of *B. coli*. It received approximately 80,000 cells in eight divided doses given at 4-days interval each. The serum was obtained a week after the last injection.

When the washed organisms were mixed with the fresh immune serum they were rapidly immobilized and lysed in a dilution of 1 : 5 in normal saline. However, when the serum was inactivated lysis did not occur and the process of immobilization was observed clearly. The inactivated antiserum in dilutions up to 1 : 10 in normal saline produced immobilization almost immediately. Inactivated normal rabbit serum used in the same dilution had no effect on the activity of *Balantidium.*

The ciliates treated with antiserum remained immobilized for 6–7 hr., after which they gradually disintegrated. Although they remained stationary during the period of this reaction, local ciliary movement continued with gradually diminishing activity, until final disintegration.

It is suggested that the reaction may be of value in the differentiation of various species of *Balantidium* and possibly in the diagnosis of Balantidial infection in man.

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Protective Action of Glycerol in the Freezing of *Shigella sonnei*

GLYCEROL has been used as a protective agent for frozen bacteria[1,2], *Leptospiræ*[3], trypanosomes[4], *Toxoplasma gondii*[5], *Entamoeba histolytica*[6], animal tissues[7], bull spermatozoa[4], and yeasts[8]. Although much work has been done on a variety of micro-organisms, little or no information is available regarding the protective action of glycerol on frozen *Shigella sonnei*. The purpose of the present investigation was to determine the effect of glycerol on frozen *S. sonnei* and also the effect of glycerol on cells frozen and thawed repeatedly. Four strains were studied: strain *CH*, obtained from Dr. Horace M. Gezon, University of Pittsburgh, Pittsburgh, Penna.; strains *B*–2501–3 and *B*–2506–3, obtained from the National Naval Medical Center, Bethesda, Md.; and strain 13–3, obtained from Dr. Arthur Abrams, Walter Reed Army Institute of Research, Washington, D.C.

Nutrient broth cultures of the four strains were grown for 48 hr. at 37° C. and mixed with sterile glycerol. The following glycerol concentrations were tested: 1, 2, 5, 10 and 20 per cent (final concentrations). The tubes containing the cells and glycerol were rotated in the palms of the hand to mix the contents thoroughly, then placed in the freezer (–20° C.), and allowed to freeze slowly. The frozen tubes were removed at weekly intervals for a period of 5 weeks; another series of tubes were frozen and thawed one, two, three, four, and five times and held frozen for a period of one week before the final thawing and plate counting. The bacterial populations were determined by removing 0·1 ml. of the cell suspensions, making an appropriate number of serial dilutions, and streaking 0·1 ml. of the final dilution over a nutrient agar plate and streaking with a bent glass-rod 'hockey stick'. These plates were inverted, incubated for 48 hr. at 37° C., and counted with the aid of a Quebec colony counter (Spencer).

All four strains of *S. sonnei* responded fairly uniformly to the freezing and thawing. A 10 per cent solution of glycerol produced maximum protection of the frozen cells. 5, 2 and 1 per cent glycerol afforded some protection, in terms of viability, but in an order of decreasing levels of protection. 20 per cent glycerol protected the cells at a level comparable with the 10 per cent level. Results of a typical experiment are given in Table 1. The bacterial population in the frozen preparations did not decrease significantly during the five-week storage period after attaining a stable population. This is in agreement with the observations of Clement[9]. Repeated freezing and thawing decreased the population of frozen cells substantially; the results are shown in Table 2.

Table 1. PROTECTIVE EFFECT OF GLYCEROL ON FROZEN *S. sonnei* CELLS*

Glycerol concentration (per cent)	Viable count (Initial population was $5 \cdot 9 \times 10^7$/ml.)	Fraction surviving (per cent)
0	$5 \cdot 8 \times 10^5$	0·98
1	$2 \cdot 2 \times 10^6$	3·7
2	$7 \cdot 7 \times 10^6$	13·0
5	$4 \cdot 8 \times 10^7$	81·3
10	$5 \cdot 4 \times 10^7$	91·4
20	$5 \cdot 0 \times 10^7$	84·8

* These data represent triplicate experiments.

Table 2. PROTECTIVE EFFECT OF GLYCEROL ON *S. sonnei* FROZEN REPEATEDLY*

No. of times frozen and thawed	Viable count (initial population was $5 \cdot 7 \times 10^7$/ml.)	
	Frozen in the absence of glycerol	Frozen in the presence of 10 per cent glycerol
1	$6 \cdot 0 \times 10^5$	$5 \cdot 5 \times 10^7$
2	$2 \cdot 1 \times 10^5$	$3 \cdot 5 \times 10^7$
3	$9 \cdot 5 \times 10^4$	$8 \cdot 9 \times 10^6$
4	$7 \cdot 7 \times 10^3$	$3 \cdot 3 \times 10^6$
5	$7 \cdot 7 \times 10^3$	$6 \cdot 6 \times 10^5$

* These data represent triplicate experiments.

Glycerol protected the cells against repeated freezing and thawing procedures; but the numbers did decrease slightly with each freezing. Additional work is being carried out to determine the effect of glycerol on the metabolic injury produced by freezing and thawing of *S. sonnei*. Metabolic injury has been reported by Straka and Stokes[10] in bacteria subjected to sub-zero temperatures. It would be interesting to follow up these investigations with the effects of freezing and thawing on the pathogenicity of *S. sonnei* in view of the findings of Szturm-Rubinsten *et al.*[11], who suspected a case of shigellosis transmitted by ice used in food which may have contained the *S. sonnei*.

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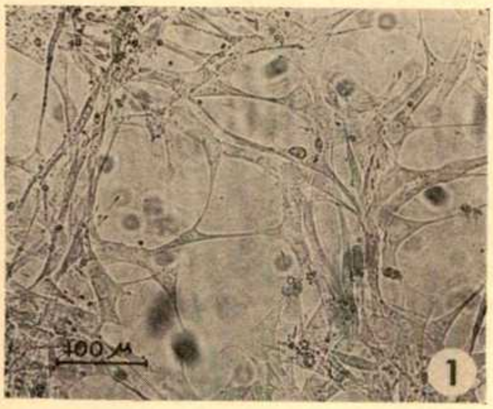
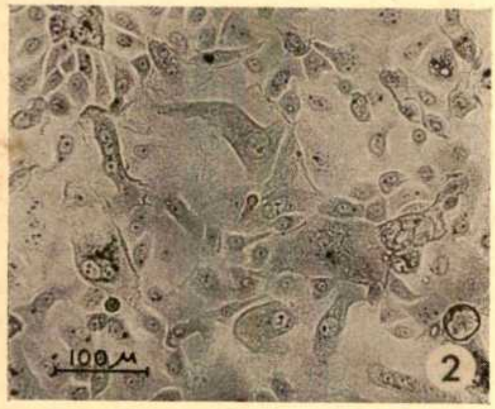
CYTOLOGY

Chromosomes in a Marsupial (*Potorous tridactylis*) Tissue Culture

The chromosome constitution of marsupial, *Potorous tridactylis*, was described by Sharman and Barber[1]. The males have five pairs of autosomes and three sex chromosomes. The low number of chromosomes plus the fact that the same investigators reported somatic pairing made this species desirable for genetic studies in tissue cultures.





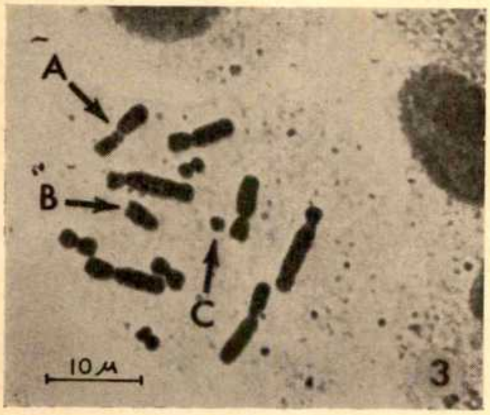


Fig. 1. Fibroblast-like testis cells of *Potorous tridactylis* in tissue culture

Fig. 2. Epithelial-like kidney cells of the same animal in tissue culture

Fig. 3. Somatic chromosome complement of a tissue culture cell of the male *Potorous tridactylis*. *A*, the *X*, *B*, the Y_1 and *C*, the Y_2 chromosomes

The animals were obtained through the courtesy of the Animals and Bird Protection Board. (We thank Dr. E. R. Guiler for help in obtaining these animals.) One male was killed and cultures were prepared from tissues of the brain, eye, lung, liver, kidney and testis with mammalian tissue culture methods[2]. Only the kidney and testis cells multiplied reasonably well; cultures of these have been actively growing through transfers for six months. The most satisfactory culture medium consisted of 90 per cent LEY (2 per cent lactalbumin enzymatic hydrolysate, 2 per cent yeast hydrolysate in Earle's salt solution) and 10 per cent fœtal calf serum. The cells adhered easily to the glass and eventually covered the surface of the culture bottle. Figs. 1 and 2 show the characteristic fibroblast-like testis cells and the epithelial-like kidney cells.

Chromosome examination of both types of cells was carried out on slides prepared in accordance with the technique reported by Ruddle *et al.*[3]. For the observation of somatic pairing pre-treatment with colchicine was not done.

In the kidney and the testis cultures both diploid and polyploid division figures were abundant. The chromosome complements were essentially similar to those reported by Sharman and Barber[1] for spermatogonial divisions. All five pairs of autosomes and the three sex chromosomes (X, Y_1, Y_2) were identifiable (Fig. 3). The X did not show the heteropycnosis described for spermatogonial divisions, but its characteristic centromeric structure was evident. Complete somatic pairing, as described for some spermatogonial metaphases, was quite rare in the tissue culture preparations; but some homologous chromosomes were frequently found close together at both prophase and metaphase. The low number of chromosomes as well as their clarity and individuality indicate that the *Potorous* material should prove suitable for cytogenetic studies with tissue cultures.

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Repression of Growth of Mammalian Cells under Agar

When disaggregated mouse embryo cells are first cultivated *in vitro*, the cells grow into a confluent layer of fibroblasts on the bottom of the Petri dish, and then begin to grow additional layers on top of the first. If the cells are transferred, subsequent subcultures will show less tendency to form multiple layers. This effect is accompanied by a diminution in growth-rate of the cells and may be described as a progressive decrease in their growth potential. This behaviour is to be contrasted with that of *L* cells, an established cell line derived from mouse fibroblasts, which show no diminution in their growth potential upon transfer, and retain their ability to grow out of the monolayer.

In order to see how growth would be affected in these two types of cultures by the application of a force which would oppose growth out of the plane of the monolayer, the following experiment was performed. Casts containing 4 per cent of agar in addition to growth medium were prepared by pouring the melted mixture into Petri plates. When cooled, these casts, which are rather stiff, though only 1–2 mm. thick, were removed with a spatula and placed over primary or secondary mouse embryo cell cultures in Petri dishes of the same size, and additional liquid medium was added. At daily intervals, the agar was removed from a plate, the cells trypsinized and counted and the counts compared with those of parallel monolayers incubated in the absence of agar. The results of such an experiment are shown in Fig. 1. The rate of cell growth under the agar is reduced, over a 6-day period, so that the final number of cells is usually only about one-third that of the control. Parallel examination of the fresh or stained cultures shows that the difference in growth-rate is most appreciable after the cultures have approached confluence. Evidently the principal action of the agar is to retard the growth of cells out of the plane of the monolayer.

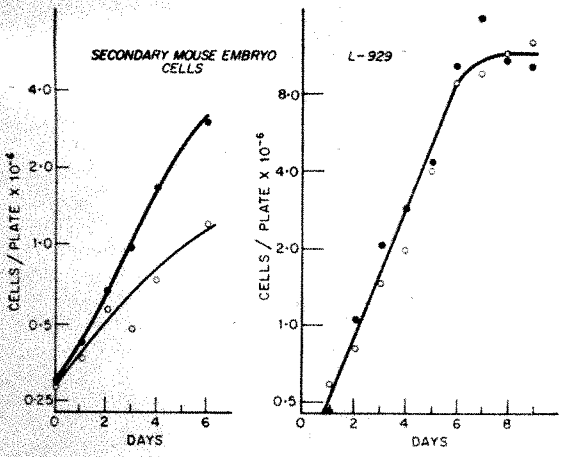

Fig. 1. Growth of secondary mouse embryo cells and *L*-929 under agar. Medium of Dulbecco and Vogt, containing 10 per cent calf serum. ●, Cells growing in absence of agar; ○, cells growing under agar layer

L cells do not show this effect. Fig. 1 demonstrates that the presence of a layer of agar does not appreciably inhibit the growth of *L* cells up to a density of 10^7 cells per plate (20 cm.^{2}). Microscopic examination shows that the cells form more than a single layer on the surface of the Petri dish as readily whether an agar layer is present or not.

Growth out of the plane of the monolayer can be prevented almost completely in the case of the *L* cells as well as the normal fibroblasts by increasing the pressure of the agar layer. Glass beads of a diameter of 0·2 mm. were found convenient for this purpose, as they are commercially available, easily washed and sterilized, and weighed aliquots in sterile tubes can be poured on to the agar layer and evenly distributed by gently tapping the Petri dish.

Growth of fibroblasts and of *L* cells under 5 or 10 gm. of glass occurs quite readily in the plane of the monolayer, but growth out of the plane of the monolayer is prevented. The cells appear somewhat flattened, small cell clumps are forced into a monolayer but few cells die, as judged cytologically. When 15 or 20 gm. of glass are placed over the agar, growth of *L* cells in the plane of the monolayer still occurs although there is more distortion of the shape of the cells than is the case under 5 or 10 gm. of glass. Normal fibroblasts, however, are not so resistant. A series of experiments was performed in which the growth of primary or secondary fibroblast cultures was followed by counting the number of fixed and stained cells on several Petri dishes just before agar and glass were applied, and three days later. In the case of the fibroblast cultures, growth was usually seriously affected under the higher quantities of glass, and 20 gm. was sometimes sufficient to prevent all growth. In some cases the number of cells actually declined, and many very distorted cell nuclei and degenerated cells could be seen. The growth-rate of *L* cells was not much affected by up to 20 gm. of glass in spite of distortion of cell shape.

It would seem most likely that the effects exerted by agar, or agar and glass, are due to the weight of these substances, though it is probably true that the agar slows the exchange of substances between the cells and the medium, especially of the larger molecules. This is also true of the glass, which immobilizes the water trapped between the beads.

The pressure exerted by the 4 per cent agar casts and the glass may be calculated from their density and that of the medium. The casts in medium weigh approximately 140 mgm. and the glass about 0·6 of its weight in air.

Under ordinary conditions of cell culture, there is no resistance to the growth of the cells, in at least one direction. This is rather different from the conditions in which animal cells grow *in vivo*. The present experiments suggest that cells in culture may differ from each other in behaviour when their growth is opposed by mechanical forces, very small forces readily inhibiting growth out of the plane of the monolayer in the case of normal fibroblasts. The inhibition of cell growth by such means may be related to the phenomenon of contact inhibition[1]. Experiments on other cell types will be necessary to determine if relative resistance to this inhibition is correlated with the 'established' or neoplastic condition.

Cell cultures have been maintained under agar and 5 gm. of glass for at least a month without transfer. Following the attainment of a confluent state, all growth was arrested, for the remainder of the period. A fairly high proportion of *L* cells maintained in the resting state in this way remained viable, judging from the growth of sub-cultures.

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VIROLOGY

Antibodies of Low Molecular Weight in Normal Human Urine

THE presence of precipitating and viral neutralizing antibodies in normal human urine has been reported previously[1-3]. Some physicochemical properties of these antibodies are described in this communication.

Urine was collected from individuals with normal renal function who had been immunized with poliovirus vaccines or with tetanus toxoid. The urines were concentrated with 'Sephadex G-25' (ref. 4) or by lyophilization following dialysis. Proteins having electrophoretic mobilities and antigenic characteristics similar to but not identical with those of serum $7S$ γ-globulins were separated from the concentrated urines either on columns of diethylaminoethyl cellulose (DEAE), using phosphate buffer (0·0175 M, pH 6·3), or by starch-block electrophoresis in a borate buffer (μ0·064, pH 8·6).

Analysis of these γ-globulin fractions by sedimentation velocity in the ultracentrifuge revealed a major component with an average $S_{w,20}$ of 1·5 and a minor component with an average $S_{w,20}$ of 5·7 (Fig. 1A). The proportion of the latter as estimated from the area under the curve in the ultracentrifuge patterns, assuming the specific refractive index increment for the two components to be the same, was 0·5, 0·7, 2·0 and 10 per cent of the total γ-globulin in four samples. Separation of these two components was achieved by sedimentation in a sucrose gradient (1–20 per cent). The top and bottom fractions obtained from these separations were reacted in agar with a rabbit antiserum prepared against human $7S$ γ_2-globulins. The material obtained from the top fraction formed a precipitin line that showed a reaction of partial identity with serum $7S$ γ-globulins, whereas the material obtained from the bottom of the gradient gave a reaction of complete identity with serum $7S$ γ-globulins (Fig. 1B). Analysis of the top fraction in the ultra-centrifuge showed a single peak with an $S_{w,20}$ of 0·92.

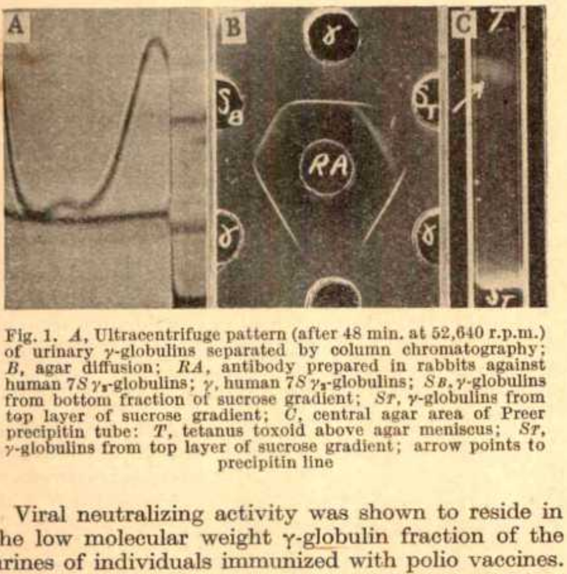

Fig. 1. A, Ultracentrifuge pattern (after 48 min. at 52,640 r.p.m.) of urinary γ-globulins separated by column chromatography; B, agar diffusion; RA, antibody prepared in rabbits against human $7S$ γ_2-globulins; γ, human $7S$ γ_2-globulins; S_B, γ-globulins from bottom fraction of sucrose gradient; S_T, γ-globulins from top layer of sucrose gradient; C, central agar area of Preer precipitin tube: T, tetanus toxoid above agar meniscus; S_T, γ-globulins from top layer of sucrose gradient; arrow points to precipitin line

Viral neutralizing activity was shown to reside in the low molecular weight γ-globulin fraction of the urines of individuals immunized with polio vaccines. The appearance or increase in antibodies to poliovirus types I and III in the serum following booster doses of vaccine was accompanied by the occurrence of measurable antibodies to the homologous viruses in the urine[3].

The presence of precipitating antibodies to tetanus toxoid was demonstrated in the low molecular weight γ-globulins separated from the urines of individuals who had been immunized with that antigen (Fig. 1C). The precipitin techniques used in these studies were those described by Preer[5] and Ouchterlony[6]. The precipitating activity of the serum of several individuals was compared with that of the corresponding low molecular weight γ-globulins prepared from their urine. Even though the amounts of γ-globulin in the serum and in the preparation of low molecular weight γ-globulins of urine appeared to be the same, 3–4 weeks were required for a visible precipitin line to appear when the urinary antibodies were used, in contrast to 4–9 days for serum antibodies, although the relative positions of the precipitin lines in the Preer tubes were the same for the antibodies derived from these two sources. The faster sedimenting component ($S_{w,20}$ 5·7) of the urinary γ-globulins did not precipitate with homologous antigen. This may have been due to the small amounts of this fraction in normal human urine.

Determination of the molecular weight of the precipitating urinary antibodies by Archibald's method[7] gave a value of 12,900. Thus, these γ-globulins have a molecular weight roughly one-third to one-fifth the molecular weight of the active fragments obtained by papain digestion of rabbit or human $7S$ γ_2-globulins[8,9], or one-thirteenth the molecular weight of human $7S$ γ_2-globulins. It is noteworthy that these naturally occurring low-molecular-weight antibodies, unlike the fragments obtained by papain digestion, have the ability to precipitate with antigen, suggesting that two antibody-combining sites are present on this relatively small unit.

This work was supported by grants from the U.S. Public Health Service (E23 and A251).

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Purification of Clover Yellow Mosaic Virus

A VIRUS occurring in a complex with white clover mosaic virus was isolated from white clover (*Trifolium repens* L.) plants growing in Montana[1]. Host-range and serological studies were made simultaneously at Vancouver[2] and Missoula[3] and the virus was named clover yellow mosaic virus. The Missoula authors at the same time made a study of the distribution of the virus in the United States. The present investigation was made using this virus maintained on clover plants.

Leaves from white clover plants infected for about 4 weeks were frozen in a deep freeze for 36 hr. Sap was extracted from this material by means of a meat

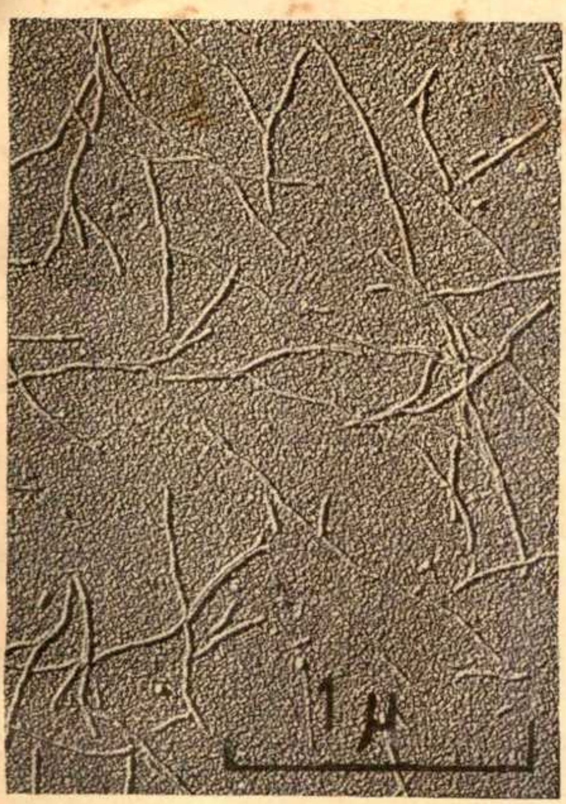

Fig. 1. Electron micrograph of purified clover yellow mosaic virus. (× 40,000)

grinder using 0·1 *M* disodium hydrogen phosphate (1 ml. to each gm. of leaf tissue). The extract was expressed through cheese-cloth and clarified by centrifuging for 60 min. at 2,500 r.p.m., after which the supernatant was again centrifuged for 30 min. at 10,000 r.p.m. in a No. 40 rotor of a Spinco model *L* ultracentrifuge. The pellet was discarded and the supernatant centrifuged for 60 min. at 40,000 r.p.m. Four such cycles of alternate low- and high-speed centrifugations were given, resuspending the pellet in 0·1 *M* phosphate buffer (*p*H 7·0) each time. This was then followed by centrifugation for 2 min. at 30,000 r.p.m. The resulting supernatant was given a final high-speed centrifugation, yielding a faintly pale yellow pellet. Further purification of this pellet was achieved utilizing cæsium chlorate according to the method described by Levintow and Darnell[4]. This gave a completely colourless virus preparation which on examination in the analytical ultracentrifuge showed a single distinct peak. Its purity was confirmed by electron microscope and ultra-violet absorption examination. No pellet was obtained with the healthy material processed in the same manner.

Electron microscope examination revealed flexuous rods (Fig. 1), measurements of which gave a peak around 525 mμ. Smaller and larger particles were also found, but these were regarded as fragments and aggregations of the regular virus particles. Pratt[5] reported an average length of about 530 mμ for his clover yellow mosaic virus.

We thank Dr. B. H. Hoyer of the National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Montana, for his assistance with the analytical ultracentrifuge. This work was supported by the U.S. Public Health Service grant No. *E*–596 (*c*).

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SOIL SCIENCE

Colloidal Silicic Acid in Terra Rossa

ACCORDING to Reifenberg[1] colloidal silicic acid has an important role in the development of terra rossa. In order to substantiate this theory the existence of water-soluble colloidal silicic acid should be proved. The present work is an attempt to estimate colloidal silicic acid in water extracts of Israel terra rossa.

The silicic acid was determined by colorimetric determination of the silicomolybdic acid[2]. Ionic silicic acid was considered to be that which enters into the silicomolybdic complex immediately, and colloidal that which enters only after treatment with sodium bicarbonate, which depolymerizes the colloid. A 0·1 per cent solution of sodium silicate was neutralized with hydrochloric acid and the presence of colloidal silicic acid proved by precipitation with gelatin[3]. From this dilutions 1 : 20–1 : 200 were prepared and the amount of ionic silicic acid was determined. On the day of preparation only about 10 per cent of the silicic acid was in ionic form. The amount of ionic acid increased steadily with the age of the solutions and after two weeks all the silicic acid was in the ionic state. It follows that in concentrations up to 500 p.p.m. colloidal silicic acid depolymerizes in neutral *p*H. In particular colloidal silicic acid may depolymerize during extraction with water. The theoretical aspects of this equilibrium have been discussed[4].

Next, the amounts of silicic acid in various natural waters in Israel were determined and found to be 10–50 p.p.m. All the silica was in ionic state.

The first problem in soil extracts is the ratio water : soil. In order to elucidate this the following experiment was carried out, summarized in the table : To 200 gm. water various amounts of Jerusalem terra rossa (column *a*) were added and allowed to equilibrate for a week. Then the amount of silica in the extract was determined (*b*) p.p.m. water and (*c*) p.p.m. soil:

(*a*)	(*b*)	(*c*)
10 gm.	5·6	112
40 gm.	9·2	46
100 gm.	13·0	26

All the silica was in ionic state and no colloidal silicic acid was detected. It appears that whereas more concentrated suspensions give more concentrated extracts, the amount of extractable silicic acid increases with the amount of water. This suggests that silicic acid is adsorbed on soil, which indeed was proved by the following experiment: To 40 gm. soil 200 c.c. of silicic acid solutions (55 and 106 p.p.m.) were added and allowed to equilibrate for a week, after which the solutions were analysed and found to contain 15 and 20 p.p.m. respectively. (All the silica was in ionic state.)

Finally the amount of soluble silicic acid in various top soils in Israel was determined by extracting 40 gm. soil with 200 c.c. water and was found to be 10–100 p.p.m., all in ionic state.

The present work can neither support Reifenberg's theory nor refute it. Though no colloidal silicic acid was found in the extracts, it may be argued that it was present in the soil samples and was depolymerized on extraction.

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FOREST PRODUCTS

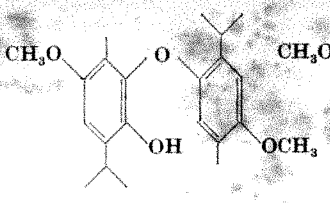
Chemistry of Decay of Heartwood on Ageing in Incense Cedar (*Librocedrus decurrens* Torrey)

IT is now generally recognized that the type of extractives present in certain woods accounts for practically all the decay resistance, and physical factors are of little significance. It has been repeatedly observed in tests of resistance to natural decay that the inner heartwood of many woods is less resistant to decay than the outer heartwood. This is particularly true in the lower trunk of larger and older trees. Moreover, it is a common observation that a hollow tree or log has sustained most loss of wood in the central portion of the trunk. The phenomenon of decreasing resistance to decay with ageing of the wood in the tree occurs in many species, so necessarily there will be a variety of chemical changes involved.

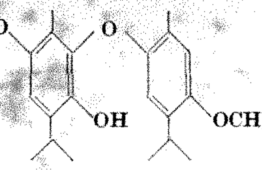
The fungitoxic properties of a number of compounds isolated from incense cedar heartwood were previously reported[1]. The observation was made that, while one of the extractive components, namely, *p*-methoxythymol, exhibited fungicidal properties against a number of wood-destroying fungi, its dimer, libocedrol (I), exhibited very little fungitoxic action.

It was suggested that this may be one type of chemical change that conceivably could account for the decrease in resistance to decay in heartwood on ageing.

Recently, two new extractive components have been isolated from incense cedar heartwood—hyderiol (0·14 per cent dry wood basis) (II) and 3-libocedroxy-thymoquinone (0·02 per cent) (III)[2]. The former is a dimeric cryptophenol ether consisting of *p*-methoxythymol and *p*-methoxycarvacrol, while the latter is a trimeric ether consisting of two molecules of *p*-methoxythymol (libocedrol) and thymoquinone.



(I)



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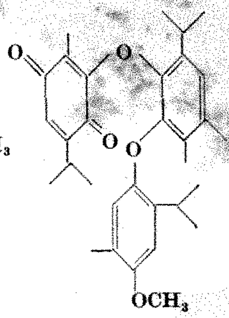

(III)

The decay-retarding characteristics of each of these compounds were determined, together with that of their monomeric counterparts, *p*-methoxythymol, *p*-methoxycarvacrol and thymoquinone. The method used, except for minor deviations, was the standard soil-block bioassay procedure ("Tentative Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures", A.S.T.M. Designation *D* 1413-56 *T*, 1956). Test blocks of ponderosa pine sapwood were impregnated with each of the aforementioned compounds and subjected to decay by a pure culture of a wood-destroying fungus. Loss in block weight during the test constituted the measure of decay. The wood-destroying fungus used was *Lentinus lepideus* (Madison, 534). The results are summarized in Table 1.

Table 1. DECAY RETARDENT BIOASSAY USING *Lentinus lepideus**

Chemical	Weight-loss (per cent)
p-Methoxythymol	15
p-Methoxycarvacrol	16
Thymoquinone	26
Heyderiol	22
Libocedrol	27
3-Libocedroxythymoquinone	33
Control	32

* Test blocks containing 1·2 per cent, by weight of chemical. Test duration, six weeks.

It will be noted that heyderiol, while exhibiting faint fungitoxic properties, was not so potent as its monomeric counterparts. Libocedrol, as previously indicated, again showed, at best, weak fungicidal action. Similarly, but even more apparent, 3-libocedroxythymoquinone had virtually no decay-inhibiting capacity against *Lentinus lepideus*. It is even less fungitoxic than the dimers heyderiol and libocedrol and apparently retains little or none of the fungitoxic qualities inherent in the parent compounds.

The present finding of the denaturing of *p*-methoxythymol, *p*-methoxycarvacrol and thymoquinone via their several polymeric phenol ethers represents a type of chemical change that conceivably could account for relatively lower decay resistance, hence greater susceptibility to heart-rot, in the central lower trunk of mature incense cedar trees. As the tree or wood ages it may be reasonable to assume that, in the biogenesis of extractives, certain enzymatic systems and/or time convert certain phenols to polymeric phenol ethers which can reduce the resistance of heartwood to decay.

Further investigations are in progress.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, April 30

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. John R. Raeburn: "Economic Development and Growth: Agricultural Aspects".*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Applications Engineer—Control Schemes and Automation".

Monday, April 30—Saturday, May 5

PRODUCTION EXHIBITION (at Olympia, London).*

Tuesday, May 1

INSTITUTION OF CHEMICAL ENGINEERS (at the Park Lane Hotel, Piccadilly, London, W.1), at 10.45 a.m.—Annual General Meeting. 12 noon—Mr. C. E. Spearing: "The Evolution of Chemical Engineering" (Presidential Address).

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 4.15 p.m.—Mr. R. E. Strange: "Sporulation and Spore Germination in *Bacillus* Species".*

UNIVERSITY OF LONDON (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. A. L. Lehninger (Johns Hopkins): "The Enzymatic and Structural Organization of Mitochondria".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. V. Dunworth. "Nuclear Power—Its Key Problems".

SCIENCE FORUM (at Friends House, Euston Road, London, N.W.1), at 7.30 p.m.—Mr. H. G. Frost: "The Individual Scientist versus the Two Cultures".

Wednesday, May 2

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. E. S. Anderson: "Phage Typing and Ecology of Bacterial Species".*

COLOUR GROUP (GREAT BRITAIN) (in Lecture Theatre No. 2 of the new Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 2.30 p.m.—Annual General Meeting, followed by Mr. S. S. Gindy: "Colour Contrast Effects and their Measurement"; and Mr. I. Nimeroff: "Variability in Colorimetry".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Wellcome Building, Euston Road, London, N.W.1), at 2.45 p.m.—Meeting on "The Determination of Sterols".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 3 p.m.—Annual General Meeting.

BRITISH NUCLEAR ENERGY SOCIETY (at the Institution of Civil Engineers, 1–7 Great George Street, London, S.W.1), at 5.30 p.m.—Paper by Dr. A. D. Maclean.

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. L. P. Bagge: "Development of the Shell Toroidal Oxygen-Liquid Fuel Burner".

UNIVERSITY OF LONDON (at the Institute of Education, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. J. S. Bruner (Harvard): "Teaching and Learning".* (Further lectures on May 3 and 4).

BRITISH INSTITUTION OF RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. M. Millett and Mr. Z. A. Kiss: "Loudspeaking Telephones".

INSTITUTION OF ELECTRICAL ENGINEERS, and ROYAL AERONAUTICAL SOCIETY, LONDON JOINT GROUP (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Aircraft Electrical Supply System Characteristics and their Effects on the Design of Electronic Equipment", opened by Mr. J. W. McPherson.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. R. Palmer: "The Texture of Polymers".

Wednesday, May 2—Thursday, May 3

IRON AND STEEL INSTITUTE (at the Royal Commonwealth Society, Northumberland Avenue, London, W.C.2; Church House, Great Smith Street, London, S.W.1; and at 4 Grosvenor Gardens, London, S.W.1)—Annual General Meeting.

Thursday, May 3

IRON AND STEEL INSTITUTE; and the SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at Denison House, 296 Vauxhall Bridge Road, London, S.W.1), at 10 a.m.—Joint Conference on "New Horizons in Stainless Steel".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Meeting for the election of Foreign Members, followed by Dr. F. P. Bowden, F.R.S., and Mr. L. T. Chadderton: "Fission Fragment Damage to Crystal Lattices: Dislocation Formation"; Mr. G. O. Harding and Mr. J. Wilks: "The Absorption of Sound in Dilute Solutions of ^{3}He in Liquid Helium II".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. Rudolf Florin (Stockholm): "The Distribution of Conifer and Taxad Genera".

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. E. Jones: "The City in Geography" (Inaugural Lecture).*

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. H. G. Wittmann (Tubingen): "Genetic Code between Nucleic Acid and Protein".*

UNIVERSITY OF LONDON (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. I. Friedmann: "Electron Microscopy in Otolaryngology".*

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting.

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at the Hoare Memorial Hall, Church House, Westminster, London, S.W.1), at 6.30 p.m.—Prof. A. B. Winterbottom: "Studying Surface Films on Metals Optically" (Spring Lecture).

Friday, May 4

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Machines that Teach" opened by Mr. H. W. French.

Monday, May 7

UNIVERSITY OF LONDON (at St. Mary's Hospital Medical School, Wright–Fleming Institute of Microbiology, Paddington, London, W.2), at 5 p.m.—Prof. Frank Fenner: "Reactivation of Animal Viruses".* (First of four Almroth Wright Lectures.)

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. I. M. Glynn: "Sodium and Potassium Movements in Living Cells".* (Further lecture on May 14.)

UNIVERSITY OF LONDON (in the Physics Lecture Theatre, Queen Mary College, London, E.1), at 5.30 p.m.—Prof. R. Righini (Florence): "The Abundance of Isotopes in the Solar Atmosphere".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. A. Winegarten: "Agriculture". (First of three Cantor Lectures on "The Development of Natural Resources".)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting. Prof. L. Jacqué (Paris): "The French Chemical Industry".

WOMEN'S ENGINEERING SOCIETY, LONDON BRANCH (at "Hope House", 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Mr. P. F. Mariner: "Micro-miniaturisation".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. John H. Hemming and Mr. Christopher S. Lambert: "The Iriri River Expedition".

Monday, May 7—Tuesday, May 8

POWDER METALLURGY JOINT GROUP of the IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS (at Church House, Great Smith Street, London, S.W.1)—Spring Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Sheffield (May 5).

LECTURER IN THE DEPARTMENT OF MORAL PHILOSOPHY—The Secretary, The University, Edinburgh (May 7).

LECTURER (registered member of the veterinary profession) IN THE DEPARTMENT OF VETERINARY MEDICINE, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (May 7).

LECTURER (graduate with medical qualifications) IN ANATOMY—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 9).

LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Secretary, The University, Edinburgh (May 12).

ERASMUS SMITH'S CHAIR OF MATHEMATICS in the University of Dublin—The Secretary, The University Council, Trinity College, Dublin 2, Republic of Ireland (May 14).

LECTURER IN THE DEPARTMENT OF MATHEMATICAL PHYSICS—The Secretary, The University, Edinburgh (May 14).

LECTURER or ASSISTANT LECTURER IN PHYSICAL OR INORGANIC CHEMISTRY—The Registrar, The University, Hull (May 14).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Leeds 2 (May 14).

UNIVERSITY CHAIR OF NATURAL PHILOSOPHY in the University of Dublin—The Secretary, The University Council, Trinity College, Dublin 2, Republic of Ireland (May 14).

LECTURER (preferably with a special interest in rock structures and sedimentary processes) IN THE DEPARTMENT OF GEOLOGY—The Registrar, The University, Edgbaston, Birmingham 15 (May 15).

DEWAR RESEARCH FELLOW, to engage in research in radiation chemistry—The Secretary, The University, Edinburgh (May 18).

LECTURER IN PSYCHOLOGY at Newcastle University College, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (May 18).

LECTURER (with a special interest in the geochemistry of sedimentary rocks) IN THE DEPARTMENT OF GEOLOGY—The Secretary, The University, Edinburgh (May 19).

RESEARCH ASSISTANT IN THEORETICAL PHYSICS in St. Salvator's College—The Secretary, The University, College Gate, St. Andrews. (May 19).

LECTURER or ASSISTANT LECTURER IN PHYSICS at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 21).

LECTURER/SENIOR LECTURER IN MATHEMATICS at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 21).

LECTURERS and ASSISTANT LECTURERS IN MATHEMATICS—The Secretary, The Royal College of Science and Technology, Glasgow (May 21).

LECTURER or ASSISTANT LECTURER (with high qualifications in physics and some research experience, preferably (though not necessarily) in the field of biophysics) IN THE ASTBURY DEPARTMENT OF BIOPHYSICS—The Registrar, The University, Leeds 2 (May 21).

UNIVERSITY DEMONSTRATOR (with an appropriate honours degree) IN ANIMAL NUTRITION—The Secretary, School of Agriculture, The University, Cambridge (May 23).

ASSISTANT LECTURER or LECTURER (preferably with an interest in extraction metallurgy or refractories) IN THE DEPARTMENT OF METALLURGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 26).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 31).

LECTURER (with a good honours degree in geology and preferably experience in teaching in a university department) IN GEOLOGY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, May 31).

DIRECTOR OF THE NUFFIELD INSTITUTE OF COMPARATIVE MEDICINE which is to be built in the Zoological Society's grounds at Regent's Park—The Secretary, The Zoological Society of London, Regent's Park, London, N.W.1 (June 1).

LECTURER (with an honours degree in psychology, and preferably an interest in experimental psychology) IN PSYCHOLOGY at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 15).

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletins of Marine Ecology (formerly *Hull Bulletins of Marine Ecology*.) Nos. 49 and 50, Vol. 5: Use of the Hardy Continuous Plankton Recorder in a Fishery Research Programme. By Robert B. Colton, Jr., and Robert R. Marak. Continuous Plankton Records—Contribution towards a Plankton Atlas of the North-Eastern Atlantic and the North Sea. Part 6: The Seasonal and Annual Distributions of the Gastropoda. By F. R. Vane and J. M. Colebrook. Pp. 231–253 + plates 33–40. (Edinburgh: Scottish Marine Biological Association, 1962.) 10*s*. [12

The Incorporated Linguist, Vol. 1, No. 1 (January 1962). Pp. 32. Subscription rates: 4 issues, 30*s*. post free. Single issues 8*s*. post free. Published quarterly in January, April, July and October. (London: The Institute of Linguists, 1962.) [12

Royal Observatory Bulletins. No. 48: Fundamental Data for Southern Stars (Fourth List). By David S. Evans, A. Menzies, R. H. Stoy and P. A. Wayman. Pp. i+E389–E406. 3*s*. net No. 44: Observations of Twenty-four Single-line Variables. By David S. Evans. Pp. i+E351-E364. 2*s*. 6*d* net. (London: H.M. Stationery Office, 1961.) [12

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 133: Commercial Egg Production on Range. Pp. 8. (Belfast: Ministry of Agriculture, 1961.) [22

Commonwealth Mycological Institute. Mycological Papers. No. 80: Coelomycetes 1. By B. C Sutton. Pp. 16+2 plates. 5*s*. net. No. 81: Host Lists of Kenya Fungi and Bacteria. By R. M Nattrass. Pp. 46. 12*s* 6*d*. net. No. 82: Dematiaceous Hyphomycetes, 3. By M B. Ellis. Pp. 55. 20*s*. net. No. 83: Studies of Pyrenomycetes, 6: *Thielavia*, with Notes on some Allied Genera. By C. Booth. Pp. 15+1 plate. 5*s*. net. (Kew: Commonwealth Mycological Institute, 1961.) [21

Other Countries

Polarforschung, Band 5, Jahrgang 30, 1960, Heft 1/2: Neue Ergebnisse der Polarforschung Symposium uber Wissenschaftliche Probleme der Grolandforschung—Bericht uber die Tagung der Deutschen Gesellschaft fur Polarforschung in Munster/Westf. Pp 74. Herausgegeben von der Deutschen Gesellschaft fur Polarforschung und dem Deutschen Archiv fur Polarforschung. (Holzminden: Weserland Verlag, 1961.) [22

Unesco. Arid Zone Research. Plant-Water Relationships in Arid and Semi-Arid Conditions: Proceedings of the Madrid Symposium. Pp. 352+30 plates. (Paris: United Nations Educational, Scientific and Cultural Organization; London H.M. Stationery Office, 1961.) Paper covers 52*s*. 6*d*.; Cloth covers 60*s*. [22

United States Department of the Interior: Geological Survey. Bulletin 1108–A: Geology of the Mount McKinley Quadrangle, Alaska. By John C. Reed, Jr. Pp. iv+36+plate 1. Bulletin 1112-E: Coal Resources of the Trinidad Coal Fields in Huerfano and Las Animas Counties, Colorado. By Ross B. Johnson. Pp. iv+129–180+plate 12. (Washington, D.C.: Government Printing Office, 1961.) [22

Makerere University College, Kampala. A Final Report of the Makerere College International Geophysical Year Expeditions to Ruwenzori, 1957–1960. Edited by P. H. Temple. Pp. iii+80. (Kampala: Department of Geography, Makerere University College, 1961.) [22

Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 126, No. 1: Salientia of Venezuela. By Juan A. Rivero. Pp. 1–208+plate 1. Vol. 126, No. 2: A Revision of the Genus *Pseudisobrachium* in the North and Central America (Hymenoptera, Bethylidae). By Howard E. Evans. Pp. 209–318+5 plates. Breviora. No. 146 (December, 1961): New Rodents from the Early Miocene Deposits of Sixty-six Mountain Wyoming. By Craig C. Black. Pp. 7. No. 147 (December, 1961): Australian Carabid Beetles VIII—*Leiradira*, especially the Tropical Species. By P. J. Darlington, Jr. Pp. 12. No. 148 (December, 1961): Australian Carabid Beetles IX—The Tropical *Notonomus*. By P. J. Darlington, Jr. Pp. 14. No. 149 (December, 1961): A Preliminary Study of the Silurian Ceratiocaridids (Crustacea: Phyllocarda) of Lesmahagow, Scotland. By W. D. Ian Rolfe and T. P. Burnaby. Pp. 9. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1961.) [22

Metropolitan Life Insurance Company. Statistical Bulletin (November 1961): Population Profile of Our Largest Cities. Illness and Deaths from Chronic Respiratory Diseases. Hospital Utilization in an Industrial Area—Nonsurgical Cases. The Accidental Gas Poisoning Hazard. Pp. 12. (New York Metropolitan Life Insurance Company, 1961.) [22

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 17, No. 8 (December, 1961): One Man's Family. By Glenn D. Everett. Pp. 153–172. (Washington, D.C.: Population Reference Bureau, Inc, 1961.) [22

Colony of Mauritius. Meteorological Observations and Climatological Summaries, 1960. July, 1960. Pp. 19. August, 1960. Pp. 19. September, 1960. Pp. 19. October, 1960. Pp. 19. November, 1960. Pp. 19. December, 1960. Pp. 21. (Port Louis: Government Printer, 1961.) [22

Commonwealth of Australia: Department of Supply. Australian Defence Scientific Service. Defence Standards Laboratories. Report 251: The Performance of Clear Finishes on Exterior Timber. By K R Bussell and P. J. Goldstraw. Pp. 14. (Maribyrnong, Vic.: Defence Standards Laboratories, 1961.) [22

State of Illinois. Department of Registration and Education Natural History Survey Division. Bulletin, Vol. 28, Article 1: The Amphibians and Reptiles of Illinois. By Philip W. Smith. Pp. vi+298 (Urbana, Ill.: Department of Registration and Education, Natural History Survey Division, 1961.) [22

Institut des Parcs Nationaux du Congo et du Ruanda-Urundi. Exploration du Parc National de la Garamba. Mission H. de Saeger, en collaboration avec P. Baert, G. Demoulin, O. Denisoff, J. Martin, M Micha, A. Noirfalise, P. Schoemaker, G. Troupin et J. Verschuren (1949–1952). Fascicule 25: Colydiidae (Coleoptera Clavicornia). By Robert D. Pope. Pp. 115. Exploration du Parc National de l'Upemba. Mission G. F. de Witte, en collaboration avec W. Adam, A. Janssens, L. Van Meel et R. Verheyen (1946–1949). Fascicule 62 Trichoptera. Par Serge Jacquemart. Pp. 46+5 planches. Fascicule 63. 1: Eucnemidae y Throscidae. Por Antonio Cobos. 2: Cassidinae By Stanley Shaw. 3: Fulgoridae. Par Henri Synave. 4: Oxynopterinae. Par L Laurent et E. Tamiauux. Pp. 50. Exploration du Parc National Albert. Mission J. de Heinzelin de Braucourt (1950). Fascicule 6: Le Paléolithique aux Abords d'Ishango Par Jean de Heinzelin de Braucourt. Pp. 34+47 planches. Exploration de Parc National Albert. Mission G F. de Witte (1933–1935). Fascicule 98 Calliphoridae (Dipreta Cyclorrhapha). Part 3: Miltogramminae. Par Dr. Fritz Zumpt. Pp. 137. Exploration du Parc National Albert. Deuxième Série. Fascicule 12. 1: Pentastomida. Par Jean Doucet. 2: Pselaphidae. Par Rene Jeannel, 3: Prioninae. By E. Forrest Gilmour. 4: Languriidae. Par André Villiers. Pp 78. (Bruxelles: Institut des Parcs Nationaux di Congo et du Ruanda-Urundi, 1961.) [22

United States Department of Commerce: National Bureau of Standards. Miscellaneous Publication No. 242: 1961 Research Highlights of the National Bureau of Standards—Annual Report, Fiscal Year 1961. Pp. vii+204. (Washington, D.C.: Government Printing Office, 1961.) 75 cents. [22

Japan. National Committee for the International Geophysical Coordination. Japanese Contribution to the International Geophysical Year 1957/8 and the International Geophysical Cooperation 1959, Vol. 3. Pp. ii+195. (Tokyo: National Committee for the International Geophysical Coordination, Science Council of Japan, 1961.) [22

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles. Comptes Rendus de Recherches, No. 27 (Janvier 1962): Travaux du Comité pour l'Etude du Fluage des Métaux aux Températures Elevées, Fascicule 1: Pp. 1–120. Fascicule 2; Pp. 121–247. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1962.) 330 francs. [22

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ASSISTANCE TO AGRICULTURE OVERSEAS

On May 17, 1961, Dr. P. V. Sukhatme, of the Food and Agriculture Organization, presented to the Royal Statistical Society a quantitative assessment of hunger in the world and of future needs for food supplies. The paper has since been published in the Society's *Journal* (124, 463): it should be obligatory reading not only for those professionally concerned with these problems but also for all interested in their political and humane implications. After giving an account of the scope, limitations and sources of error in the available data, Dr. Sukhatme shows that 300–500 million people (10–15 per cent of the world population) are undernourished (that is, their calorie intake is insufficient for normal health and activity), particularly in the Far East, where the gap between the average levels of consumption *per caput* and the requirement is 11 per cent, and he examines the distribution within populations, in various regions of the world, of undernourishment. Malnutrition (qualitative inadequacy of diet, with particular reference to intake of protein) and undernourishment probably affect from one-third to one-half of the human species. Linear programming is then used to calculate the quantities of additional food required to meet the nutritional needs of the estimated populations at various dates up to the year A.D. 2000, taking cost into account. To achieve this objective by the year 2000, cereal supplies in the Far East must be more than doubled, and those of animal products multiplied six times. For the world as a whole, the United Nations medium population forecast requires that food supplies be more than doubled by 1980 and trebled by the year 2000 to achieve a moderate improvement in the level of nutrition of mankind. This would mean (as Mr. Colin Clark pointed out in the discussion) a continuing increase of 3 per cent per annum in agricultural production. While this was, in fact, achieved even in the Far East in the period 1948–58, it cannot be expected to continue without special efforts to increase the yields of agricultural produce per acre, and wherever possible, to extend the area of agricultural land.

In the United States (as in some other industrialized countries) agricultural technology is progressing so fast that agricultural surpluses and enforced restriction of production are the order of the day, and great areas of potentially useful land lie uncultivated. The air traveller to New York may see below him, as he flies down the eastern seaboard, evidence of the great contraction of agricultural acreage in the New England States during the past eighty years. In the under-developed countries, by contrast, production methods and yields are still in the medieval or early neolithic stages of development, particularly in the tropics. There is ample technical evidence, much of which is marshalled in the British Association's pamphlet, *Hunger: Can it be Averted?* (edited by Sir John Russell and Dr. Norman Wright, August 1961), and in the papers read at a recent meeting of the Nutrition Society (*Nature*, 192, 1028; 1961) that while there are innumerable problems still to be resolved, and many social and economic obstacles, the application of known technical means can lead to very considerable advances in the output of agriculture in these areas. The social and economic difficulties call for political, educational and administrative action by Governments, most of which are now independent, but there remains a large and growing need for technical aid in research, agricultural education and extension.

For eighteen years past the Rockefeller Foundation has been supplying this need most effectively, first in Mexico, later in Colombia and Chile, and in the past few years in other American countries and in India. The first annual report of these activities, which brought the story up to the year 1959–60, was reviewed in *Nature* last year (191, 539; 1961). The second report has now been published*.

The Program is based on operating programmes in various countries, together with a fellowship and scholarship programme and a grants programme. The territorial programmes are intended to develop indigenous organizations for agricultural progress. The Foundation enters into an agreement with the national Government to establish an appropriate organization, included in, and co-operating with, the existing official structure, and staffed so far as possible by local scientists and technicians. The Foundation provides such senior staff as are required, to direct or participate in the planning and execution of the work. It helps to develop local institutions for higher agricultural and scientific education, and awards fellowships and scholarships for advanced study and training (mostly, but by no means entirely, in the United States), so that a higher cadre is built up to replace Foundation staff as soon as possible. Research stations and institutes are planned and developed, and particular attention is given to extension services. In Mexico, the Office of Special Studies, established in 1943 within the Ministry of Agriculture, was fused in 1960 with the Institute of Agricultural Research to form a National Institute, with a professional staff of nearly 300, of whom 11 only are Rockefeller staff acting as advisers or heads of sections. Many of the 136 Mexicans who have obtained their higher training under Rockefeller auspices now carry senior responsibilities in the Institute. Steady progress in the same direction is reported from Colombia and Chile, where the Rockefeller staffs number 17 and 5, respectively.

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* The Rockefeller Foundation, 1961. Program in the Agricultural Sciences. Annual Report 1960–1. Pp. ix+326+many plates and diagrams. (New York: Office of Publications, The Rockefeller Foundation, 1961.) n.p.

Work on maize, potatoes and wheat is extended co-operatively to other Latin American countries by the Inter-American Crop Improvement Program, which also co-ordinates more basic research activities on these crops throughout the Americas, from Canada to Chile and the Argentine. Much fundamental work of great importance is being done through this agency on maize and potatoes, the two main indigenous American food crops, and clearly the Rockefeller team is accumulating unparalleled experience of the methods of agricultural improvement on a hemisphere scale. The programme is also providing training, under an arrangement with the Food and Agriculture Organization, for visiting crop technologists from the Middle East who are concerned with wheat, and the maize work now includes studies of the varieties in use in Europe, India and South-East Asia. The potato programme is providing material, especially of the less-well-known species of *Solanum*, for basic research and crop improvement work all over the world.

The steady extension of the Foundation's interests on a world scale is shown by the Indian Program and the support given to the operation of the new International Rice Institute in the Philippines. In India, where the Foundation started work in 1956, co-operation has been established with 26 Federal and State experiment stations in work on maize, sorghum and millets. Four maize double-cross hybrids, partly derived from inbreds previously produced in India, and partly from U.S. and South American material, were put into production in 1960. More than 3,300 samples have been collected in a study of Indian sorghums and millets, and hybrid sorghum varieties are being developed. Special attention is being given to the development of seed production. In many ways the most striking advance is the development, with Rockefeller help, of the Postgraduate School of the Indian Agricultural Research Institute. In October 1960 the first degrees (4 Ph.D., 78 M.Sc.) were awarded, and the third graduate class was admitted; in April 1961 there were 182 M.Sc. and 169 Ph.D. students. The teaching staff numbers 129, of whom almost all are Indian nationals, while the total Rockefeller staff in India numbers 7.

Most of the cost of all this work is borne by the Governments concerned. The total cost to the Foundation of the Rockefeller Agricultural Program, including operations, scholarships, fellowships and grants, for twenty years 1941–60, is given as less than 43 million U.S. dollars, and the total staff of the Program (including headquarters staff) in 1960–1 was only 57. This work, which is accumulating respect and experience of the greatest importance for the advancement of world agriculture, appears to be conducted with economy both of men and of money.

During the same period the British contribution to the agricultural services of under-developed countries has declined in the process of general disengagement from colonial responsibilities. Independence usually produces conditions of instability, and by the time the emergent Government realizes that intolerance of foreign rule is not incompatible with the continued service of individual expatriate scientists, the technical services may have collapsed. Usually there has been no one to replace departing officers, and continual staff changes, career uncertainty, and the shortage of senior experienced workers, have combined to reduce the effectiveness of the services, often to vanishing point. The employment of non-British expatriates has not, on the whole, succeeded, while the new university faculties of agriculture and science are only just beginning to produce a trickle of indigenous technologists genuinely capable of assuming the burden. Yet in many countries, once the tensions of the actual attainment of independence have passed, a solid residue of goodwill and a common educational system and language have led to a renewed demand for British agricultural science and scientists.

The central problem before us is to develop an economical method of deploying the British potential in the service of overseas agriculture. The objectives, *inter alia*, would be to maintain technical liaison with overseas Governments and agricultural organizations, to organize the provision of advice and the recruitment of staff for them, while seeking means of retaining staff at present working overseas, to catalyse agricultural development, to advise the United Kingdom Government on overseas financial aid in the agricultural field, and to co-operate with the international agencies like the Food and Agriculture Organization and Unesco. These activities are at present dispersed into many channels, including the British Council, the Ministry of Agriculture, the Department of Technical Co-operation, technical officers attached to overseas diplomatic missions, the Inter-University Council for Higher Education Overseas, the Commonwealth Agricultural Bureaux, the tropical commodity research organizations, commercial firms and demi-official and private contacts. Means must be found of bringing these efforts together and of encouraging active and continuing interest in overseas agriculture. The Department of Technical Co-operation, which is clearly the central official body in the field, has established a commendably small and distinguished advisory committee for agriculture, animal health, forestry and fisheries, with a supporting panel of consultants. From this arrangement might well develop a broadly based joint council for overseas agriculture, with which all official and unofficial interests capable of contributing effectively (including those in the Commonwealth) could be encouraged to co-operate. Such a body would not itself direct research and development, though it might help to develop programmes and to find the men who could carry them out as servants or consultants of overseas Governments.

The mode of action of such a joint council might well resemble that of the Inter-University Council (which also is now part of the charge of the Department of Technical Co-operation), the quiet but extremely effective activities of which have done so much for overseas territories during the past sixteen years. Its physical organization would be modest: it would need no technical headquarters; and much of its work could be done by an executive (perhaps the existing Advisory Committee) and a small permanent secretariat. Where it needed scientific and technical staff of its own, they might well (following some existing precedents) be based on university departments and research stations rather than on a headquarters office. It would, however, require mobility, and should have a reasonable budget for travelling. The autonomy of its operations should be sufficient to minimize the imputation of 'neo-colonialism', and to allow flexibility and directness in the relations between home and overseas institutions and individuals.

A great deal of the work of such a body, like that of the Inter-University Council, would be concerned with personnel for longer or shorter term assignments. Most overseas appointments at present are for a limited number of years, and this is likely to continue. Though the agricultural science curricula of the universities need to be strengthened, particularly in respect of studies of comparative agriculture on a crop physiological and ecological basis, there is no doubt that a sufficient number of suitable young recruits could be found in both applied and pure science, ready to devote a number of their earlier years to overseas service. They must, however, be offered some assistance in continuing their careers when they return. The really serious shortage is of more senior personnel, particularly of men with appropriate experience of agricultural science and research in tropical or continental environments. No effective way of conserving staff of this sort has been found during the past fifteen years: in this time a valuable asset has been dispersed and in part lost. It is clearly very difficult for a relatively senior man, who usually has family responsibilities, to give up post and prospects in order to serve abroad for a limited term.

An effective solution must involve an element of continuing responsibility in Britain for such staff. Secondment offers one solution to these problems, as Prof. P. M. S. Blackett pointed out in his Irvine Memorial Lecture at St. Andrews (*Nature*, 193, 416; 1962). It has been used sporadically both for universities and research institutions during the past fifteen years. Direct secondment is usually inexpensive, since the overseas institution normally has funds, and for larger departments or institutes it offers no real difficulties. Where staffs are small, as in most university agricultural science departments, direct secondment is seldom possible, and the creation of supernumerary posts is unlikely to commend itself. In these circumstances some form of joint recruitment with a guaranteed return is necessary. The University of Reading has developed a general secondment scheme with the University of Khartoum, based on joint recruitment, which provides a guaranteed period on the staff at Reading after the overseas service ends†. The scheme is essentially simple and it could readily be adapted for the use of other institutions. The Ministry of Education has a somewhat similar scheme for school teachers. An important recent report‡ on the future of research services in East Africa, of which the Secretary of the Agricultural Research Council was a signatory, proposes an even more broadly conceived arrangement involving the co-operation of the appropriate Research Councils in Britain. The Department of Technical Co-operation has set up a Committee on University Secondment, which, working in co-operation with the British Council and the Inter-University Council, may be expected to contribute effectively to thought and action in this general field.

In the years ahead, leadership and knowledge in agricultural science must come to be regarded as among our most important exports, so that periods of service abroad are normal in the careers of many, even most, British agricultural scientists. The benefits will not by any means be one-sided. A continuing interest, through a succession of visits and secondments, in tropical problems cannot but enrich a university department or research institute and raise the stature and professional value of its staff. Such a policy will meet many administrative obstacles; but this is the common hazard of all forward-looking policies, and it is the task of official and academic administrators to deal with them. Given policy and leadership, Britain can deliver the goods in this increasingly competitive field.

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† See *Nature*, 194, 337 (1962).

‡ Report of the Commission on the most suitable structure for the management, direction and financing of research on an East African basis. Under the chairmanship of Prof A. C. Frazer. Pp. (12+) 76. (Nairobi, East Africa High Commission, 1961) 5s.

THE DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

The Department of Scientific and Industrial Research
By Sir Harry Melville. (The New Whitehall Series, No. 9.) Pp. 200. (London: George Allen and Unwin, Ltd.; New York: Oxford University Press, Inc., 1962.) 25*s*. net.

THIS latest addition to the Whitehall series gives a comprehensive picture of the activities of the Department of Scientific and Industrial Research obtainable from no other work that has hitherto described the activities or functions of the Department. Its appearance is the more welcome at a time when the scope of the Department and its place in the organization of civil research generally is again the subject of some discussion. To that discussion it would be unreasonable to expect any direct contribution, and the only respect in which the book disappoints is in failing to lift appreciably the veil which the Department's own reports keep drawn over the activities of the Information Division.

On this function Sir Harry Melville touches in two chapters dealing with the dissemination and use of the results of research and with international relations, chapters which well display the Department's potentialities in this field both in Britain and abroad. Clearly, it would have been difficult for Sir Harry to have written more without disturbing the balance of the book or entering on questions of policy, but a simple comparison of this volume or of an annual report from the Department with an annual report of the U.S. National Science Foundation demonstrates what is lacking and the root of the trouble lies outside the Department and at a higher level.

For the rest the book, lucid and authoritative, is admirably conceived. After setting forth first the constitution and functions of the Department as re-stated in the Act of 1956 and outlining the extent and magnitude of its existing activities, Sir Harry gives an excellent summary of the origin and development of the Department from 1915 to the present time. This account, which pays deserved tribute to those who played so large a part in getting the Department started and steering it safely through the early years, is supplemented in the appendix by lists of successive Lord Presidents of the Council, Chairman of the Advisory Council and Secretaries of the Department. A brief chapter on the organization and control of the Department follows, and there is one describing the Department's system of grants for postgraduate training and research.

In this chapter, as in that on the co-operative research associations, Sir Harry attempts to assess impartially the benefits to Britain which have flowed from these activities, and at the same time to indicate some of the implications and questions of policy which arise. Four chapters on the Department's own research establishment, apart from a brief concluding survey, complete the volume and these chapters cover the stations which have now been closed, or for which the responsibility has been transferred to other Departments or Research Councils. In his impressive picture of the benefits which the Nation draws from the activities of all these research establishments, it is curious, however, that Sir Harry makes no reference to work done, notably at the Road Research Station and the Building Research Station, on behalf of overseas territories. Some work for overseas is also done by the Pest Infestation Laboratory, now the responsibility of the Agricultural Research Council, and by the Tropical Products Institute for which the Department became responsible in 1959, and in assessing the significance of the work of the Department as a whole it should be recognized that the overseas territories benefit as well as Britain herself.

R. BRIGHTMAN

TEXT-BOOK OF VIROLOGY

Wirusologia Ogolna

Edited by Dr. E. Mikulaszek and Dr. W. Dobrzanski. Pp. 580. (Warszawa: Panstwowe Wydawnictwo Naukowe, 1960.) Cena zl. 70.

IN the world literature there are relatively few text-books on general virology; this book is the first and only one on the subject written in Polish by Polish authors.

The editors are obviously in step with present-day trends in virology which, after a period of interest in practical application of the existing knowledge, is now entering a phase of very rapid theoretical advance due largely to general progress in all biological sciences and to the active interest in virology of scientists specializing in other biological disciplines.

A young and vigorously developing science like virology is often faced with divergences not only in observations but also in their interpretation; this makes adequate presentation in a text-book no easy matter. Fortunately, this problem seems to have been overcome in this book.

It is never easy to arrange for uniform presentation in a book written by numerous authors. In this instance it is clear from the references that not all the chapters were written at the same time; some are two or three years older than others, and this has made the problem even more difficult. Some chapters are rather reminiscent of monographs, while others—elaborated in masterly fashion—uphold the tradition of the high quality university text-books.

The book is evidently intended for science students and graduates who wish to become acquainted with virology or to broaden their knowledge of the subject. It attains its object on the whole, but suffers from the fact that much of it is based on the literature up to 1956 or 1957—only in some chapters are references from 1959 quoted—and that some important publications from the period summarized are not referred to.

These criticisms, however, do not alter the fact that the book is of great value in that it attempts to give a general conspectus of virology, and to integrate all our knowledge of viruses into one biological discipline. There is a chapter on "Viruses in Biocenotic Complexes" which 'enlivens' the whole subject, and also a chapter on "Plant Viruses" skilfully written in an introductory form; similar chapters on animal and bacterial viruses would have been very valuable.

The chapters are well planned, the illustrations are good and the discussion is lucid; there are numerous tables. The indexes are good. What is needed, besides what we have already noted, is further integration of the contents, uniformity of presentation and avoidance of repetition; a chapter outlining technical methods would be desirable. A new edition revised on these lines would be well worth while, and it would be such a good book that a translation into English would be of the greatest value.

J. W. CZEKALOWSKI

VIBRATIONS AND WAVES

An Introduction to the Physics of Vibrations and Waves

By Prof. Norman Feather. (Science and Mathematics Texts, No. 4.) Pp. viii + 330. (Edinburgh: At the University Press, 1961. Distributed by Thomas Nelson and Sons, Ltd.) 30*s.* net.

WITH *Mass, Length, and Time*, Prof. Feather gave us a book which went quite deeply into the foundations of physics, and traced the historical emergence of the concepts; this was for the "beginning specialist" in physics, and the mathematical treatment was elementary. The present volume, based on lectures for second-year honours students who had worked through the previous course, deals with strings and rods and pipes, the propagation of plane waves in an infinite medium, water waves, interference and diffraction, and the wave-particle duality. The mathematics is appropriate to this more advanced level.

The author speaks in the preface of his "unorthodox" approach. This really means that he proceeds by a careful analysis of a number of particular cases, and has selected a limited field for detailed study. Thus, in Chapter 3 on coupled vibrations, he discusses the loaded string, Barton's pendulums, and two interesting experiments by Searle and Wilberforce, and goes very fully into the incidence of damping in a system of limited energy content. Earlier, in considering the dissipation of energy from a vibrating string, he arrives at the logarithmic decrement without benefit of a differential equation. This may be unorthodox, and a fluent mathematician might find it slow; but the average student would, one feels, really understand that he is dealing with a physical process rather than a mathematical solution.

The difficulty of explaining one-way propagation by Huygens's construction is faced but not completely resolved; there is a reference forward from this section to Fresnel's treatment of diffraction, and it is there implied that this is the justification for accepting Huygens's construction. But can one do much more at this stage ?

Inevitably, the book swings over to physical optics in the treatment of interference and diffraction, rather from the point of view of the major historical experiments: and the chapter on wave-particle duality is little more than an outline sketch of the origins of modern ideas. I learned a great deal from the earlier chapters, and found the whole enjoyable to read. Its great merit is that it introduces students to wave-motion slowly and deliberately, and prepares them for the forthcoming dive into deep waters; for it is re-assuringly fathomable and clear.

G. R. NOAKES

ANALYSIS, CLASSICAL AND FUNCTIONAL

Riesz Frigyes :

Ósszegyűjtött Munkái. A Magyar Tudományos Akadémia Megbizásából sajtó alá rendezte Császár Akos. (Friedrich Riesz: Gesammelte Arbeiten.) Elso Kötet: Pp. 1–760. Második Kotet: Pp. 761–1601. (Budapest: Akadémiai Kiado, Publishing House of the Hungarian Academy of Sciences, 1960.) I, II kötet ára: 400,-Ft.

THE publication of the collected works of F. Riesz in two large volumes is due to the Hungarian Academy of Sciences. He wrote in Hungarian, French, German, English and Italian. Of the papers which appeared only in Hungarian, a translation into French is supplied. The tract (1913) "Les systèmes d'équations linéaires à une infinité d'inconnues" is reproduced in Volume 2. (The much longer Riesz–Nagy, "Leçons d'analyse fonctionnelle" (1952) is naturally not reprinted here.)

Riesz was born in 1880 and studied at Zürich, Budapest and Göttingen. Most of his life was spent at Szeged (1920–46) and Budapest (1946–56). Together with Haar he made Szeged a centre of mathematical activity and founded the flourishing journal *Acta Scientiarum Mathematicarum*.

Riesz's papers are classified in this collection under nine headings: topology, real functions, function spaces, analytic functions, harmonic and subharmonic functions, functional analysis, ergodic theory, geometry, miscellaneous. To the first seven of these subjects his contributions were of fundamental significance. He was one of the chief architects of function spaces and functional analysis and he created the theory of subharmonic functions.

Riesz's influence on mathematical analysis is illustrated by his early papers. The Riesz Fischer theorem (*C*2 in the collection) appeared in 1907. The expression of a linear functional operation by a Stieltjes integral $\int f(x)d\alpha(x)$ followed in 1909 (*C*7). We see here classical analysis broadening into functional analysis. Concurrently, Riesz was working at general topology and he formulated in 1908 a set of axioms for a general topological space (*A*8). In 1910 followed the long paper on the class of functions L^p with the notions of strong and weak convergence (*C*10). This fertilization of analysis by topology was due to F. Riesz more than to any one else.

Among the many later papers of major importance which are characteristic of Riesz may be mentioned those on the boundary values of analytic functions (*D*7), the Young–Hausdorff theorems (*C*13) and linear operations (*F*16).

Riesz's papers are models of elegance and deceptive simplicity. The clarity of his vision enabled him on one hand to see existing theorems in different and more general settings and, on the other, to present radically simplified versions of complicated arguments. The reader will find instances in the exposition of the integral (*B*15), proofs of the differentiability of monotonic functions (*B*12, 13) and of some inequalities of Hardy and Littlewood (*B*8, 9), and the work on ergodic theorems (*G*1–4).

The books are a fitting memorial to a great mathematician.

J. C. BURKILL

CHROMATOGRAPHY OF STEROIDS

The Chromatography of Steroids

By Prof. I. E. Bush. (International Series of Monographs on Pure and Applied Biology. Division: Biochemistry, Vol. 2.) Pp. xxi + 437. (London and New York: Pergamon Press, 1961.) 80*s*. net.

THE author set out to link the general theory and background of partition chromatography with the practical aspects of steroid chromatography. He has succeeded. In the first chapter he describes with masterly clarity the basic theory of chromatography, the derivation of R_F and R_M values, the interactions between solvent, and solvent structure, and solute; the effect of chemical structure of simple compounds on their chromatographic behaviour is dealt with fully and numerous examples from the non-steroid field are included. In Chapter 2 a general survey of adsorption and partition chromatography as applied to steroids is given. The extent of the field is indicated by a table that quotes published examples of partition chromatography of steroids. This table extends to 15 pages. In Section 2 of this chapter, the quantitative treatment, based on R_M values, of steroid behaviour on chromatograms is exhaustively developed. The next chapter describes techniques and equipment. No effort is spared to give details that are essential for successful chromatography. A useful and authoritative contribution is a description of the preparation of extracts suitable for chromatography. Chapter 4 is devoted to quantitative chromatography, including colorimetric and radioisotopic techniques. The technique of direct scanning of stained chromatograms occupies, deservedly, 24 pages in which modifications are fully described and possible sources of errors indicated. In Chapter 5, the identification of steroids by chromatography and the procedure for structural analysis is described with commendable clarity. The generalizations in this chapter appear to be well founded and are based on the extensive experience of the author. The final chapter deals with some specific problems, such as chromatography of cortisol in blood, œstrogens and aldosterone in urine, the procedures dealing with conjugates of urinary steroids and fractionation of mixtures of steroids. Since, as the author says, "there is more than one way to skin a cat", the techniques are critically evaluated and guidance given as to the best procedure to be adopted. A useful contribution is in the appendixes that contain technical information necessary for the chromatographist.

Prof. Bush is to be congratulated on having written this massive book that will not only be an important source of information to the expert but also a guide to newcomers in this complex field of biochemistry. The lively style makes easy reading; the print, tables, illustrations and indexes are impeccable.

E. Kodicek

KERATIN

Keratin and Keratinization

An Essay in Molecular Biology. By Dr. E. H. Mercer. (International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 12.) Pp. xiv+316+24 plates. (London and New York: Pergamon Press, 1961.) 70*s.* net.

The year 1961 saw the untimely death of Prof. W. T. Astbury, to whose inspiration the development of molecular biology and in particular the study of keratin structure owes so much, and it is fitting that the year should also see the publication of a book on this subject dedicated to his memory. The main ideas of the molecular structure were outlined by Astbury and his co-workers thirty years ago as a result of X-ray analysis and investigation of the mechanical properties of hard keratins, and the extension of the work to the soft keratins so confirmed the similarities at this level that these ideas still form the framework of our theories. The older study of the cytology and histology was well advanced at this time, but the biological and the physical lines of attack were separated by a size-factor of 100–1,000, and it remained for electron microscopy to provide a link between them. During the past 15 years the author has taken a considerable part in forging this link and is well qualified to 'look both ways' into this subject.

In the early chapters he deals with the two lines of attack, partially outlining the physical methods, as well as their results, for the benefit of the biologist and explaining the relevant biological findings for the benefit of the physicist. Unfortunately, this process has produced a lot of forward cross-references which are commendable but irritating. The later chapters are devoted to recent developments, culminating in a discussion of keratinization, and here the author has had a difficult task because of the rapidity of these developments. As a result the treatment is uneven, for example, as between electron microscopy, where much recent work is reported, and the mechanical properties of wool fibres where the work of the Australian school has been neglected almost completely. It seems also that the presentation has suffered because of the necessary haste of writing, and it is perhaps symptomatic of the present state of this subject that the book should end in mid-page, and mid-air, with a sentence in parentheses. However, while this work is scarcely an 'essay' with a steady progression from introduction through development to conclusion, it is to be recommended as a comprehensive review, filling, as its bibliography shows, an important gap in the formal literature.

The reproduction of electron micrographs is adequate, although the descriptive keys for these and the many sketches are not always comprehensive, but the X-ray photographs are marred by poor printing —indeed, they are better printed on the dust jacket, although here mis-orientation gives then a slightly inebriated air.

N. Peacock

THE FATHER OF BRITISH DENTISTRY

Sir John Tomes

A Pioneer of British Dentistry. By Sir Zachary Cope. Pp. ix+108+11 plates. (London: Dawsons of Pall Mall, 1961.) 25*s.* net.

When John Tomes was born in 1815 dentistry was no more a profession than it had been when an ancestral John Tomes of Longmarston, near Stratford, gave a night's shelter to the fugitive Charles Stuart after the battle of Worcester.

The young Tomes, born three months before the Battle of Waterloo, went to the grammar school at Stratford on Avon, and at the age of sixteen became apprenticed to an apothecary of Evesham. At the end of his apprenticeship he came to London to walk the hospitals with the intention of taking a medical diploma. Very soon he became interested in the structure of the teeth and of bone, an interest that led him to the practice of dentistry as a career, and ultimately to his high place in the hall of dental fame as the 'father of British dentistry'.

Though there are many records of Tomes's public life and actions, very little evidence has remained to tell us much of the man himself. Nevertheless, the author, with his great skill and experience as a medical historian, succeeds admirably in this necessarily short account in conveying the essence of Tomes's character. Tomes's inventive and scientific genius led to many contributions to dentistry, including his classical description of the dentinal fibrils, which is reproduced in the book in full and which, as the author says, deserves the designation "classic" more than many writings to which that term has been applied. However, Tomes's greatest achievements lay in the key part he played in the raising of dentistry from the level of a trade plied by any who wished, without restriction or supervision, to the status of an honourable profession.

The record of the struggle for recognition by dentistry, with Tomes always at the forefront, with the 'memorials' to the Royal College of Surgeons, the formation of the abortive College of Dentists, the eventual institution of the Licentiate in Dental Surgery and the passing of the Dental Act establishing the registration of dentists, makes fascinating reading for the dentist or doctor. The book is also sufficiently concise and so well written as to constitute a well-worth-while excursion for the more general reader into, perhaps, an unfamiliar field.

R. B. Lucas

Notes on Operations Research

Assembled by the Operations Research Center, Massachusetts Institute of Technology. Pp. viii+256. (London: Crosby Lockwood and Son, Ltd., 1959.) 30*s*.

THIS book consists of eleven chapters by various authors, and covers the programme of lectures on operations research, given in August 1959 at Brussels, for members of European NATO countries. It starts with a chapter on probability, then one on search, and one on Markov processes. The latter is by Prof. Morse. There are chapters on queueing, control processes, organization, sequential decisions, reliability, information theory, production scheduling and simulation. Several of the chapters are followed by a list of exercises, and there is an adequate bibliography.

This work, modest in price and good from the expository point of view, will make an excellent text-book for courses on operations research. It represents the sort of publication which we should see more of—books which can be used as solid texts for the training of future personnel for operation research, rather than books which merely review or comment on the content of the subject.

L. S. GODDARD

Experiments for Young Scientists

By Don Herbert. Pp. 160. (London: G. Bell and Sons, Ltd.) 15*s*. net.

EACH of the thirteen chapters of this book contains one or two experiments referring to a particular branch of science, together with some brief comments on the work of a scientist in that field—astronomy, physical chemistry, meteorology, zoology, etc. Some good modern photographs are included.

The directions, accompanied by clear diagrams, are easy to follow, but a few of the experiments demand a fair degree of skill for their successful completion; the construction of a barometer using a 'plumber's friend' a rubber plunger, and the illustration of a Gaussian distribution with marbles running to rest down a bagatelle-type board, are two cases in point. The making of a microscope using a bead of water as a lens could be less elaborate, but the details given would appeal to the mechanically minded boy. Some of the other experiments are very simple, and the general level of the book is for the young person coming into first real contact with scientific matters. The style of the American author is pleasantly informal, and the elements of scientific method are unobtrusively emphasized throughout.

The book should find its best use in awakening interest and providing a useful starting point from which an absorbing hobby or professional interest could develop.

D. C. PRICE

The Nerve of Some Animals

By Robert Froman. Pp. 250. (London: Victor Gollancz, Ltd., 1961.) 21*s*. net.

THIS book is a collection of true incidents about certain animals that can be found in the American Continent—namely, the wolf, mustang, mountain lion, buffalo, coyote, beaver, llama, bear, jaguar, rat and dolphin.

It is the belief of the author that in all species there are far greater variances between individuals than is usually realized. Therefore, many of the animals mentioned do not conform to generally accepted ideas and theories on pattern behaviour, and are not necessarily typical of their species. All show relentless spirit and unfailing nerve in dealing with particular problems. For example, there is the wolf that defies all attempts at capture; the mustang that resists every would-be rider; the friendly dolphins that adapt themselves to befriending man.

The book will appeal to a wide range of people. Each chapter reads as an interesting story, or group of stories. At the same time, much information is gained on the history, habitat and environment of the animals. There are many surprises and contrasts, made all the more fascinating by dealing with the exception rather than the rule.

B. J. G. MAITLAND

Government and Science

Their Dynamic Relation in American Democracy. By Don K. Price. (Galaxy Book, No. 72.) Pp. ix+203. (London and New York: Oxford University Press, 1962.) 10*s*. 6*d*. net.; 1.65 dollars.

MR. PRICE'S penetrating study of Government and science deserved a reprint, but it is a pity that after eight years it should have been reprinted unchanged and without addition of either index or bibliography to repair the two conspicuous defects of the first edition. In the interval the relations between Government and science in the United States have somewhat altered and a bibliography would have helped to keep the book up to date without significant revision of its two most valuable chapters on the machinery of advice and the structure of policy. A bibliography, moreover, could have extended its appeal and relevance to the British reader.

R. BRIGHTMAN

The Moon

A Complete Description of the Surface of the Moon, containing the 300-inch Wilkins Lunar Map. By Dr. H. Percy Wilkins and Patrick Moore. Pp. 388+15 plates. (London: Faber and Faber, Ltd., 1961.) 63*s*. net.

SINCE its publication nearly seven years ago *The Moon* has remained one of the greatest accomplishments of modern amateur astronomy. Containing a reduced form of Wilkins's lunar map and comprehensive descriptions of all named lunar formations this book far surpasses the previous similar works by Elger and Goodacre in describing the intricacies of the lunar surface.

In the new edition Moore has aimed at bringing *The Moon* more into line with contemporary developments. The Soviet chart of the averted side of the moon has been included, together with short explanatory comments. Descriptions are provided of the few additional named formations the names of which had not been assigned at the time of the first edition.

The major part of the book, the description of lunar features, has not been significantly changed from the first edition. The only major alteration is in the description of the crater Alphonsus, which now contains a mention of Kozyrev's reported outbreak near the central mountain of this crater. It is a pity that the text could not have been more thoroughly overhauled as a very large amount of work has been carried out by amateurs all over the world since the original publication date. As it stands, the text is rather inhomogeneous; it often contains elaborate descriptions of insignificant pits and craterlets and omits detailed description of the more important topographical features.

BRIAN WARNER

THE CHANGING SCENE IN UNIVERSITY EDUCATION

By PROF. F. C. STEWARD, F.R.S.
Cornell University, Ithaca, New York

WITHIN the life-time of anyone now in his mid-fifties, universities have been subject to radical change. This is particularly true in science. The impact of two World Wars and the increasing dependence of modern society on technology have played their part. It is safe to say that the pressures in the future are to be even greater, if the universities of Britain and the United States are to meet the needs of their respective societies for educated and trained personnel. In both countries expansion is essential. In the United States the pressure is to meet the vociferous demands of an increasing population. The already high percentage of the population which either submits to, or profits from, higher education must continue to do so, or this percentage may even need to be increased. In Britain the pressure is to close the gap between the two countries and to pass a much larger percentage of the population through the universities than hitherto. In the United States existing university establishments will be and are being expanded and their resources strained; in Britain the slow trend to increase the number of universities by conferring university status on a number of pre-existing institutions will now be accelerated by creating a somewhat surprising number of new universities. All this presupposes that in the past only a few of those in the population who were fitted by genetics, by interest and by motivation to profit from a higher education were in fact able to receive it and to reach the highest levels of their capability. One suspects that these untapped scholastic reservoirs, which are obviously calculable, are not as great as are often supposed. In all this somewhat phrenetic activity to increase the quantity of university education the important question is: What is to happen to its quality ?

These observations come from one who has spent about half his career in the universities of Britain and the other half in the American universities, and who happens to believe that if each system can learn from the other the ideal might not be far to seek. Thus, as Britain expands its university system, it is well to be alert to some of the warnings, as well as to the stimulus, that may come from the United States.

One may ask first, what is the distinguishing characteristic of a true university ? Size alone does not suffice, and age and tradition may enrich or enfeeble a university community. There is perhaps no generally acceptable answer to this question; but the often very different character of universities in Britain and in the United States suggests that the answers, if formulated, would in the past at least have been very different. The university is clearly not and should not be merely an enlarged school, in the sense that school is the proper place where students acquire general, and often their terminal, education. Similarly, the university is not and should not be only a trade school to supply a flow of trained, but not necessarily creative, personnel to industry. Ideally, the university should still be the guardian of standards of scholarship in a world preoccupied with the superficial things; in this competition the university should provide the environment and the way of life in which the knowledge inherited from the past is not only disseminated but also, more important, is extended by scholarly research. Universities are first places where knowledge is sought; schools where it is taught. While in a measure these ideas would be approved in most universities, it is the degree of emphasis given to them which has imparted to universities in Britain and in the United States much of their respective characteristics.

In the United States, and in the past, the general education achieved in the schools fell short—often far short—even of those which were generally deemed necessary for a terminal education. Therefore, in that country the average university undergraduate curriculum has had much more of the character of a school than its British counterpart. Conversely, because in the past the British school was, rightly or wrongly, believed to furnish a sufficient general and basic education, except for fields of specialization, the British university became primarily a place for specialized study and training. (One went 'up' to the university from the school, whereas the American student continues to go to 'school' throughout his university life.) But the difference is deeper than this. A general education in a given area of subject-matter for large masses of the population can only be provided in a standardized way and cannot be given solely by those engaged in scholarly or creative work in these fields, for there simply are not enough of them to go round. Hence, in the United States more than in Britain, much undergraduate instruction has tended to become routine, and the prerogative of teachers who may not be productive scholars and for whom teaching is an end in itself. Thus, effective contact of the student with the more creative aspects of his subject, or subjects, is normally deferred until postgraduate work. (There are, of course, notable exceptions, especially in some of the smaller and private institutions where personal contact between a research-minded staff and their students is decidedly more intimate.) Thus, the undergraduate tuition in most of the large American universities has assumed more and more of the traditional role of the school, in that it imparts information over a broad front rather than fostering understanding and ideas in a limited area. In turn this has, inevitably, had an effect on the postgraduate schools which, by British standards, have become almost frighteningly large. One has had the experience of lecturing in the United States to much larger groups of graduate students studying for the Ph.D. than the number in a British university who might be reading even for the honours degrees. These are, therefore, two different ways of achieving what may be the same ultimate goals.

In Britain it was largely between the two World Wars that the Ph.D. became a natural sequel to the bachelor's degree. In practice, however, the relatively few who embarked on this very specialized road were believed to be already equipped by knowledge and training from their bachelor's degrees with honours to do original research for the doctor's degree. In the United States, however, the shortcomings of the great variety of bachelor's degrees, which qualified so many candidates for entry into the graduate school, needed to be met by courses which, even at the graduate-level, again communicated information. Thus, even the Ph.D. became at least in part a degree for which a student was 'taught' rather than being trained through the medium of original inquiry. Therefore, what happens to the school curriculum inevitably changes the face of the undergraduate university, and what happens to the undergraduate determines what sort of higher postgraduate education he may receive, the kind of work he may do and the degree of maturity he may reach. Thus, as new universities spring into being, care should be taken to preserve those values that have contributed to the essential quality of the distinctively British higher education.

Among these values the integrity of the honours schools in the universities should rank high, and this should be preserved against all the pressures for more general training for more people. Unless the system of selecting able students has in the past been completely inefficient, it is inevitable that as more students enter the university there may not be a proportional increase in those able to reach the highest standards; and, at a distance, one wonders whether the standards of the honours degrees have not, in consequence, already suffered. It is sad, but true, that whenever the appeal is to process numbers the standards go down, and when this insidious decline begins at one level in the school, the undergraduate curriculum, or the postgraduate school, it has repercussions throughout the rest of the system.

There is obviously a very subtle balance between having quality on one hand and size and quantity on the other: it is very difficult to have both. The great advantage of the smaller universities is that, for them, undiluted quality is more attainable.

In the United States, with its different standards of democracy, there has always been, and no doubt will continue to be, a greater respect for numbers; the United Kingdom, at least in the past, has laid the emphasis on quality. As the winds of change blow through the British universities, their obvious problem is to retain their quality while they produce more in quantity; the problem in the United States is more often to regain quality and standards without indefinitely prolonging the education of the able few into ever-lengthening Ph.D. courses and post-doctoral training. The latter is now almost as common as the ubiquitous Ph.D. When standards are lost at the lower levels, courses of university postgraduate instruction may be diluted and prescribed study is unwholesomely prolonged. Indeed, the most creative years of many a student in the United States may, even now, be spent in learning his subject from others instead of becoming a practising scientist or a scholar. This takes so long that the zest for discovery may be quenched, and often all that is left is to teach someone else how to learn to do the same in even longer time.

If even a fraction of the present annual crop of Ph.D.s in the United States (10,500 in 1960–61, of whom at least 45 per cent were in the physical and biological sciences) retained any real contact with creative work, it would seem that even this addition to the already large body of trained research personnel (*American Men of Science* in the tenth edition will list about 100,000 names in the physical and biological sciences and 24,000 in the social and behavioural sciences) should equip the United States to solve almost any scientific problem. But these are measures of quantity: they have little bearing on quality. When outstanding abilities are needed, an infinite number of the merely competent will not suffice. As universities are being 'tooled-up' to more quantity production of graduates, they should, above all, preserve the milieu in which outstanding ability may flourish and be recognized.

It is true that the British graduate, and even the British Ph.D.s, were often not as mature as the system supposed. Thus, both postgraduate courses of study and even post-doctoral training can be usefully, if judiciously, interpolated into the existing system. But the dangers of doing this at the cost of the essentially research and original character of the work for advanced degrees should be guarded against; higher degrees earned very largely by undergraduate techniques of study, as may too often occur in the United States, should certainly not be encouraged. In fact, one of the outstanding problems in the ever-increasing American graduate schools is to bring the students to an adequate state of preparation for research in their field without making them into perennial undergraduates. The formal graduate courses that need to be taught to achieve this should be quite different from courses in the undergraduate school; the first should not merely be an extension or enlargement of the latter, as they too often are. Instruction as it is, or used to be, offered in a British honours school may be a better model here.

As new universities come into being, they may well be, at first, ill-equipped with research resources and productive research scholars on their staffs, for the supply can scarcely be equal to the increased demand. The obvious temptation will be to stress teaching through courses and not through creative work; but if this practice were to be widely adopted in a seeming imitation of a pattern often found in the United States, it could easily undermine the chief strength of the British system. Obviously, what is needed to-day are larger numbers educated to a higher general level, and particularly more of the skilled technicians that industry and a technological society needs. If, to meet these needs, all this extra traffic is passed through the university, this may threaten its chief strength, for creativity, scholarship and research may be submerged beneath the need to impart information and to teach skills. Special steps should be taken to guard against this outcome.

But perhaps another consequence of size and numbers is not always appreciated in Britain. While a minimum size is necessary to give a university the balance and diversity it needs, excessive size often leads to a separation of instruction by teaching and through scholarly research. As the communication of information becomes an end for larger and larger numbers, teachers and research scholars become separately identified; they are more rarely found in the same person. Moreover, as departments become larger, staffs more specialized, rarely are scholarship and knowledge so comprehensive as they used to be. Since numbers of students sway administrative policies, it is a short step to the predominance and ascendancy of a teaching cult rather than of a creative, essentially scholarly outlook in the policy-making of the universities. Anti-intellectualism is not unknown even in the university itself, and there is no surer way of engendering this than the fostering of a teaching staff which is divorced from creative scholarship and research; and this happens all too easily under the pressure of numbers and large classes. With the increasing support for research and the need for larger and more specialized research teams, it is often the case that teaching and research lose their essentially complementary nature and even university faculties become divided on their relative importance. (One may note that it is wise to locate, wherever possible, such essential research functions as those which are supported by the Agricultural or Medical Research Councils or the Department of Scientific and Industrial Research in selected units which are placed alongside and affiliated with the universities. These arrangements undoubtedly enrich the universities by contact with a highly specialized research staff, but they also help the research worker. This happens by keeping the research investigator in some contact with the teaching profession and with students, and even by providing him with an otherwise much-needed opportunity to expound his speciality in the class-room with consequential benefits to the quality and range of the research done.)

There are, however, two important safeguards in the British system which should be jealously guarded; one is academic, the other is administrative. First, the external examiner system (virtually unknown in the United States) should prevent any university, school or department from too blatantly 'branding its own herrings'. The second is inherent in the very different concepts of university administration in Britain and in the United States.

The average American university runs like a corporation; and the trustees, like a board of directors, either collectively or in executive committee, make over-all policy. The president of the university, like the board chairman of a corporation, 'runs the show'; and often the chief finance officer, by exercising the power of the purse, exercises influence even over academic policy. A whole 'chain of command' also, often of erstwhile academicians, become professional and career administrators, carry the administrative policy down to the lower levels via vice-presidents, deans, assistant deans, directors of this and that, and often heads of departments, the last-named often being selected more for their performance of routine administrative chores than for their mastery, or direction, of an area of academic subject-matter. By contrast, the senate of the average British university, stuffy or conservative though it may sometimes seem to be, at least places the destinies of the university in the hands of those who, if any, are dedicated to the preservation of its academic and scholarly values, and who do not, or should not, mistake or glorify the organization at the expense of its objectives. In Britain, fortunately, there has been much less vested interest in university administration than in the United States.

As universities expand in Britain, both in number and size, there may well be a trend to create more and more organization, with the inevitable dichotomy between administrative and academic functions; it is this dichotomy which often creates problems in the average American university and results from the way it is administered and the way it has grown in size and in complexity. It is often difficult for those in American universities to realize that one rarely used to hear in Britain about difficulties between the 'faculty' and the 'administration', for in very large part the senior faculty of a British university really administers the university. The corporation, on which the American university is so closely modelled, may be judged by a balance sheet, and similarly the American university by its buildings, its endowments, its numbers of student body, of graduates, etc. But these are all criteria of quantity, not quality. Though necessary enough in a modern society, they need to be tempered by some criteria that measure whether the university of to-morrow will continue to create the milieu in which discoverers are nurtured and discoveries are made; discoveries like those of chromatography or the advances in the ultra-structure of large molecules like hæmoglobin and deoxyribonucleic acid (to mention but two distinctively British advances). Where shall these ends be met if not in the university or its immediate environs ?

Moreover, in times of crisis, numbers alone rarely suffice. A relatively few aircraft and pilots of superior quality saved the country in the Battle of Britain; probably an equally good case can be made for the relatively few highly able scholars and scientists, who in radar, anti-submarine activities and numerous other ways also played a decisive part. The one remaining natural resource is the British reservoir of brains; this should be jealously guarded. If it is an avowed objective to increase the quantity of scientifically trained personnel, the university system should not be geared to this end alone in ways that retard, or reduce the role of, those few with the outstanding qualities on which progress really depends. It should be remembered also that the American system has, at least in the past, been able to recruit heavily from the countries of Europe and thus acquire, ready trained, people with unique knowledge or attributes; Britain presumably must grow her own, even enough to compensate for the inevitable losses to the United States. The American university, especially the larger State ones, often tends to work on a pattern that reflects the American concept of democracy, and it therefore tends to treat all alike, at least in theory. There are many reasons, however, for the belief that to attain its highest standards and developments, a university cannot conform to this idea of democracy, for it must be an autocracy, or oligarchy, of scholarship. Otherwise, the larger the percentage of the population that passes through the university, the more it will come to represent an average intellectual sample of the whole. Obviously, however, the real strength of the university is not with the average, but with those who are far from the mean at the extreme of the distribution curve of ability.

Perhaps some of the new universities of Britain should take on something of the role of the junior colleges of some American States, and the older and more expensively equipped centres provide more for advanced studies and scholarly research and discovery ? But can any new university continue to assume indefinitely what will seem to be a secondary role ? Will not its staff begin to feel that they are academically second-class citizens ? Moreover, with more centres of advanced education and more departments of this and that, the total resources available for the support of scholarly work, without which these new universities cannot fulfil their true function, will prove increasingly inadequate. In fact, one may ask whether all universities really need to offer all courses of study, for there might well be a measure of specialization with resultant efficiency. (If all the new universities are to have, for example, a department of botany, they can at least develop different aspects in agreement and at the advanced level make their specialists available to each other. Otherwise there will simply not be enough qualified people to go round, at least for some time to come.) It seems inevitable, however, that the existing governmental sources to support research and advanced study in science will need to be vastly enlarged to nourish, on something approaching the American scale, the greatly increased numbers of academic personnel who will be engaged in university work—otherwise most of these will be condemned to the uncreative imparting of information which is the proper function of the school, or the trade school, and is not the role of a true university. Some may wonder whether the governmental largess to support research in the United States, at least in science, is always as wise as it is lavish. However, the fact remains that single American investigators may now receive funds that in the recent past would have had to suffice in Britain for a whole department or a major school of research. Admittedly, money cannot, and does not, purchase creativity; but the ideal must surely be somewhere between the two systems.

On the American scene, it is an ideal combination when the unrivalled resources of the United States for research and the advancement of knowledge can be used in the truly creative fashion which is more often fostered by the training in the universities in Europe. On the British scene, the goal should be that resources more nearly on the American scale should be made more generally available to those university scholars with proved ability to utilize them effectively. Thus, without vastly increased funds for the support of research, an increase in the number of universities and in the volume of students may not result in a proportionate increase in the fully qualified personnel that the university should produce nor in the rate of that advancement of knowledge on which industry and technology will ultimately depend.

Another topic is also relevant; this is the proper place of the 'applied' rather than the 'basic' studies in the modern university—new or old. Such essentially applied disciplines as medicine or engineering and many aspects of technology find a proper place in the instruction offered by the universities of to-day; but there is a real question, which the newer universities of Britain will need to face, to determine how far they need to, or should, go in the direction of technological instruction. The more subtle point is, however, to determine what part of the applied discipline is a proper subject for a university education or a degree.

This is a sensitive subject, especially in the United States, where the professional and technical schools are so strong and where there has been a tendency to proliferate university education into all manner of applied areas. Obviously, however, the university should not do what the industry can do better, or the profession by 'on the job' training. It is a needless dissipation of the resources of the universities and an unwise use of their limited effect on youth in the formative period to allow departments or colleges to become so pre-occupied with the problems peculiar to a trade or profession that they cease to make scholarly contact with other branches of learning. Thus, the role of the university should be to bring to all technological disciplines within its shelter the resources of academic personnel who will, within its area of subject-matter, make scholarly contributions to knowledge which can be recognized as such by those in other branches of learning. As soon as the work of the applied department becomes so obsessed with developments from existing knowledge, with screening tests or with other aspects of commercial operation (as may so easily happen in response to the pressure of industry), then the question should be asked whether this type of activity is not more proper for industry or the technological research institute.

In other words, it is only in as much as the applied disciplines add to basic knowledge, which may be recognized as such by those outside the particular technology, that they are suitable areas of university study or of work for advanced degrees. But when this is not so and when they become pre-occupied with the affairs of the industry they represent and become, in effect, 'trade schools', their continued presence in the university system ceases to be a source of strength and may even become debilitating.

One may note here that it is generally unwise to place basic areas of subject-matter under the control of an applied discipline to which they relate. Mathematics obviously should not be controlled by engineering, biology by medicine or agriculture, chemistry by chemical engineering, etc. When this happens, the basic subject assumes a service role to the technological department. This is bad for both, for development tends to loom larger than basic research. The newer universities in Britain, as they spring up to meet the needs of an increasingly industrial and technological society, will need to be protected from these hazards.

Obviously, neither system—British or American—has a monopoly of wisdom. In fact, individual variations in each country make it perhaps unwise to speak of characteristics which define either system. It is, however, interesting to note how in our time the two countries adopted from the German universities the idea of the Ph.D. degree and of more general postgraduate education than either had previously practised. From the same roots very different structures have grown. One could feel sure, however, of the future of Western scientific education if there were more signs that some of the best features of both systems—British and American—could now be blended. On balance, however, the Ph.D. degree seems to have fared better academically in Britain than in the United States, where it has become the essential status symbol for entry into a profession, or as a qualification for a post, merely because the bachelor's degree has lost its significance in this regard. In the United States, however, even the Ph.D. ceases to suffice, and postdoctoral training is now the vogue. One may wonder how many of the annual, and increasing, crop of Ph.D.s, produced in the modern quantity really do become the 'practitioners of the love of learning' which the symbols of their degree imply. Or are the Ph.D. degrees like the 1962 mass-produced cars: temporarily useful, but with their own brand of inevitable 'build-in obsolescence' like the rest of the products of mass production ? Especially at the levels of the graduate school, the supply of scholars cannot be turned on and off at will, and numbers here are no substitute for quality of training. Unless the university student is brought to a sense of the historical development of his subject, he cannot really understand its present; unless from this he gains a sense of philosophy, he cannot hope to anticipate its future. If to achieve mere quantity of trained personnel, technically equipped to meet the needs of the moment, these essential values are lost, the price to be paid is too high. The hope is, however, that the university training, like other products that bear the symbol 'Made in Great Britain', shall continue to stand first for quality, however much pressure there may be to yield, in competition with the United States and the U.S.S.R., to the need for quantity production of university graduates.

This article is obviously and admittedly written mainly from the point of view of science. Few men of science are to-day unaware of the need to foster the humanities, especially as so much greater support now comes to the aid of science. The more alarming trend, however, is not toward an imbalance between the humanities and the sciences but rather between the basic, fundamental or creative studies on one hand and the applied or technological subjects on the other. Contributions which are made through the application of knowledge, that is by development without necessarily making any ultimate additions to knowledge, can readily be dramatized. For this reason these functions can be, and should be, left largely to the industries that will profit from them or to their technological research institutes. The limited resources of the universities should, however, be fully geared to the work for which they alone provide the proper milieu which fosters the creativity on which the flow of new knowledge depends. It will be interesting to see how successfully the universities in Britain can be preserved for this function, while they respond to the inevitable pressure to produce greater numbers of graduates as in the United States.

OUTER VAN ALLEN BELTS AND NEUTRAL POINTS ON INTERFACE BETWEEN SOLAR WIND AND GEOMAGNETIC FIELD

By Prof. C. C. CHANG
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ALTHOUGH many contributions[1-3] have been made to the nature and origin of Van Allen belts, the mechanism of trapping high-energy particles from solar wind in the outer belts is still not understood. This article suggests a physical model of such a trapping mechanism.

Beard[4] obtained analytically the interface between the steady solar wind and geomagnetic field (essentially being a vacuum, the region is called hollow), with some approximate boundary conditions. The general features of the interface, although approximate, are consistent with the exact two-dimensional theory[5-7]. Beard's theory was recently generalized to some other geomagnetic orientations by Spreiter and Briggs[8]. One interesting result of their calculations yields a pair of neutral points (called N-1 hereafter), on the interface or discontinuous surface between the plasma of solar wind entirely on one side and the geomagnetic field entirely on the other, as shown in Fig. 1*a*. They are located opposite to the geomagnetic poles, each leaning about 19° with vertical polar axis toward the upstream of the horizontal solar wind. This type of singularity on a discontinuous surface is new in mathematical physics. Its instability and physical significance have not yet been recognized. Some features will be discussed here qualitatively. On the other hand, Aström[9], Dungey[10,11], Chapman and Akasofu[12,13], and Grad and Norton[14] studied the particle trajectories and plasma instability near another type of neutral point (called N-2 hereafter), which lies entirely in a continuous magnetic field. The N-2 point is a mathematical saddle point. There is some similarity between the two types of singularities, as shown in Fig. 1*b*. Their treatments can be interpreted to shed some light on the nature of the new type of singularity.

Trapping Mechanisms of Charged Particles

With the Chapman–Ferraro model of particle specular reflexion of solar wind on the interface, it has been shown[8,15,16] that across the interface, magnetic pressure $\frac{H^2}{8\pi}$ or the equivalent effect of surface current sheath J balances to approximately one-half Newtonian dynamic pressure $mnv^2\cos^2\psi$ as follows:

$$\frac{H^2}{8\pi} = 2\pi J^2 \approx mnv^2 \cos^2\psi$$

Here m is assumed essentially the mass of one proton, and n is the number of electrons or protons in the

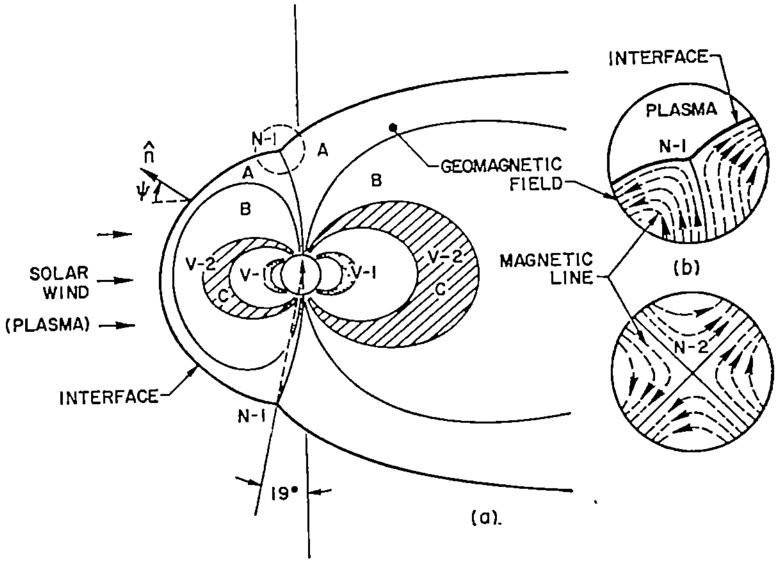

Fig. 1. *a*, Two neutral points (N-1) on the interface between solar wind plasma and geomagnetic field. Orbit zones A, B, and C in the hollow and Van Allen belts are also roughly indicated. *b*, Comparison of types of neutral points, N-1 and N-2

neutral plasma stream, which possesses a horizontal constant velocity v at an angle ψ with the normal n to the interface surface, as indicated. The magnetic field H can be shown to be parallel locally to the interface. At N-1 points, $\psi = \pm \frac{\pi}{2}$. Consequently, both $\frac{H^2}{8\pi}$ and J have to be zero. Near each N-1 point, the magnetic field has to be very weak and non-homogeneous (Fig. 1*b*) and energetic protons and electrons of the solar wind can penetrate much deeper into the hollow. Furthermore, the trajectories of these particles are non-adiabatic[14] and somewhat irregular because of drifting through field gradient and curvature. Thus, they become trapped instead of reflecting back to the solar wind, as conceived before[4,13,15]. In other words, the interface has two local weak spots of the geomagnetic field near the N-1 points, through which charged particles can seep into the hollow instead of undergoing total reflexion.

As the charged particles are trapped inside the geomagnetic field, three possible trajectory zones, A, B and C, in the hollow can be classified according to their locations, which are related to the N-1 points and the adiabaticity of particle magnetic moments. In zone A, the particle trajectories are at some instants very near to the N-1 points, unstable, and highly non-adiabatic[14]. As soon as many charged particles leak continuously into this zone, their accumulations and orbital motions demagnetize and weaken the field further. Three consequences arise: (1) The leading regions near the N-1 points are enlarged and more plasma from solar wind can be trapped in zone A. (2) Some of the particles diffuse into neighbouring zone B, according to Fick's law of diffusion on particle concentration gradient[17]. (3) The mirror reflexion points of each orbiting particle along a magnetic line move closer to geomagnetic poles. Some of the particles may possess sufficiently high velocity components along the magnetic lines to penetrate deeply into the atmosphere and become lost through colliding with low-energy hydrogen atoms and other species. The resulting ionization phenomena will first be observed as auroræ in polar regions[12]. In zone B, the particle trajectories are slightly non-adiabatic and the magnetic moment can change[14] to the order of 20 per cent or less during one round trip reflecting between the two mirror end-points of the geomagnetic poles. If too many charged particles crowd in zone B, some may similarly diffuse into zone C. In zone C, the particle trajectory is practically adiabatically invariant, and the particles are trapped for a long period between mirror reflexion points, until charged particles are accumulated excessively. This accumulation weakens the magnetic field further and some particles are finally also lost with the auroral display. In the case of exceptionally strong solar wind (10^3–10^4 particles/cm.^{3}), they can diffuse even beyond zone C, as identified from some aurora seen in Mexico[12]. In short, with the mechanism described here, the solar wind particles can leak into the hollow of the geomagnetic field passing through a cascade of feeding processes into the above different zones. It is estimated that zone C is the region of the second Van Allen belt (V-2). Zones A and B may be the region of the third Van Allen belt[3,12].

Mechanism of Boosting Particle Energy

Now we can investigate the possible mechanism of boosting up the energy-levels of the trapped particles in the outer belts. According to Teller's principle[18,19] of interchange (flute) instability in plasma physics, it is briefly stated: the plasma region is stable against disturbance, if the curvature of the boundary or interface next to the magnetic field is concave, and unstable if convex. In the present case, stream plasma interface is concave everywhere, except convex in the vicinity of the N-1 points. Therefore, the N-1 points and surrounding areas of interface are unstable, while the main portion of the interface is stable. The interface, being parallel to the magnetic field, possesses surface tension. The combination of the interface and two N-1 points act mechanically as a springy elastic membrane with two holes leaking plasma. The membrane-like interface is supported by plasma dynamic pressure outside and geomagnetic pressure inside. In case of a pressure disturbance, there is always an interplay among the two pressures and surface tension in the interface. The N-1 points, and neighbouring unstable areas of interface, start to move out of place, but the remaining stable interface resists moving along with them. The spring action in the interface and the pressure disturbance cause the local oscillations to travel continuously up and down with the N-1 points (Fig. 2).

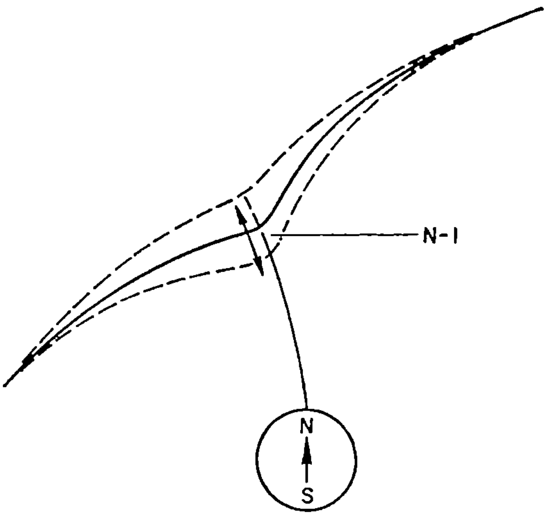

Fig. 2. Probable interface oscillation region near neutral point N-1 due to instability (exaggerated scale)

The two N-1 points, although unstable and oscillatory, always appear on the interface, so long as solar wind is present. This is quite different from the instability of N-2 points. The N-2 points move farther away with time from initial position and may finally collapse[10,12]. The fields in the two geomagnetic polar regions act as oscillatory magnetic mirrors or pistons to accelerate the charge particles. The locally oscillating interface will alternately compress and relax the geomagnetic field and thus induce magnetohydrodynamic waves in the hollow. The period of the magnetic fluctuation is much smaller than the period of the particle trajectory between the two polar regions. Therefore, the longitudinal invariant of particle trajectory is no longer valid as pointed out by Northrop and Teller[20]. This scheme of generating magnetohydrodynamic waves may be related to the so-called Fermi mechanism accelerating electrons in the outer Van Allen belt as discussed in Parker's theory[21]. As the interface oscillates, the plasma particles in the solar wind also reflect an uneven rate, thereby inducing plasma oscillation in the solar wind.

These oscillatory mirror fields can step by step transform the low-energy particles (proton energy ≈ 20 keV., electron energy ≈ 2 eV.) (ref. 12) into higher energy-levels, passing succeedingly through zones A, B and C of the outer Van Allen belts. The energy-levels in the second Van Allen belt is not known exactly. Some recent measurements gave the estimated values of proton energy ≈ 1 MeV. and electron energy ≈ 40 keV. This process of stepping up particle energy-levels is consistent with the observations reported by Jastrow[22]. In other words, a part of the kinetic energy of the solar wind feeds to the interface: (1) to maintain the oscillations of the N-1 points and local interface; (2) to create the magnetohydrodynamic waves, which boost the energy-levels of the trapped particles higher and higher in succeeding zones; (3) to induce plasma waves in solar wind.

The existence of solar winds and the interface seems to be confirmed by recent measurements of the United States Satellite *Explorer XII*; but the local oscillations of the interface and existence of magnetohydrodynamic waves have not been verified. It will be of scientific interest to have satellite measurements checking the above postulations.

Helium ions, being abundant on the Sun, are also likely to be trapped in the solar flare and wind. They can leak into the hollow through vicinities of N-1 points by a similar process. This may be the main source of atomic helium zone recently found between zones of atomic oxygen and atomic hydrogen of the magnetosphere[22], although the amount of helium released from decaying radioactive materials in the earth should also be considered.

Conclusions

In conclusion, the two N-1 points are believed to be the key mechanisms of trapping high-energy particles in the outer Van Allen belts. Their neighbourhoods allow the solar wind plasma to seep into the geomagnetic field without reflexion. Their instability causes the connected interface to oscillate locally, inducing magnetohydrodynamic waves, which increases the energy of the trapped particles in succeeding steps to much higher levels. Even when the solar wind is not steady, the above conclusions are expected to be valid, except the interface may change its position. Furthermore, those mechanisms of trapped particles and boosting energy become more effective.

As a result of Prof. S. Chapman's lectures, I was inspired to further study of this problem. I thank Drs. L. Davis, R. Jastrow, S. F. Singer and E. Smith for private discussions. I also thank Drs. C. F. Curtiss, R. Huddlestone, L. Johnston, V. Josephson, R. Meyer, F. S. Mozer, G. A. Paulikas and A. R. Sims for criticisms and valuable discussions. This work was performed under the auspices of the U.S. Air Force through Deputy Commander, Aerospace Systems Air Force Systems Command.

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STIMULATED EMISSION OF RADIATION AND ITS PRACTICAL APPLICATION TO MASERS AND LASERS*

By PROF. R. A. SMITH, C.B.E.

THE realization that light is a form of electromagnetic radiation must be regarded as one of the most outstanding consequences of Maxwell's theory of the electromagnetic field. On many previous occasions it had been postulated that light is a form of wave-motion, but no one had dared to suggest what was the quantity that varied in the motion. The experimental verification by Hertz of the existence of electromagnetic waves in what is now known as the short-wave region of the radio spectrum was indeed a triumph since this was a phenomenon which had not previously been observed. Light was, however, familiar to all, and an explanation of its properties in terms of electromagnetic waves gave a 'new look' to a familiar phenomenon. It was mainly measurements in the infra-red, for example by H. Rubens *et al.*, which showed that the theory could account quantitatively for a number of observations such as the reflectivity of metals.

The initial success of the electromagnetic theory of light was, however, short-lived, and it was not long before it was in very serious difficulty. The new century had scarcely begun when the first major trouble arose in connexion with the spectral distribution of the light from a glowing black-body. The spectral intensity has a marked peak at a wave-length which depends on the temperature, whereas an application of statistical consideration to the wave theory shows that the intensity should increase indefinitely towards the short-wave end of the spectrum. This would lead to what was known as the

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* Substance of one of the lectures delivered at King's College, London, to mark the centenary of the publication of Clark Maxwell's papers on electromagnetic production. The lectures will be published in book form in due course.

'violet catastrophe'—radiation would tend to have shorter and shorter wave-lengths. This difficulty was overcome by Planck by introducing the assumption that radiation can exchange energy with atoms only in discrete amounts or quanta. The quantum of energy exchanged at frequency ν has a value $h\nu$, and was, as a result of the work of Bohr, shown to be equal to the energy difference between two allowed atomic levels of energy E_1 and E_2, so that if $E_2 > E_1$:

$$h\nu = E_2 - E_1$$

This quantum hypothesis led also to the explanation of many other phenomena, such as the photoelectric effect, in which the energy given to an electron depends on the frequency of the incident light and not on its intensity—an observation which is very difficult to explain in terms of a wave theory of light. A tendency grew up to regard light quanta as like billiard balls flying through space, but one was never quite able to forget the wave nature of light as evidenced by the beautiful interference phenomena which it exhibits. It was not until the arrival of the new quantum mechanics and the Uncertainty Principle that the duality of light in having both the properties of waves and of particles was appreciated. Prof. R. E. Peierls has described not only the similarity of light quanta and electrons but also their essential difference, in this connexion.

There are a number of phenomena in which both the wave nature and particle nature of the light must be appreciated. If we regard a light beam as a stream of 'classical' particles each carrying energy $h\nu$ so that the mean number passing a point in the beam in unit time is n we may calculate the fluctuation $\overline{\Delta n^2}$ in this number. It is given by $\overline{\Delta n^2} = n$. The fluctuation $\overline{\Delta W^2}$ in the light power W is thus given by $\overline{\Delta W^2} = h^2\nu^2 \, \overline{\Delta n^2} = h\nu \, \overline{W}$ where W is the mean power. If, however, we regard the beam as made up of a number of modes of wave motion and calculate the power, assuming each mode has energy given by the classical equipartition law, namely kT, then it may be shown that the fluctuation $\overline{\Delta W^2}$ is given by $\overline{\Delta W^2} = kT\overline{W}$, where T is the absolute temperature. A correct quantum mechanical calculation, on the other hand, gives:

$$\overline{\Delta W^2} = h\nu \, \overline{W}\left[1 + \frac{1}{e^{h\nu/kT} - 1}\right]$$

The first term corresponds to the fluctuation given by the particle picture and is the dominant term when $h\nu \gg kT$; the second term is dominant when $h\nu \ll kT$ and then reduces to $kT \, \overline{W}$. It therefore corresponds to the fluctuation given by the wave picture. An interesting alternative interpretation of the fluctuation can be given in terms of the bunching of quanta into groups of two, three, etc.

There are a number of other phenomena which are subtly linked with the dual property of light, for example, the correlation between photo-electrons produced by different parts of a coherent beam of light as shown in the experiment of Hanbury Brown and Twiss.

Here, we shall be concerned with the processes involved in the exchange of energy between radiation and matter. As we have already seen, radiation is absorbed in quanta, the absorption process consisting of the raising of an atom from a state (1) to a state (2), with the disappearance of a quantum. (The term 'atom' is used here to denote any atomic system such as a molecule or paramagnetic ion). In the reverse process, in which radiation is emitted by an atom, it drops from an upper energy-level (2) to a lower level (1) with emission of a quantum of radiation, the frequency being given also by Planck's law. This process is frequently regarded as a random process, the time in which the atom remains in an excited state being determined only by statistical considerations, assuming that it is not disturbed by collisions with other atomic systems. This process indeed is one which occurs in the emission of light. The excitation of atoms to higher states may be accomplished by thermal vibrations, as in a hot metal, or by electron impact, as in a low-pressure gas discharge. That there is a second process of emission was shown by Einstein early in the century. This is the process of stimulated emission in which an atom is induced to fall down from a state (2) to state (1) by the presence of radiation of frequency ν also given by $h\nu = E_1 - E_2$.

The probability of this process occurring is, under normal conditions of light excitation, small compared with the probability of spontaneous emission and is frequently overlooked except when some of the subtler properties of radiation, such as those discussed earlier, are under discussion. In the microwave region of the spectrum, however, it turns out that stimulated emission may be much more probable than spontaneous emission, especially at low temperatures. It was shown by Einstein that, for a given intensity of radiation of appropriate frequency, the probability of stimulating an atom already in an upper state (2) to fall to a lower energy state (1) with emission of a quantum is exactly equal to the probability of exciting such an atom in the lower state into the upper with absorption of a quantum. Stimulated emission can therefore be regarded as the converse of absorption.

It may be shown that in conditions of thermal equilibrium the ratio of the probabilities of spontaneous emission to stimulated emission is equal to $[\exp(h\nu/kT) - 1]$. For red light $h\nu \simeq 1\cdot5$ eV. and for $T = 300°$ K., kT is about 1/40 eV., so the ratio is very large.

When $h\nu \ll kT$, however, the ratio is approximately equal to $h\nu/kT$. For the microwave radiation of wave-length 10 cm. $h\nu$ is approximately equal to 10^{-5} eV., so that the ratio is 4×10^{-4}. In this case stimulated emission clearly predominates.

On the classical wave theory of the interaction of radiation with matter, absorption and both processes of emission occur naturally. Spontaneous emission corresponds to radiation damping of the atomic system regarded as a harmonic oscillator. Absorption and stimulated emission correspond to forced oscillations induced by the incident waves. Whether one obtains emission or absorption depends on the state of oscillation of the system when the wave is incident on it. It is in the quantitative description of the energy exchange that the wave-theory fails.

Let us now consider the absorption of a beam of microwave radiation by material in which we have N atomic systems in state (1) and N_2 in state (2). We shall consider only these two states for simplicity, taking their energies as E_1 and E_2 with $E_2 > E_1$. We shall also neglect spontaneous emission and consider the effect of absorption and stimulated emission. The number of quanta absorbed will clearly be proportional to N, and the number emitted which, as may be shown, must travel in the same direction as the beam, will be proportional to N_2, with the same constant of proportionality. The net absorption will therefore be proportional to $(N_1 - N_2)$, that is, to the differences in populations. The ratio N_2/N_1, in equilibrium, is given by Boltzmann's law, namely:

$$N_2/N_1 = e^{(E_1 - E_2)/kT} = e^{-h\nu/kT}$$

If $h\nu k \ll T$ we have approximately, if $N = N_1 + N_2$:

$$N_1 - N_2 = Nh\nu/2kT$$

In this case, the population difference is small and increases as the temperature is lowered. The net absorption is simply a balance between absorption and stimulated emission, and if N_2 is nearly equal to N_1 they very nearly cancel each other. This equivalence of the two processes leads to a far-reaching conclusion on the nature of stimulated emission. The absorption process is a coherent one; a beam retains its direction and coherence even when a considerable part of it is absorbed. We can therefore conclude that the same applies to stimulated emission, the incident beam grows in intensity and retains its coherence. The process can be regarded, therefore, as a true amplification of the beam. Spontaneous emission, on the other hand, is a random process, the emitted quanta travelling in all directions.

When microwave radiation is absorbed by a system of atoms as described above, the number N_2 in the upper level will begin to increase and the number N_1 in the lower level to decrease, since more make the upward transition than the downward one. If no other processes were occurring the numbers of atoms in the two states would tend to equality and the absorption would be reduced to zero. In fact, other processes do take place, for example, interactions with the lattice vibrations in a solid or collisions with other atoms or with the walls of the containing vessel in a gas, and these will tend to restore the equilibrium values N_{10} and N_{20} with a relaxation time τ. If τ is very short the departure from the equilibrium values will be small. In many instances sufficiently large values of τ are found so that N_1 can be made very nearly equal to N_2 with microwave signals of reasonable power. This phenomenon is known as 'saturation' of the two levels.

Since it is possible to tamper with the populations of atomic energy-levels in this way the question arises as to whether one can go further and make N_2 greater than N_1. If this could be done a very interesting situation would arise, since stimulated emission would exceed absorption and we should have a net amplification. It turns out that this also can be done. The possibility of the use of this process to obtain microwave amplification was realized about the same time by a number of people in different places, but was first successfully demonstrated by Prof. C. H. Townes and his colleagues at Columbia University in 1955. An amplifier based on the process is known as a 'maser' (*m*icrowave *a*mplification through *s*timulated *e*mission of *r*adiation).

Under normal conditions there will always be more atoms in the lower state than in the upper ($N_1 > N_2$), because of Boltzmann's Law. Suppose by some means we could distinguish between the two states and could filter out most of those in the lower state leaving only those in the upper state. The material would then be in what is known as an emissive condition, that is, it could be stimulated to produce coherent radiation. Let us imagine for example that we had an atomic (or molecular) beam consisting of an admixture of atoms (or molecules) in states (1) and (2) separated by a small energy difference corresponding to a microwave frequency. Now let us imagine that the beam passes a gate at which is seated a demon, such as envisaged by Maxwell, who recognizes the two states by means of a label which each atom carries. He rejects those labelled (1) and passes those labelled (2). The beam consisting only of atoms in state (2) then can be passed into a resonant cavity to interact with microwaves. This is exactly what Prof. Townes and his colleagues did. The beam consisted of ammonia molecules which have two states separated by a small energy corresponding to microwave radiation of wave-length about 3 cm. The label used for distinguishing the two states was the sign of the induced dipole moment in an electric field and the gate simply consisted of an inhomogeneous electric field which focused those in the upper state on a slit of a resonant cavity and dispersed those in the lower state. By this means both microwave amplification and oscillation were obtained. This experiment would have delighted the heart of Maxwell.

It is mainly as an oscillator that the ammonia maser has found a practical use. The frequency band-width over which amplification is obtainable is very narrow, and the amplifier is not tunable although there are a number of adjacent absorption lines which can be used. As an oscillator it produces a very stable frequency determined largely by the natural frequencies of the ammonia molecule. An even more sharply determined frequency can be obtained from a maser of this type using hydrogen as the active material. An oscillator of this type has been developed at Harvard University and may have important applications as an 'atomic clock'.

Various other tricks have been used to obtain 'inversion' of the population of two levels. For example, a single unpaired electron in a paramagnetic ion has two levels separated by an amount $2\ \mu H$ when placed in a magnetic field of strength H, μ being the magnetic moment of the electron. For values of H of the order of 10^4 œrsteds the energy separation of the levels is such as to give radiation of the order of 1 cm. in wave-length. In principle, two such levels could be 'inverted' simply by reversing the magnetic field. In practice, this takes too long a time so that the populations can relax back to their equilibrium values; but the same effect can be obtained by sweeping in frequency a strong microwave signal so that it passes through the resonant frequency. The frequency band-width of the microwave absorption lines in paramagnetic solids is considerably greater than for a gas and amplifiers of reasonable band-width can be made in this way. These would, however, be pulsed amplifiers, since the population inversion would relax soon after the inversion process and could have to be regenerated.

A method of obtaining continuous inversion of the population of two levels and of maintaining a crystal containing paramagnetic ions in an emissive condition was proposed by Prof. Bloembergen of Harvard and has been made the basis of practical microwave amplifiers. For this, three energy-levels are required so that paramagnetic ions with magnetic moment greater than that corresponding to a single unpaired electron are necessary. Cr^{3+} ions diluted in alumina are commonly used for this purpose in the form of a ruby crystal.

If we have three levels with energies E_1, E_2, E_3 ($E_1 < E_2 < E_3$) and their populations are N_{10}, N_{20}, N_{30} in equilibrium then by Boltzmann's Law, $N_{10} > N_{20} > N_{30}$. Now if we apply a strong microwave signal of frequency ν_p given by $h\nu_p = E_3 - E_1$ we can 'saturate' the levels (1) and (3), as explained earlier, and so make $N_1 \simeq N_3$. Now if level (2) and level (3) are closely coupled thermally, and no disturbing signal is applied, we must have $N_2 > N_3$. This implies that when the levels (1) and (3) are 'saturated' $N_2 > N_1$ and we have an emissive condition set up between levels (1) and (2). We can then obtain amplification at a frequency ν_s given by $h\nu_s = E_2 - E_1$. The frequency ν_p is generally called the pump frequency and ν_s the signal frequency. We note that $\nu_s < \nu_p$.

Two types of practical amplifier have been made using this principle. In the first the paramagnetic material is contained in a resonant cavity, which is generally cooled to liquid helium temperature, and is placed in a magnetic field. Both the incoming signal and pump-power are fed to the cavity and the amplified signal, 'reflected' from the cavity, is separated from the incoming signal by means of an arrangement of wave-guides called an 'isolator'. An amplifier of this kind is known as a cavity maser. An alternative, and in many ways preferable, arrangement is to have the paramagnetic crystal set along the length of a wave-guide. The signal is fed in at one end and taken out at the other end of the wave-guide, which also carries the pump power. The wave-guide is of rather special construction in order to slow up the signal wave so as to give it as long a time as possible to interact with the 'emissive' material. In this case no 'isolator' is required. This type is known as a travelling-wave maser. Its main advantages over the cavity types are greater band-width and greater stability in operation.

The chief advantage of maser amplifiers over conventional microwave amplifiers using crystal mixers or travelling-wave tubes is that they generate very much less electrical 'noise' and so can receive weaker signals without their loss in the noise background. There are two main reasons for this. First, in these amplifiers no streams of electrons are involved in the amplification process so that shot noise, due to the discrete nature of the charges making up a current of electrons, is avoided. Secondly, they are operated at very low temperatures so that thermal fluctuation noise (Johnson noise) is very small. It is of interest to note that the equivalent of this kind of noise in the maser amplifier comes from the random spontaneous emission of radiation.

In order to appreciate the marked improvement in signal-to-noise ratio which may be obtained with maser amplifiers we must say a word about the way in which amplifier performance is specified. Let us take for granted that adequate gain is available and attend only to the noise problem. The noise-level generated by an amplifier is generally described by means of a noise temperature T_n measured in degrees absolute. When we say that an amplifier has a noise temperature T_n we mean that it generates noise-power equivalent to the maximum noise-power available from a resistor at temperature T_n at its input. For example, a conventional microwave amplifier might have a noise temperature of 3,000° K.; this would mean that it generates about ten times as much noise-power as a matched resistive input load at room temperature. With maser amplifiers on the other hand, noise temperatures of the order of 3° K. may be obtained. Such an amplifier would really be wasted in working with an input which is effectively at room temperature.

When a nearly loss-free microwave aerial has its beam directed well above the horizon it behaves like a low-temperature load, receiving very little noise from outer space. When pointing nearly horizontally so as to receive an appreciable amount of noise from the ground (which is at a temperature of about 300° K.) such an aerial behaves like a resistive load having a temperature of the order of 300° K. Maser amplifiers are therefore mainly of use in receiving signals from elevated objects. They have been used successfully in radio astronomy and in reception of reflected signals from artificial satellites. For example, a maser receiver was used in conjunction with the recent experiment known as 'Project Echo' in which trans-continental communication was obtained by reflecting signals from a satellite in the form of a large spherical balloon. Figures given for the performance of this receiving system operated at the Bell Telephone Laboratories in the United States are quite remarkable.

The overall noise temperature of the receiving system was only 18·5° K. Of this the maser amplifier contributed 10·5° K. with a power gain of 3,000 at a wave-length of 5·3 cm. The aerial and feeder system contributed 5·5° K., the remaining 2·5° K. being attributed to noise coming in from the sky. This should be compared with the noise temperature obtainable with the best conventional microwave receivers for this wave-length, which is not much less than 1,000° K.

When we come to consider the use of stimulated emission to obtain coherent amplification of a light beam the situation looks far from promising, in view of the very small ratio of the probability of stimulated emission to that of spontaneous emission. In the usual light sources, either in the form of hot solids, fluorescent solids or gas discharges, by far the greatest number of light quanta are generated as a result of spontaneous emission, and the light has a rather similar nature to random noise. In spite of this, conditions have now been found in which stimulated emission of light may predominate, and the phenomenon has been used to produce narrow coherent beams of light which approximate more clearly to the plane wave trains envisaged by Maxwell than one would have ever imagined to be possible some years ago.

The method used to obtain such beams depends on the fact that stimulated emission is not a random process. If we have a large number N of atoms radiating with random phases they produce at any point an intensity NI where I is the intensity each would produce by itself. If on the other hand they radiate with phases such that, in a specified direction, their radiations are in phase, then they produce an intensity equal to N^2I. If N is a very large number, a very great intensity may be obtained in a closely specified direction. Some device must be used to ensure that the radiations from the individual atoms are in phase. The device normally used consists of two accurately parallel highly reflecting plates as in a Fabry–Perot interferometer. If the active material is placed between the plates only light waves travelling in a direction accurately at right-angles to the plates will be reflected many times through the active material and will be amplified by stimulated emission if the material is in an emissive condition. If such a condition could be achieved, and amplification sufficient to overcome the losses on reflexion could be obtained, an intense standing-wave light pattern would be set up between the plates and if one plate were slightly transparent an accurately parallel beam could be made to emerge. The main difficulty is to produce the emissive condition. This was first achieved by Maiman and soon after by a group at the Bell Telephone Laboratories, again using ruby as the active material. In the latter case the parallel plates were made by grinding optical flats on the ends of a ruby rod about 0·5 cm. in diameter and 4 cm. in length and then coating them with silver.

The emitted light was red and had wave-lengths corresponding to the red fluorescence of ruby. A very intense source of light of much shorter wave-length was used to depopulate the ground-state of the Cr^{3+} ions, so that originally only short pulses were used. Electrons then fell back into the metastable states (a doublet) from which the fluorescent radiation originates so that these acqured a population in excess of that of the ground state, so producing an emissive condition. A large part of the radiation absorbed as pump-power is re-emitted in all directions through spontaneous emission, only a small fraction going into the coherent radiation in the beam. Nevertheless very high intensities can be produced; for example, a light pulse lasting 0·5 m.sec. and having energy 0·2 joule has been achieved. The beam is only partly coherent due to defects in the ruby but it may be focused to a spot of about 0·1 mm. diameter. The mean output is thus 0·4 kW. and the power flux of the order of 4×10^6 W./cm.^{2}. This should be compared with a value of the order of 500 W./cm.^{2} obtainable by focusing the Sun with an *f*1 lens. A device of this kind is known as a 'laser' the *l* standing for light. Many possible uses have been proposed including signalling from the Moon. The most practical use made so far would appear to be in flash photomicrography. If continuous operation could be obtained, and we understand that this has now been achieved with a ruby laser although no details are available, the higher power flux could be used to melt refractory materials.

Lasers may also be made to operate in the near infra-red using U^{3+} ions in calcium fluoride. In this case the final state for the emission lies a little above the ground-state and can be effectively emptied by cooling the sample with liquid helium. Much less pump power is now required. Continuous operation of a laser has been obtained by Javan *et al.* using a mixture of hydrogen and helium as the active medium. The parallel plates in this case are placed in the gas, which is excited by a high-frequency discharge, exchange of excitation between the hydrogen and a metastable state of helium producing the emissive condition. The Fabry–Perot plates need to be flat to a very high degree of accuracy and have a multi-layer coating to give high reflectivity and low absorption. This is required because many passages through the gas are needed because of its low density compared with that of the ions in ruby. A much more accurately phase coherent beam is obtained, however, interferometric experiments showing coherence over the whole diameter of the beam, which was about 1·5 cm.

These devices are clearly in an early stage, and many new developments may be expected in the near future. Monochromatic sources in the infra-red will be of the greatest value and intense coherent sources of light open up many possibilities for optical experiments. Already lasers have been used for demonstrating beats between light waves and the generation of harmonies at optical frequencies.

An important new chapter has been started in the study and use of light and radio waves. It is curious how long it has taken in this case to find this new application of theory now more than half a century old.

There was, of course, also quite a gap between the first publication of Maxwell's theory of the electromagnetic field and its practical use in radio communication. These developments in the use of stimulated emission are also likely to lead to exciting new episodes in the story started by Maxwell in King's College, London, about a century ago.

OBITUARY

Prof. F. Richter

BEILSTEIN'S *Handbuch der Organischen Chemie* is now in its fourth edition and has been extended by two complete supplements so that with comparatively few exceptions all compounds prepared up to 1929 are included. A third supplement is now appearing. If those who use this great work had time to look back to 1881, when the first edition appeared, they would be conscious of a simpler and less-hurried existence. The devotees of chemical science were few, their laboratories by present-day standards were poorly equipped and the physical aids now so valuable had never been imagined. Nevertheless, thankfulness for the privilege of being a chemist or a student of chemistry and the slow, sometimes painful, but always exciting process of acquiring at the laboratory bench that invaluable 'feel' for organic compounds produced the results and laid the foundations on which the modern edifice of carbon chemistry has been constructed.

Four names have been specially associated with the development of the *Handbuch*: Konrad Friedrich Beilstein, Paul Jacobson, Bernhard Prager and Friedrich Richter. At the time of his death on November 22, 1961, at the age of sixty-five, Richter was director of the Beilstein Institut fur Literatur der Organischen Chemie in Frankfurt a/d Main. Since 1933 he had been responsible for the production of *Beilstein* during the most difficult period of its history and had seen eighty volumes through the press. On many occasions he recorded or lectured on the history of this great undertaking. It is remarkable that long-continued work on a production of this kind should, nevertheless, have given rise to so much loyalty and self-sacrifice, to so much real joy and justifiable pride in achievement and to such deep satisfaction in serving the needs of chemical science. Those who have read Richter's writings on *Beilstein*—the man and the work—realize that this description of his own outlook and that of his staff and predecessors is not exaggerated.

Friedrich Richter was born in Berlin on September 1, 1896, and educated at the humanistic Friedrichs-Gymnasium, where he was a remarkably outstanding pupil. At the University of Berlin he first studied classical and semitic philology, but soon decided that his interests lay in natural science. His command of languages dates from this time, however, and he read English, French, Italian, Spanish, modern Greek and

Russian with ease. His written and spoken English were of a high order. His teachers in Berlin were Emil Fischer, Leuchs, Traube and also Tiede, with whom he worked on the phosphorescence of magnesium sulphide. It is interesting that Richter, whose whole life was to be devoted to organic chemistry, should have carried out his first research on such a subject. It may be mentioned, however, that even Ramsay's early researches were concerned with the toluic acids and then with pyridine bases. Richter left Tiede in 1917 and worked for a year with Haber on methods for the analysis and counteraction of 'war gases'. He also began to abstract papers for the *Chemisches Central Blatt* and soon afterwards became associated with the production of the first volume of the fourth edition of *Beilstein* which was published in 1918; his name appears on page xxviii.

Shortly after the death of Paul Jacobson in 1923 the German Chemical Society asked him to undertake the preparation of the first and second *Ergänzungswerke* which, with the *Hauptwerk* of the fourth edition, would provide a survey of the literature up to 1929. Bernhard Prager, who had succeeded Jacobson, retired in 1933 and from that time Richter was responsible for the production of the whole work.

The years that followed were marked by ever-increasing difficulties. Many members of his staff were lost owing to the racial policy of the Government; during the Second World War the Hofmann Haus was destroyed and the editorial office transferred first to Silesia and then to Tharandt in Saxony. It was finally re-established as the Beilstein Institut in Frankfort a/d Main. The next fifteen years, though presenting many problems for Richter and his colleagues, were characterized by steady progress, confidence in the value of their work and by wide recognition of Richter's outstanding qualities not only as an organizer with a unique knowledge of chemical literature but also as a dedicated scholar and a cultivated man. The success with which he carried out an almost superhuman labour was largely due to what his brother, Dr. Hans Richter, has described as his economy of mental effort (*geistige Ökonomie*). Without this capacity the magnitude of his task, both in its broad conception and in detail, might have become overwhelming.

In 1956 the University of Frankfurt elected Richter to an honorary professorship, and there, and in the University of Mainz, he lectured on nomenclature and documentation. Heidelberg made him an honorary doctor and the Gesellschaft Deutscher Chemiker awarded him the Gmelin-Beilstein Medal. His lecture on this occasion has been published.

Between 1923 and 1931 Richter found time for research on aspects of terpene chemistry and related topics, in the laboratory of the Hofmann Haus. He studied first the bromination of thymol in glacial acetic acid and showed that one of the products, tribromomenthadienone, readily loses the isopropyl group when treated with sulphuric acid. In later papers he found that unexpected structural changes may occur during the hydrogenation of various unsaturated compounds of the terpene series. Thus β-pinene gave α-pinene and finally pinane in presence of palladium black. Sabinene under similar conditions suffered fission of the three-membered ring to give an unsaturated and then a saturated derivative of *cyclo*-pentane. Hydrogenation with platinum black, however, proceeded normally giving thujane. α- and γ-terpinene in a nitrogen atmosphere with palladium saturated with hydrogen reacted violently, giving cymene as well as menthene and menthane. This work on terpinene led him directly to a study of the physical properties of cymene and the reactions of ascaridole.

About 1930 Richter's laboratory was dismantled owing to the need of the Hofmann Haus for more library space. For a time he secured accommodation in the laboratory of Prof. Rosenheim, but after 1932 his publications were confined to articles dealing with stereochemistry, the life of von Baeyer and especially the life and work of Beilstein and the history of his *Handbuch*.

In 1952 Richter visited the Department of Organic Chemistry in the University of Leeds. A research student had just synthesized the solid isomer of thiophthen and felt greatly honoured that the first chemist from outside the Department to whom he showed his specimen was the head of the Beilstein organization.

Richter was no mean pianist and organist; he was an authority on the art of East Asia, especially on Chinese and Korean ceramics, and steadily refused to allow his heavy professional work to interfere with these interests. His appreciation of Japanese wood-carving led him to study the language, with the result that during the long evenings spent in Silesia he translated a collection of ghost stories from Japanese into both German and Russian. He also utilized his knowledge of Japanese in his professional work.

In spite of Richter's deep knowledge of his chosen science and his wide cultural interests, he was never pedantic or merely erudite. He was always ready to discuss, with a characteristic clarity, professional, literary or artistic matters and to answer questions. To have solved a problem, whether in discussion or in the laboratory was to him a source of deep satisfaction. Whenever he learned a new language Richter studied its literature. He loved books, and his library and art collections were a treasure house. Their destruction during the War was a grievous blow to him which he bore without complaint.

Richter's physique was not robust, but he inherited from his mother a great tenacity of spirit. Moreover, like St. Paul, whose epistles he and a student translated from the original text, he learned to come to terms with his physical limitations. He was never married but lived for many years with his brother in ideal understanding. Though always courteous, he was very diffident and reserved and at times may have appeared aloof. Not many people knew him well. This was not due to conceit or to any kind of arrogance on his part but rather to his very independent and restrained judgment of men and things.

On the other hand, he possessed great social gifts and when among congenial people he was a brilliant, polished and, at times, humorous conversationalist. with a light and gracious touch which gave great pleasure to those who understood him. Richter had a love for southern Europe and its people, to whom his agile and imaginative nature made him akin. Those for whom he lifted his curtain of reserve and gave of his friendship were permanently enriched by the experience.

I am grateful to Dr. Hans Richter and to Frau R. Knoll for sending me much valuable information about Richter's scientific work and cultural interests and a striking assessment of his character.

FREDERICK CHALLENGER

NEWS and VIEWS

First Anglo-American Satellite, *Ariel*

At 18.00 U.T. on April 26 an American *Thor–Delta* Rocket was launched from Cape Canaveral carrying the first Anglo-American Satellite, *Ariel* (*U.K.*1). This Satellite is the first of a series, which arose from an offer to the Committee on Space Research of the International Council of Scientific Unions in March 1959 by the National Aeronautics and Space Administration to launch satellites instrumented by other countries. *Ariel* has a higher orbit than intended; its height above the Earth varies between 242 and 754 miles, and its initial period was 100·86 min. The orbit is inclined to the equator at 53·87°. *Ariel* passes over Britain and is visible through binoculars at certain times. The Satellite, which weighs 132 lb., is a cylinder with rounded ends, being 23 in. in diameter and 21 in. long. Extending from this cylinder are sensors and paddles, giving an overall width of 10 ft. *Ariel* has been designated 1962 o 1, and its carrier rocket, which is in a similar orbit, has been designated 1962 o 2. The equipment aboard *Ariel* is intended to send back information for a year, though the Satellite itself is expected to remain in orbit for 20–30 years. The main purpose of the experiments is to investigate the properties of the ionosphere. Instruments provided by University College, London, are intended to measure the electron temperature and density, the ion mass spectrum and the intensity of solar radiation. Equipment designed by the Imperial College of Science and Technology is to measure cosmic rays, while that provided by the University of Birmingham is to measure electron density.

Royal Naval Scientific Service :

Dr. H. F. Willis, C.B.

Dr. H. F. Willis, who has been Chief of the Royal Naval Scientific Service since 1954, has joined the Ministry of Defence as a scientific adviser. Dr. Willis, who is fifty-three, is a graduate of University College, Cardiff, where he obtained first-class honours in mathematics in 1930 and physics in 1931. In the following year he gained the M.Sc. degree for research on relativity, and became a Fellow of the University of Wales in 1933. He spent the next two years at Trinity College, Cambridge, engaged on experimental research into the optical properties of molecules, and for this work was awarded the Ph.D. degree. In 1935 he joined the British Cotton Industry Research Association and carried out fundamental research on the diffusion of dye-stuffs into cellulose fibres. These early researches were described in papers published in the *Philosophical Magazine* and in the *Proceedings of the Royal Society* and of the *Faraday Society*. Dr. Willis entered Admiralty service in 1938, and until 1945 was engaged on underwater acoustics research in the anti-submarine experimental group, where he served with notable success. He was then transferred to the Admiralty Research Laboratory, and until 1953 was leader of the group concerned with problems in electromagnetics and underwater ballistics. He was promoted to the rank of deputy chief scientific officer in January 1950, and became superintendent of the Admiralty Research Laboratory in 1953.

Dr. R. H. Purcell, C.B.

Dr. R. H. Purcell, chief scientific adviser to the Home Office, has been appointed to succeed Dr. H. F. Willis as Chief of the Royal Naval Scientific Service. Dr. Purcell, born on July 14, 1904, graduated at the Royal College of Science, South Kensington, with first-class honours in chemistry in 1924. He then worked under Prof. H. B. Baker and gained the Ph.D. degree in 1927 for a thesis on the influence of intensive drying on the velocity of heterogeneous reactions. Obtaining first a University of London Travelling Fellowship and then a Ramsay Memorial Fellowship, he spent some time working under Prof. A. Smits in Amsterdam on physico-chemical topics. He was appointed lecturer in physical chemistry at the Royal College of Science in 1929 and later assumed charge of the teaching laboratories in that subject. From 1939 onwards he was, for an increasingly high proportion of his time, engaged on defence research. He acted as scientific adviser to the then Department of Miscellaneous Weapons and Devices, Admiralty, and his services to the Admiralty became full-time from 1944 onwards. After the War, when the Royal Naval Scientific Service was formed, he was appointed to the Department of Aeronautical and Engineering Research, and was responsible for the organization of materials research and development work. Periods of attachment to the Admiralty Materials Laboratory and then to the Admiralty Research Laboratory followed. His work was characterized by the great range of his scientific interests and this was reflected in his appointment in 1951 as deputy director of the Department of Physical Research, Admiralty. He left in March 1954 in order to become chief scientific adviser to the Home Office, and his appointment as chief of the Royal Naval Scientific Service is a return to a field of research and development of which he possesses wide experience and where he has already achieved conspicuous success.

Soil Survey of England and Wales :

Dr. D. A. Osmond

Dr. Donald Arthur Osmond, formerly deputy director of the Soil Survey of England and Wales, has been appointed director, in succession to Dr. A. Muir, who died on February 1 (*Nature*, 194, 135; 1962). Dr. Osmond, who has been with the Soil Survey since it was constituted under the late Prof. G. W. Robinson in 1939, had previously been engaged on soil survey while on the staff of the Long Ashton Research Station, Bristol, and made notable contributions to the study of the soils of the Vale of Evesham, Shropshire and Somersetshire, where he investigated the 'teart' condition of cattle, caused by excess of molybdenum in the herbage. During the War, when work on soil surveying was suspended, he was engaged on advisory work, and later took a major part in land-classification projects in the West Midlands, Gloucestershire, Somersetshire and Wiltshire. He has been largely responsible for the introduction of micro-morphological methods into English soil surveying. He is the British member of the Food and Agriculture Organization team which is preparing a soil map of Europe. For some years he was assistant editor of the *Journal of Soil Science*.

New U.S. Division of Research Facilities and Resources at the National Institutes of Health

A NEW Division of Research Facilities and Resources at the National Institutes of Health, Bethesda, is to be established, taking effect from July 15. Dr. F. L. Stone, at present acting chief of the Division of General Medical Sciences, will be in charge of the new Division, and Dr. C. C. Powell, assistant director of the National Institute of Allergy and Infectious Diseases, will become chief of the Division of General Medical Sciences. The new Division will centralize the administration of some of the major activities and programmes of the National Institutes of Health which provide broad support to biomedical research institutions in the United States. The responsibilities of the new Division will include the following programmes and activities: the Health Research Facilities Construction Programme; the Programme of Support for Primate Centers; the General Clinical Research Centers Programme; the Special Resource Centers Programme; and the General Research Support Grant Programme. Dr. Powell will take charge of the programmes for the support of fundamental research and research training. The Center for Aging Research and the Center for Research in Child Health will come within the Division of General Medical Sciences.

Dr. F. L. Stone

BORN in Biloxi, Mississippi, in 1915, Dr. Stone received a Ph.D. degree at the University of Rochester in 1948. He was commissioned in the U.S. Public Health Service at that time and served at the National Institutes of Health until 1954. He was chief of the Research Fellowships Branch in the Division of Research Grants until 1951 and chief of the Extramural Programs in the National Institute of Neurological Diseases and Blindness from 1951 until 1954. During 1954–55, Dr. Stone was assistant vice-chancellor for professional services in the Schools of the Health Professions at the University of Pittsburgh, and during 1955–56 was director, Medical and Scientific Department, National Multiple Sclerosis Society, New York City. Dr. Stone returned to the National Institutes of Health in 1956, becoming assistant to the Associate Director in 1956, assistant chief, Division of Research Grants, in 1957, and assistant chief, Division of General Medical Sciences, and chief of its Research Training Branch in 1958.

Dr. C. C. Powell

DR. POWELL's work in the Public Health Service has been in the areas of general programme development and grants management—both in the Division of Research Grants and in the National Institute of Allergy and Infectious Diseases. From 1958 until 1960, he was in the Division of Research Grants, National Institutes of Health, first as an executive secretary and later as assistant chief of the Research Grants Review Branch, and as deputy chief of the Division. He has been assistant director of the National Institute of Allergy and Infectious Diseases. Dr. Powell was born in Hartford, Connecticut, in 1918, and received a B.S. degree from the Massachusetts Institute of Technology in 1940, and an M.D. degree from Boston University in 1944. He was commissioned in the U.S. Public Health Service in 1946, and from 1951 was a resident in radiology at the U.S. Public Health Service Hospital, Baltimore. During 1952–54 he was a fellow in radiology at the University of Pennsylvania Hospital, but returned to the National Institutes of Health in 1954. In 1955 he served in the National Cancer Institute's Research Grants and Fellowship Branch, and from 1956 until 1958 Dr. Powell was chief of the Radiological Health Medical Program in the Division of Special Health Services.

Expansion Trends in British Universities

ON April 10, Sir Edward Boyle said that in the academic years 1959–60, 1960–61 and 1961–62, university entrants as a percentage of the relevant age groups were 4·5, 4·8 and 4·6, respectively. For the years 1962–63, 1963–64, 1964–65 and 1965–66 the corresponding percentages were estimated at 4·6, 4·6, 5·3 and 4·6, respectively. The figures excluded British students going to universities overseas, including Ireland. In reply to a question in the House of Commons on April 12, the Chief Secretary to the Treasury, Mr. H. Brooke, said that allocations from public funds to the universities had been doubled in the past five years and would increase by 50 per cent in the next five. Excluding expenditure by research councils towards university research and postgraduate awards, public expenditure, including recurrent and non-recurrent Exchequer grants, public expenditure on student awards, endowment income and fees, had risen from £56·3 million in the financial year 1957–58, or 0·29 per cent of the gross National Product, to £67·3 million, or 0·33 per cent in 1958–59; £76·7 million, or 0·36 per cent in 1959–60; £85·8 million, or 0·4 per cent in 1960–61; and £109·7 million, or 0·46 per cent in 1961–62. It was expected to reach about £150 million by 1965–66.

1961 Conference of the Universities of the United Kingdom

THE 1961 report of proceedings of the Conference of the Universities of the United Kingdom, held in London during December 15–16, provides the usual concise record of the main speeches and discussions at the Conference (Pp. 82. The Association of the Universities of the British Commonwealth, 1962. 5*s*.). The Conference was concerned with the structure and organization of the university teaching profession and with problems of the maturity of students, particularly their welfare and discipline. The speeches of Dr. C. E. F. Chilver and Prof. R. L. Brett opening the former discussion are given in full, as are those of the Rev. J. C. Blackie and Dr. N. Mallison, who opened the second discussion.

Manpower of the British Scientific Civil Service

IN answer to a question in the House of Commons on April 3, Sir Edward Boyle said that in 1960, 3,235 members of the scientific officer class of the Scientific Civil Service were mainly concerned with research and its direction in England and Wales, compared with 2,903 in 1955 and 3,303 in 1950; for Scotland, the comparable figures were 172, 145 and 109. On the same day, the Secretary for Technical Co-operation, Mr. D. Vosper, said that the results of the campaign launched in November for filling key teaching posts abroad were disappointing. Britain was willing to subsidize up to 400 such posts, but so far only 147 out of more than 200 applications dealt with had been approved, and 19 out of another 70 which were still being examined.

The Institution of Professional Civil Servants

The annual report for 1961 of the Institution of Professional Civil Servants, published in *State Service* for April 1962, records a membership at December 31, 1961, of 56,927, compared with 53,923 the previous year, but uneasiness about future methods of settling pay in the Civil Service. Government policy over the 'pay pause' is regarded as jeopardizing the work of the Pay Research Unit, but a fresh campaign is to be planned for the recognition of temporary service in full for pension purposes. The recommendations of the Zuckerman Report are still being examined by the Institution, but while any effort to implement the recommendation regarding the transfer of scientists to administrative posts would be welcomed, some of the recommendations are regarded as inconsistent with the concept of a stable career in the Civil Service. Satisfaction is expressed that most of the features in the plans for the new National Reference Library for Science and Invention to which the Institution had objected have now been removed.

The Manchester Museum

The most important event of the year 1960–61 at the Manchester Museum was the opening of the gallery containing the extensive and important R. W. Lloyd Japanese collection. The lovely ivories, lacquers, examples of sword furniture, etc., have been displayed and lighted so that the incredibly detailed workmanship can be seen to the best advantage. The most interesting temporary exhibition was entitled "Pioneer Research around Manchester", in which an endeavour was made to show, by means of actual apparatus, the experiments and advances made in science and engineering in and near Manchester during the past 200 years. Apparatus of Dalton the chemist, Joule the physicist, Osborne-Reynolds the engineer was placed with the "Logical Machine" of Jevons the economist. The small chamber in which Rutherford first 'split the atom' was displayed, together with Whitworths' gauges and Royce's first automobile engine.

Colloque Ampère and the *Archives des Sciences*

The *Colloque Ampère* issue, which in former years has been a special supplement of the *Archives des Sciences*, is this year to become a separate publication and will no longer be incorporated in the *Archives*. The subscription price for the *Archives* has been reduced accordingly to 50 Swiss francs per annum, starting with Volume 15, 1962. Subscription forms for the *Colloque Ampère* issue can be obtained from Prof. G. Béné, Institut de Physique de l'Université, Boulevard d'Yvoy, Geneva, and for the *Archives* from the Muséum d'Histoire naturelle, Geneva.

The British Society for Parasitology

At its annual general meeting during April 3–4, the Parasitology Group (Institute of Biology) was dissolved and the British Society for Parasitology was formed. Membership is open to anyone actively interested in parasitology, and a scheme for founder membership is in operation whereby persons can apply for membership before July 31, 1962, without having to obtain the signatures of a proposer and seconder. The annual subscription is £1. Anyone interested in applying for founder membership should write to the honorary general secretary, Dr. J. R. Baker, Department of Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

The Granada Lectures 1961

The annual lectures organized by the British Association for the Advancement of Science and sponsored by Granada Television network are given in Guildhall, London, before an invited audience. A shortened version of each lecture is transmitted on television. During October 1961 the three lectures were given by Sir James Gray on "The Language of Animals", Prof. H. Bondi on "Why Scientists Talk", and Sir John Wolfenden on "The Gap and the Bridge". The lectures have now been published in booklet form (University of London Press. 4*s*. 6*d*.).

Society of Chemical Industry : Food Group

The following have been elected officers for the Food Group of the Society of Chemical Industry: *Chairman*, Dr. J. G. Davis; *Vice-Chairman*, Mr. T. McLachlan; *Honorary Treasurer*, Mr. R. De Giacomi; *Joint Honorary Secretaries*, Mr. G. Clewlow and Dr. B. R. J. Thomas; *Honorary Recorder*, Mr. L. C. Dutton. The following were elected members of the Committee: Mr. W. Price Davis, Dr. H. G. Smith, Mr. H. W. Symons and Mr. G. R. A. Short.

Television Society Premiums

The Television Society has awarded the following premiums, for outstanding papers read before the Society in 1960–61: the *Electronic Engineering Premium*, to Mr. I. J. P. James (Research Laboratories, E.M.I., Ltd.) for his paper on "Colour Television Camera Problems"; the *E.M.I. Premium*, to Dr. W. E. Glenn (General Electric Research Laboratory, Schenectady, New York) for his paper on "Thermoplastic Recording"; the *Mervyn Premium*, to Dr. D. E. N. King (the Hirst Research Centre, G.E.C.) for his paper on "Transparent Phosphor Screens"; the *Mullard Premium*, to Mr. D. C. Brothers (B.B.C.) for his paper on "Contrast Law Correction in Television Picture Generators"; the *Pye Premium*, to Messrs. E. R. Rout and R. F. Vigurs (B.B.C.) for their paper on "A Wide Range Standards Converter"; the *T.T.C. Premium*, to Dr. H. Barnes (the Marine Station, Millport) for his paper on "Underwater Television in Marine Biology"; the *Wireless World Premium*, to Mr. J. Roizen (Ampex International, Switzerland) for his paper on "The Use of Video Tape for Colour Television Recording".

Gordon Research Conferences, 1962

The Gordon Research Conferences, which are aimed at fostering the exchange of ideas and information between research scientists from industrial, Government and academic laboratories, are to be held this year in four residential schools in New Hampshire during the period June 11–August 31. Forty-two informal conferences, each lasting a week, will be held, their subjects covering a wide range of topics in the physical and biological sciences. Residence and registration charges for a week amount to 100 dollars. Applications should be addressed to, and further information can be obtained from, Dr. W. George Parks, University of Rhode Island, Kingston, Rhode Island.

International Visiting Professorships in Oceanography and Fisheries

The Institute of Marine Science, University of Miami, has established a programme of visiting professorships in all branches of oceanography. Each visitor will offer special lectures and provide instruction and advice to graduate students, for periods ranging from six months to a year. Visiting professors will not be limited to United States citizens, but will include scientists from overseas. The programme is partially supported by the National Science Foundation. Advance notice will be provided, giving information as to the names of visiting professors and the special fields of study in which they will lecture. Students from other institutes and universities will be invited to engage in the research and to study under the supervision of the visiting professors. Further information can be obtained from Dr. C. Richard Robins, Institute of Marine Science, University of Miami, 1 Rickenbacker Causeway, Miami, 49.

Course on High Polymer Chemistry

A postgraduate course on "High Polymer Chemistry" has been arranged by the Bradford Institute of Technology. The course, which will last for one academic year, is primarily designed for graduates entering, or already engaged in, research or production in those industries connected with plastics, synthetic fibres, surface coating and rubber. The course will include detailed treatment of the principles of high polymer chemistry together with aspects of polymer physics and a survey of polymer technology, laboratory work introducing research techniques, research projects and colloquia, and general and economic studies. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

University News : East Anglia

Prof. T. A. Bennet-Clark, professor of botany in King's College, University of London, has been appointed professor of botany and dean of the School of Biological Studies.

London

The title of professor of oral pathology has been conferred on Mr. I. H. R. Kramer, in respect of his post at the Institute of Dental Surgery, and that of professor of industrial relations on Mr. B. C. Roberts, in respect of his post at the London School of Economics and Political Science. Dr. J. N. Hunt, reader in physiology at Guy's Hospital Medical School, has been appointed to the chair of physiology tenable at that School, and Mr. J. L. Stafford, senior lecturer in hæmatology at St. George's Hospital Medical School, has been appointed to the readership in hæmatology tenable at that School.

Yale

The following have been appointed to the Department of Botany: Theodore Delevoryas, formerly of the University of Illinois, as associate professor (palæobotany); Bruce C. Carlton, formerly of Stanford University, as assistant professor (biochemical genetics); James Cronshaw, formerly of the C.S.I.R.O., Australia, as assistant professor (electron microscopy); G. Benjamin Bouck, formerly of Harvard University, as instructor (electron microscopy); Beatrice M. Sweeney, formerly of Scripps Institute, La Jolla, California, as lecturer (algal physiology).

Announcements

Under the terms of the scheme to commemorate the late Lord Rutherford of Nelson, Sir Nevill Mott, Master of Gonville and Caius College and Cavendish professor of experimental physics in the University of Cambridge, has been appointed Rutherford Memorial Lecturer for 1962, to lecture during October–November in Uganda (Makerere University College), Kenya (Royal College, Nairobi), Southern Rhodesia (University College of Rhodesia and Nyasaland, Salisbury), Nigeria (University College, Ibadan, and Zaria) and Ghana (Accra and Kumasi).

Dr. G. E. R. Deacon, director of the National Institute of Oceanography, has been awarded the Agassiz Medal by the U.S. National Academy of Sciences for his contribution to the development of oceanography in the exploration and interpretation of the hydrography of the Southern Ocean.

Dr. P. Gross, principal scientist of the Fulmer Research Institute, has been appointed to the Board of Directors of the Institute. Mr. E. A. G. Liddiard, director of research, remains as senior executive director with responsibility for administration.

Prof. J. B. Speakman, head of the Department of Textile Industries in the University of Leeds, has been elected president of the Textile Institute.

A *Neurospora Newsletter* has been initiated to facilitate communication among research workers interested in this organism. The *Newsletter*, the first of which appeared on May 1, will be sent, free of charge, to all investigators requesting it from the editor, B. J. Bachmann, Department of Microbiology, Yale University, New Haven 11, Connecticut.

A field meeting on the theme "Soil Organic Matter", the last in the present session of the Welsh Soils Discussion Group, will be held at Aber, near Bangor, on May 9. Further information can be obtained from Mr. James A. Taylor, Department of Geography, University College of Wales, Alexandra Road, Aberystwyth.

A special short course on "Technology of the Newer Plastics and Polymers" will be held in the Bradford Institute of Technology during June 22–23. Subjects under discussion will include: newer plastics and polymers; polyvinyl chloride; polypropylene; acetal resins; polyurethanes. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

The second congress of the International Federation of Societies of Cosmetic Chemists, organized by the Society of Cosmetic Chemists of Great Britain, will be held in the School of Pharmacy, University of London, during July 2–5. Subjects under discussion will include: surface and physical chemistry; experimental methods; properties of skin; treatment of skin and mouth. Further information can be obtained from A. Herzka, Pressurized Packaging Consultants, Ltd., Ashbourne House, Alberon Gardens, London, N.W.11.

An international symposium on "Problems of Man in Space", organized jointly by the International Astronautical Federation and the International Academy of Astronautics and supported by Unesco, the International Atomic Energy Agency and the World Health Organization, is to be held in Paris during October 29–November 2. The programme will be devoted mainly to the problems of ecophysiology, psychophysiology and data acquisition, analysis and control, and there will be an exhibition showing the techniques used to study these problems and the equipment developed for survival in space. Further information can be obtained from Unesco, Place de Fontenoy, Paris 7e.

FIRST SCANDINAVIAN SYMPOSIUM ON CARCINOGENESIS

THE first Scandinavian symposium on "Carcinogenesis" was held at the Norsk Hydro's Institute for Cancer Research during November 16–18, 1961, being sponsored by the World Health Organization.

The symposium was intended as an informal meeting to create a closer contact between the Scandinavian research workers and to give them an opportunity to present their recent results and research projects, to co-ordinate the possible future work as well as to evaluate who is where doing what in Scandinavian carcinogenesis research. A total of 51 members participated: Denmark, 2; Finland, 3; Iceland, 1; Norway, 22; and Sweden, 23. There were 38 papers and 10 review lectures.

The symposium was divided into three parts. The first was a presentation of the present work and research projects of the various laboratories (38 papers). A short summary of these papers—arranged in alphabetical order after cities—follows first.

Copenhagen was represented by Dr. J. V. Spärck (the Tuberculosis Immunization Research Centre, the State Serum Institute), who reported investigations on transplanted tumours in mice treated with X-rays and with cortisone. It was shown that irradiation chimæras possess particular features within various growth facets, for example, an inhibition of isologous tumour implants. Treatment of mice with high dosages of cortisone results in a marked inhibition of the progressive growth of tumours that is obtained after implantation of spontaneous mammary tumours, isologous to the recipients.

Helsinki was represented by Prof. K. Setälä and Drs. E. E. Niskanen and L. Stjernvall (Department of Pathology, University of Helsinki). These investigators reported results from investigations on the mechanism of artificial skin carcinogenesis in the mouse obtained by different techniques—chemical, physicochemical, light-, polarization- and electron-microscopical, biophysical. Particular attention was paid to the fact that it is possible to visualize dormant cellular features in mouse epidermis correlated with skin tumour evolution.

Lund was represented by Prof. C. G. Ahlstrom and Dr. N. Jonsson (Institute for Pathology), Prof. A. Levan and Drs. Audrey Fjelde, Eva Hansen-Melander and G. Östergren (Institute for Genetics), and Dr. B. Källén (Institute for Embryology)—all from the University of Lund. Prof. Ahlström and Dr. Jonsson presented *in vivo* and *in vitro* studies with two variants of Rous sarcoma virus as well as the characteristics of the resulting tumours. Mice, rats, hamsters, guinea pigs and rabbits of various age-groups served as experimental animals. There were significant differences between the oncological effects of the two variants or mutants of the virological materials tested. Prof. Levan had analysed the chromosomes in sarcomas provoked by the Rous agent. Results were given indicating that the karyotypes had lost four chromosomes or gained one or three, respectively. It was concluded, for example, that there may be some similarity to the case of chronic myeloid leukæmia in man. Finally, the relations between carcinogenesis and chromosomal alterations were discussed. Dr. Fjelde had studied the different types of changes of cell populations of human cancer cell strains *HEp* 1, *HEp* 2 and *HS* 1 on the basis of tissue culture experiments. Dr. Hansen-Melander reported results of studies on the relation of chromosomes of the *MD* mouse lymphosarcoma to carcinogenesis. Dr. Kallén demonstrated tumour formation in the embryonic chicken brain, stimulated by surgically provoked disturbances in embryonic induction processes.

Oslo was represented by Drs. R. Eker, N. Arley, P. Fritzson and K. F. Nakken (Norsk Hydro's Institute for Cancer Research, the Norwegian Radium Hospital, Montebello), Prof. O. H. Lahelle and Dr. J. Jonsen (Virus Laboratories, the State Health Institute), Dr. J. Ulstrup (Ullevål Hospital), Prof. O. Torgersen and Dr. B. Öystese (Institute for Pathology, the State Hospital), and Prof. L. Kreyberg and and Drs. E. Evensen, O. H. Iversen and E. Mylius (Institute for General and Experimental Pathology, the State Hospital). Dr. Eker presented results from experiments on renal adenomas in rats. The results strongly support the view that the evolution of these tumours depends on a dominant gene. The effects on the tumorous growth of unilateral nephrectomy, castration, high dosages of 'Primoteston' and 'Estrafurin' were given. Dr. Arley gave a review of theoretical analyses of experimentally induced carcinogenesis, adding to the evidence that the evolution of both benign and malignant states can be started by a single gene-mutation, that is, a specific atomic substitution or rearrangement in a specific deoxyribonucleic acid (DNA) molecule. Dr. Fritzson had studied the effect of acetylaminofluorene (AAF) feeding on the uracil-degrading activity of rat liver. The results showed that addition of AAF to the diet caused a pronounced decrease in the activity of dihydrouracil dehydrogenase. The effect produced was dependent on the dosage of the carcinogen fed. It was concluded that the reduced activity of the uracil-degrading system should be a cancer-promoting factor. Dr. Nakken dealt with the role and mechanism of oxidation of aromatic amines to *o*-hydroxy-amines: *p*-amino-benzoacid reacted 3–6 times quicker with OH-radicals than the radiation-sensitive pyridoxine and cysteamine derivatives. Prof. Lahelle and Dr. Jonsen presented new refinements concerning production and cultivation of the polyoma virus. It was possible to produce viruses in media that did not contain glutamine or serum. Dr. Ulstrup, too, presented new techniques for quantitative determination of polyoma virus. Prof. Torgersen presented certain experimental results on precursors of gastric cancer in mice. Iron–dextran was administered intragastrically to animals having artificially induced gastric ulcers. This resulted in a marked penetration of the compound into the ulcerated area, vascular walls, etc., whereas normal gastric mucosa remained unaffected. Dr. Öystese gave results of studies on the pathogenesis of gastric cancer, carried out with fluorescence-antibody techniques. Dr. Evensen had studied the duration of cell division in the epidermis of hairless mice after exposure of the skin to methylcholanthrene and croton oil. It was shown that the carcinogen initially decreased both the mitotic duration and the observed number of mitotic cells. After the first two days there was a marked increase in the number of dividing cells together with an increase of the mitotic duration. On the other hand, exposure of the skin to croton oil increased the number of mitotic cells without affecting the duration of cell division. Dr. Iversen dealt with the effects of different carcinogens on the mouse epidermis. It was shown that carcinogens initially lowered the DNA synthesis, more or less inhibited the mitotic activity and destroyed the mitochondria. The damage to the mitochondria was demonstrated by means of a tetrazolium reduction method, which revealed increased amount of formazan deposits in carcinogen-treated epidermis. A blind test involving twenty substances gave support to the theory that the abovementioned changes in mitochondrial function is a specific effect of carcinogens. Dr. Mylius reported ultrastructural alterations of the epidermal mitochondria in hairless mice provoked by a single local application of methylcholanthrene. Electron micrographs were shown which indicated that mitochondrial changes occurred within 24 hr. after the exposure to carcinogen. The alterations were more pronounced after eleven days. It was concluded that the changes observed may interfere with the activity and functions of the mitochondria, altering the latter in a specific manner.

Reykjavik was represented by Prof. N. Dungal (Institute for Pathology, University of Iceland), who reported results from experiments concerning the correlation between the amount of polycyclic hydrocarbons in smoked food and development of various types of tumours in rats.

Stockholm was represented by Prof. L. Ehrenberg (Institute for Organic Chemistry), Drs. T. Gustafson and T. Hultin (the Wenner-Gren Institute) from the University of Stockholm, Prof. G. Klein, Drs. Ingegerd Hellström, K. E. Hellström, Eva Klein, L. Révész, H. O. Sjögren and Britta Slettenmark (Institute for Tumour Biology, Karolinska Institute), Drs. B. Lagerlöf and B. Thorell (Institute for Pathology, Karolinska Sjukhuset), and Dr. A. Nilsson (Defence Research Institute). Prof. Ehrenberg dealt with the effect on rat lymphocytes of free radicals in irradiated foodstuffs and discussed the necessity of testing the possible carcinogenicity of irradiated foodstuffs in general. Drs. Gustafson and Hultin reported, respectively, on studies concerning the formation of enzymes induced by the substrate in animal cells growing *in vitro*. and of some effects of liver carcinogenesis in short-time experiments. Prof. Klein reviewed some recent work on the antigenicity of tumours induced by polyoma virus. Dr. Ingegerd Hellström dealt with *in vitro* experiments with tumours provoked by polyoma virus. Dr. K. E. Hellström reported results from studies on interstitial testicular tumours in mice induced by œstrogens. Dr. Eva Klein dealt with the antigenicity of induced tumours. Dr. Révész reported on transplantation resistancy against induced tumours in isologous tumour-host systems. Dr. Sjögren had studied the interrelationship between transplantation characteristics of tumours provoked by polyoma virus. Dr. Britta Slettenmark reported on studies concerning the tumour-specific antigenicity of leukæmia induced by virus of the Gross type. Dr. Lagerlof gave some results of leukæmia transformation *in vitro*. It was shown that the cells of normal bone marrow, by the presence of leukæmia virus, are able to change into malignant cells: the shortest latency was fourteen days. Dr. Thorell had studied, by means of microspectrography, the chemistry of cells undergoing leukæmia transformation. Dr. Nilsson reported on results obtained during experiments with strontium-90 in mice. There developed various types of bone tumours in certain preferential sites of the skeleton.

Uppsala was represented by Drs. J. Hultén, L. Landerholm, J. Pontén and B. Stenkvist (Institute for Pathology, University of Uppsala). Dr. Hultén reported on studies of staining of normal and of malignant colon epithelium with fluorescein–protein conjugates. Dr. Landerholm had studied the various titres and morphology of the bone marrow during the development of virus-induced chicken erythroleukæmia. Dr. Pontén dealt with sex chromosomes as markers in transplanted chicken leukæmia cells. Dr. Stenkvist (together with Dr. Pontén) reported on relationship between virus-content, morphology and the phase of growth in progressing and in regressing Rous sarcoma.

The purpose of the second and third parts of the symposium was a general review and discussion of what is known and, especially, of what is not known to-day about the nature of experimental carcinogenesis, and to try, on the basis of these discussions, to plan to co-ordinate future research in the Scandinavian countries in this field. The following ten reviews were then given, followed by comprehensive discussions: the present situation in tumour virology (Prof. C. G. Ahlström); the role in carcinogenesis of purified nucleic acids and similar extracts (Dr. Audrey Fjelde); pyrimidine metabolism during rat-liver regeneration and liver carcinogenesis (Dr. P. Fritzson); fluorescent antibodies applied in carcinogenesis research (Dr. E. Glück); theoretical models of growth and their importance in theories of carcinogenesis (Dr. O. H. Iversen); the implications of variation and selection for carcinogenesis and for the continued progression of tumours (Prof. G. Klein); considerations on endocrine carcinogenesis (Dr. B. Källén); the chemistry and biochemistry of aromatic amines in relation to carcinogenesis (Dr. K. F. Nakken); the role of the chromosomes in carcinogenesis (Prof. A. Levan); mechanism of experimental tumorigenesis in the mouse skin (Prof. K. Setälä).

In the discussion that followed the last paper, Dr. Kirstine Borum (Copenhagen) presented a short communication on the importance of the hair cycle in epidermal carcinogenesis in mice.

Since the symposium was an informal contact meeting, during which only the Scandinavian languages were used, no papers or proceedings will be published. However, the review lectures will be translated into English and, together with the abstracts of the thirty-eight papers cited above, filed with the World Health Organization's head office in Geneva and its regional office in Copenhagen, where they may be studied by anyone interested.

It was decided to hold the second Scandinavian symposium on basic cancer research in 1963 in Stockholm.

The participants wish to thank the World Health Organization and its officers for the grant which made this inspiring gathering possible.

NIELS ARLEY
KAI SETÄLÄ

MATHEMATICS FOR PHYSICISTS AND ENGINEERS

MATHEMATICS FOR PHYSICISTS AND ENGINEERS is the fourth of the Organization for Economic Co-operation and Development's *New Thinking* series*. Its predecessor dealt with school mathematics, expressing some wildly revolutionary proposals. The present report is by comparison mild.

For the engineer and physicist, mathematics must be concrete at all levels, the syllabus must be motivated by technical relevance, exercises should be drawn from real-life applications, and, if necessary, breadth of coverage must yield to depth of understanding. Topics in modern mathematics will find a place as soon as their technical applications prove important; as for logical rigour, the truth must be told, but perhaps not the whole truth. To achieve these objectives, there must be much greater co-ordination between mathematical, scientific and engineering teachers at all levels. "If the teacher is a mathematician, he must know something about the problems and methods of the physicist and engineer, and vice versa."

So much is common ground; it is in the mode of implementing these suggestions that the report exhibits a variety of opinions. If new topics are to go in, must old and valued material go out, or can room be found by using improved teaching methods? How far up the school or university can a common course in mathematics be taken? Should the elementary topics be taught by the mathematician and the advanced subjects by the scientist, or vice versa? Is it true that the spirit of contemporary abstract mathematics "no longer concerns only those who used to be called geometers . . . but concerns in fact the whole scientific community"? Will engineers thrive on Bourbaki?

The papers and discussions are full of valuable information on conditions in Europe and North America, and on experiments that are being made; they also contain speculations and suggestions about what the teacher of to-morrow may need to know and to do. So far as Britain is concerned, it seems that the technical mathematical teaching is kept close to physical reality, but is slow in making use of advanced methods and techniques in pure mathematics.

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MALARIA EPIDEMIC IN MAURITIUS

MALARIA was hyperendemic in Mauritius until a large-scale control programme was started in 1949. The programme was based on a general attack by residual insecticide (DDT) and was conspicuously successful within a short time. By 1953 malaria-rates were reduced to a negligible level and the principal vector, *Anopheles funestus*, was eradicated. Total coverage spraying was then abandoned, and, until 1960, antimalaria measures consisted only of a limited programme of case-finding and parasite surveys, which did not come up to the standards now universally accepted for surveillance operations in malaria programmes.

In 1959, the Government of Mauritius and the World Health Organization agreed on a plan for the complete eradication of malaria from the island. Under this plan, full-scale surveillance operations were started in a small district in March of that year, chiefly for training purposes, and a surprisingly high number of fresh cases of malaria was detected. Because of this alarming situation, active case detection was rapidly extended over the whole island, and, as a result, 1,001 cases were found during the period April–June. The majority of the cases occurred in limited areas with an over-all total of about 70,000 inhabitants, the incidence of malaria being approximately 14·2 per 1,000, which, in an area where malaria had been practically eradicated, had to be considered as an epidemic.

It is possible that a low degree of malaria transmission had been going on continuously since the withdrawal of total coverage spraying in 1953, and that a parasite reservoir—previously undetected owing to the limited degree of surveillance—was slowly building up until, by spring 1960, it had reached a sufficiently high level to start an epidemic. Although respraying of the foci was undertaken in an attempt to remedy the situation, it became obvious in 1961 that this measure was inadequate. In the first three quarters of 1961, 190, 702 and 83 cases of malaria were detected, mainly in areas adjacent to the foci treated in 1960. This has necessitated reverting to the attack phase in areas with a population of more than 200,000.

The work being carried out by the Government of Mauritius and the World Health Organization to combat these epidemics is reported in a recent issue of the *WHO Chronicle* (16, No. 2; February 1962).

ATTENUATED MEASLES VACCINE

DR. HUANG-CHEN-HSIANG, Chia Ping-yi, Chu Fu-T'ang, Kuo Ke-Ch'ien, Wang Hui-Ying, Wu Tsung-Lin and Wu Hsueh-Hsin have examined the clinical reactions of an attenuated measles virus of vaccine L_4 (37 passages in human amnion cells). They were considerably milder than those of vaccine M_{60-5} (5 passages in human amnion cells). The difference was chiefly limited to catarrhal symptoms and signs, rash and Filatov–Koplik's spots. There was, however, no appreciable difference if the average intensity and duration of fever were compared. Dr. Huang-Chen-Hsiang *et al.* report their findings in the

January issue of the *Chinese Medical Journal* (81, No. 1; 1961).

In a small number of cases, both vaccines gave 100 per cent antibody response even when the half tissue culture infectious dose ($TCID_{50}$) was as small as 6. The average titre of neutralizing antibody acquired from M_{60-5} vaccine was somewhat higher than that from L_4 vaccine.

There was no significant difference in the degree of temperature and duration of febrile reaction between the different doses of vaccine L_4 (600, 60, 6, 0·6, 0·06 and 0·006 $TCID_{50}$), but the percentage of children with febrile reaction decreased when the infective dose was lower than 0·6 $TCID_{50}$.

Studies on the influence of different infective doses on clinical reactions showed that man is at least 100 times more sensitive to the attenuated virus of vaccine L_4 than to that of the tissue culture system. The possibility of active immunization in infants below 6 months of age is suggested.

In another article in the same journal, Huang Chen-Hsiang, Chu Fu-T'ang, Chia Ping-Yi, Lin Ch'uan-Chia and Tseng Yi report their studies on the influence of placental globulin on the clinical and immunological response of 130 children previously inoculated with 6 $TCID_{50}$ virus vaccine L_4.

Experimental results indicate that the degree and percentage of febrile reaction to the vaccine decreased with increased dose of placental globulin. The average maximum temperature and the duration of fever showed regular decrease with increase in the dose of placental globulin. There was, however, no difference in the degree and extent of reactions when the same dose of placental globulin was given 1–4 days after inoculation of the vaccine.

The antibody-level 6–7 months after immunization was found to be about the same as when the vaccine was given alone or was followed by placental globulin. The average antibody titre of children with clinical reactions to the combined use of vaccine and placental globulin was found to be slightly higher than that of those showing no reactions, but the difference is not significant.

Twenty-eight immunized children had intimate contact with children with measles following immunization 1 day or 4 months previously. They lived and played together in the same room for a period of 3–5 weeks, but none of them had measles. Among these 28 immunized children, 4 received vaccine alone and 24 received both the vaccine and placental globulin. By contrast, living in the same environment, 39 out of 41 unvaccinated children caught the disease.

SIMPLE EXPERIMENTAL METHOD FOR VERIFICATION OF THE GIBBS ADSORPTION EQUATION

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THE derivation of the quantitative relation between adsorption and surface tension from thermodynamics by Gibbs[1] provoked attempts at an experimental verification of this equation. Such methods as the bubble method, the microtome method and, more recently, radioactive measurements have been used with varying but qualified success[2–8]. In general, those methods which attacked the problem directly, that is, skimming the surface layer from the bulk solution, followed by analysis of the surface-active components, have involved very elaborate apparatus and experimental procedures. In particular, McBain's microtome[4] involved a long trough with precision ground rails on which rode a catapulted microtome blade, automatic trap doors to permit passage of the blade through the controlled atmosphere surrounding the trough, and other complicated arrangements for collecting the skimmed solution. As described by Adam[9], " . . . the whole apparatus constitutes a triumph of mechanical skill."

The following describes a simple and inexpensive apparatus, using the same principle as McBain's microtome, but in which the solution, rather than the cutting edge, is the moving component. In addition to the simplicity, the design permits investigation of such parameters as time to reach equilibrium between surface and bulk, and the effect of electrostatic fields impressed across the interface. In fact, the apparatus was specifically designed for use in a programme for investigation of the effects of electrostatic fields on the surface properties of salt water[10].

The 'skimmer' (Fig. 1) consists of a small-diameter metal tube with one end carefully ground to a sharp edge. It is readily made from a stainless steel hypodermic needle. The hole is hit squarely by a jet of slightly larger diameter, flowing under pressure (~ 100 cm. of water) out of a rigidly fixed pipette. The hypodermic needle tip is joined to larger-diameter tubing to allow the liquid coming through the base of the needle to flow off freely. This tubing carries a collar to collect the liquid which flows down the outside of the apparatus. The needle assembly is mounted on a microscope stage so that its position relative to that of the pipette can be controlled precisely. The skimmer is enclosed in a lucite box in which 100 per cent relative humidity is maintained to prevent losses by evaporation.

The surfactant used was *n*-decylbenzene sodium sulphonate supplied by Continental Oil Co. This compound was selected because of its large $(\partial\gamma/\partial c)$ coefficient, and the existence of a strong absorption peak at 223 mμ, which permits accurate concentration determination by differential ultra-violet spectrophotometer measurements. The surface tension–concentration relationship was obtained for aqueous solutions from 5×10^{-6} to 1×10^{-3} molar, using the capillary rise method.

Theoretical surface excess values, Γ, were calculated, using the approximate form of the Gibbs equation:

$$\Gamma = \frac{c}{RT}\frac{\partial\gamma}{\partial c} \qquad (1)$$

where c = concentration of surfactant.

From the surface-tension measurement, $\partial\gamma/\partial c$ was found to be $-4{\cdot}2 \times 10^3$ dynes l./cm. mole. The

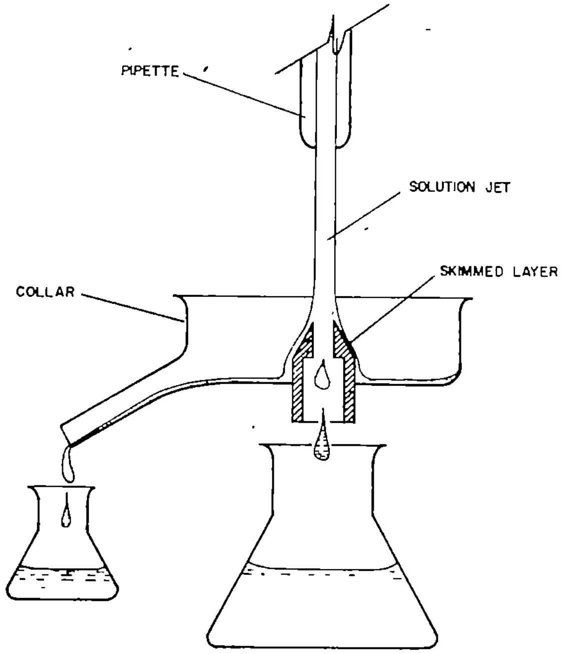

Fig. 1. Schematic diagram of skimmer

theoretical values of Γ calculated from equation 1 are represented by the line in Fig. 3.

Experimental Γ values were obtained in the following way: The flow of the jet was started and the ratio, R, of solution flowing through the hole to that flowing down the outside of the metal cone and collected by the collar was measured volumetrically. Since the diameter of the jet varies slightly with distance from the pipette tip, different values of this ratio could be obtained by simply varying the distance between the pipette tip and the ground edge of the needle. This distance was approximately 10 cm. in these experiments, with variations of less than 1 cm. to cover the ratios studied. The upper limit of distance between pipette tip and needle is that at which the jet of solution breaks up into droplets.

The thickness, x, of the skimmed layer, assuming the jet strikes the ground opening concentrically, is given in terms of the ratio, R, and the radius, r, of the opening by:

$$x \approx \frac{r}{2R} \tag{2}$$

which follows from the exact expression:

$$\frac{r^2 - (r - x)^2}{(r - x)^2} = \frac{1}{R} \tag{3}$$

when R is greater than 20 for $r = 0{\cdot}029$ cm., as used in these experiments. For ratios of 100, which could be obtained fairly easily, the thickness of the skimmed layer is $1{\cdot}5 \times 10^{-4}$ cm. It is believed that layers of 1000 Å. could be skimmed by a bit more sophistication in the construction of the apparatus.

It is not necessary for the jet to strike the opening concentrically in order to use the method. If it strikes off centre, provided only that the edge does not cut across or into the region where the concentration begins to deviate from that in the bulk (probably < 100 Å. from the surface), the value of x as obtained from equation 2, though an average, is here just as valid as if the jet would strike concentrically.

From the definition of surface excess, Γ, we can write:

$$\Gamma = (c_s - c_B)x \tag{4}$$

where c_s and c_B are the concentrations (in moles/cm.^{3}) of the skimmed layer and of the bulk solution. Equations 2 and 4 give:

$$(c_s - c_B) = \Gamma \frac{2R}{r} \tag{5}$$

so that a plot of $(c_s - c_B)$ versus $2R/r$ yields a straight line with slope Γ. Values obtained in this manner for one set of experiments are shown in Fig. 2. The scatter in the data is believed due to the edge cutting across the surface some fraction of the time during the course of the experiment.

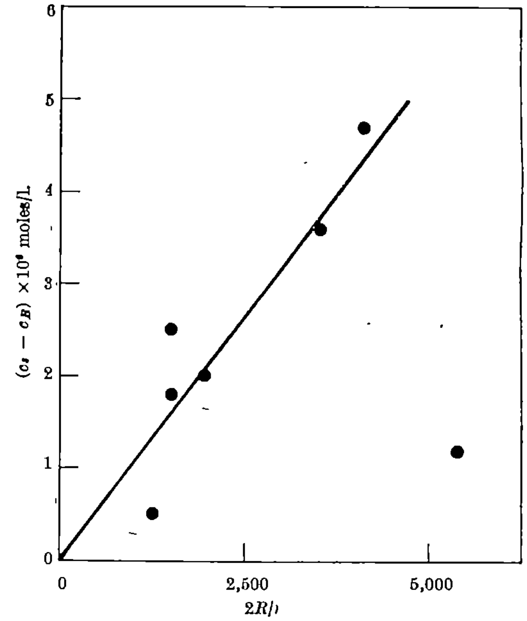

Fig. 2. Plot of $(c_s - c_B)$ versus $2R/r$ for n-decylbenzene sodium sulphonate
$c_B = 5{\cdot}5 \times 10^{-6}$ moles/l. $\Gamma = 10{\cdot}2 \times 10^{-13}$ moles/cm.^{2}; $\Gamma_{calc} = 9{\cdot}3 \times 10^{-13}$ moles/cm.^{2}

The agreement with theory is within less than 10 per cent over two decades of concentration-change. The experimental values are shown as points in Fig. 3, and are to be compared with the line which is calculated from the Gibbs equation. Appreciable deviation occurs for 10^{-3} M solutions. Here the limit of solu-

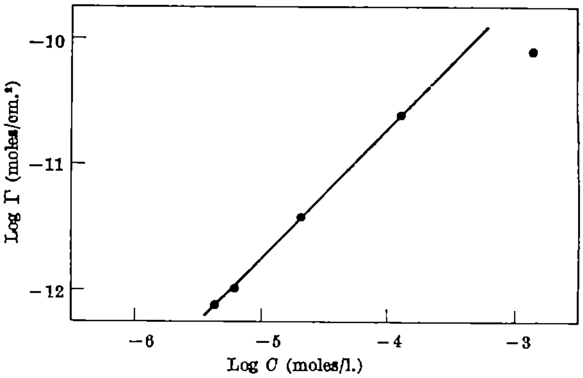

Fig. 3. Theoretical and experimental Γ values for n-decylbenzene sodium sulphonate
——, Calculated; ●, experimental

bility is almost reached, and one suspects micelle formation and/or surface film formation.

It has been implicitly assumed that the time during which the jet was in contact with air was sufficient to reach equilibrium at the jet-air interface. For 10 cm. distance between pipette and cutting edge, and 100 cm. of water pressure, this time is about 0·05 sec. The good agreement with theory indicates that, for these solutions, equilibrium is established in this interval, except possibly at 10^{-3} *M*, where the deviation may be in part due to a lack of equilibrium. However, the ability to vary the distance from capillary tip to cutting edge makes it possible to look for effects of time to equilibrium. Experiments to investigate these and effects of electrostatic fields on the surface excess concentrations are now in progress.

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SENSITIVE THERMAL ANALYSIS ESTABLISHING FORMATION OF THE INCONGRUENTLY MELTING COMPOUND $LiNaCO_3$

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THE liquid–solid equilibrium diagram for the system Li_2CO_3/Na_2CO_3 has been investigated at least four times[1–4], without general agreement among the investigators, except on the Na_2CO_3-rich side of the diagram. The point of greatest significance perhaps is that it has not been established whether or not a congruently melting compound, $LiNaCO_3$, forms. Eitel and Skaliks[1] determined the general shape of the phase diagram by cooling curves. On the basis of X-ray and index of refraction data at room temperature, they proposed the congruently melting compound $LiNaCO_3$ melting at 514° C., which was said to form eutectics with both Na_2CO_3 and Li_2CO_3. Both eutectics were found to melt at 510° C. The scatter in the cooling curve data was such that no definite conclusions could be reached about the phase diagram in the composition range 48–59 mole per cent Li_2CO_3.

Volkova[2] investigated this system by viewing the melt during cooling and noting the temperature of initial crystal formation, evidently without an atmosphere of carbon dioxide to prevent decomposition. The conclusion was reached that a compound was formed at a temperature of 501° C. The considerable uncertainty in the data did not lend much support to this proposal. Most recently, Janz and Lorenz[4] reported that this system is of the simple eutectic type based on cooling-curve evidence. However, Lorenz[5] suggested the possibility of the occurrence of an incongruently melting compound, based on the fact that X-ray results suggested the formation of another phase in the central region of the diagram. However, these X-ray results were not in complete agreement with those of Eitel and Skaliks, leading to additional uncertainty about the existence of a compound. Cooling-curve evidence was insufficient to establish the compound formation.

Because of the insensitivity of the thermal analysis equipment generally used in phase diagram determinations (± 2–3° C.) it has not been possible to detect the formation of small amounts of a new phase in a melt. This serious limitation has made it very difficult to obtain thermal analysis results in important regions (for example, eutectic, peritectic, compound formation) of phase diagrams. The usual procedure has been to obtain results well away from the region of interest, and then to extrapolate, leading to large uncertainty in the region of greatest interest.

An important object of the present work was to develop highly sensitive (± 0·003° C.) thermal analysis equipment capable of detecting very small heat effects associated with the formation of small amounts of new phases, or of phases limited to very narrow composition ranges. A recording potentiometer with zero suppression was constructed such that a full 10-in. chart deflexion could represent 5, 10, 20, 25, 40, 50, up to 10^4 μV. The highest sensitivity then corresponded to about 0·003° C., making possible the accurate measurement of previously insignificant thermal effects.

Platinum–platinum 10 per cent rhodium thermocouples were placed in a high purity alumina thermocouple well with a gold–palladium casing. The well was placed near the stirrer in order to minimize temperature lags. The thermocouples were used in pairs such that they could be checked against one another frequently. The differences never exceeded 0·025° C. at any temperature in the range investigated.

Reagent grade Li_2CO_3 and Na_2CO_3 were purified up to 99·99 per cent. The carbonates were dried at 440° C. under flowing dry carbon dioxide and stored over phosphorus pentoxide until used. All weighings were carried out to 0·1 mgm. so that composition errors were negligible (the total amount of each carbonate present was more than 100 gm.).

Cooling curves were taken at rates of 0·1–0·4° C./min. using an electrically heated furnace with a sealed quartz liner containing a crucible of 80 per cent gold–20 per cent palladium alloy. The charge of melt was in the range of 250 gm. (this large amount of melt increased sensitivity) and was always under a flowing stream of 'bone dry' carbon dioxide. Stirring was accomplished by a rotating gold–palladium alloy paddle running at 600 r.p.m.

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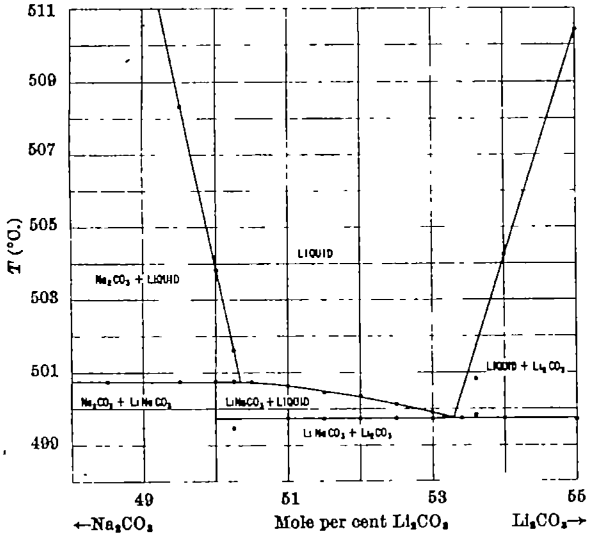

Fig. 1. Central region of the Li_2CO_3/Na_2CO_3 phase diagram showing compound formation

Cooling curve data were reduced by a modified method similar to that of Taylor and Rossini[6]. All data reported are corrected to zero impurity.

Fig. 1 shows the phase diagram in the range 48–55 moles per cent Li_2CO_3. It is clear that neither a simple eutectic nor a congruently melting compound is formed. An incongruently melting compound $LiNaCO_3$ occurs, which decomposes at 500·757 ± 0·005° C. The above limits express the repeatability of the result at various compositions of 48·5–50·25 per cent Li_2CO_3. The absolute accuracy of the results is not known precisely; however, Sigmund Cohn Corp. reference grade thermocouple wire was used, stated to be accurate to ± 0·4° C. at about 500° C. In addition, as stated above, pairs of thermocouples always agreed to within 0·025° C.

The products of the decomposition of $LiNaCO_3$ are solid Na_2CO_3 and a liquid of 50·34 moles per cent Li_2CO_3 (Fig. 1). A eutectic was found at 53·30 moles per cent Li_2CO_3, composed of $LiNaCO_3$ and Li_2CO_3, melting at 499·754 ± 0·057° C. (again, limits represent repeatability, in the range 50·5–55·0 moles per cent Li_2CO_3). The point for the liquidus at 53·6 per cent Li_2CO_3 is low because a cooling rate of 0·4° C. was too fast to allow thermal equilibrium to be established before the eutectic break was encountered. Data taken at a rate of 0·15° C./min. would surely fall closer to the line. The Li_2CO_3 liquidus line is further supported by many data taken at compositions above 55 per cent.

The three points at 50·25 moles per cent Li_2CO_3 are of special interest. They show definitely that there is formation of an incongruently melting compound via reaction between solid Na_2CO_3 (deposited starting at 503·6° C.) and the melt. After all the solid has been consumed, the remaining melt deposits additional $LiNaCO_3$ until the eutectic composition is reached, whereupon complete solidification takes place. These results rule out the formation of a congruently melting compound and simple eutectic behaviour. The fact that only two breaks were encountered in the cooling curves on the Na_2CO_3-rich side of the 50 per cent point eliminates the possibility of two enantiotropic forms of Na_2CO_3 being responsible for the behaviour encountered at 50·25 per cent. The present data thus conclusively establish the presence of the incongruently melting compound $LiNaCO_3$.

The small difference between the eutectic temperature and the decomposition temperature of $LiNaCO_3$, and the very narrow composition region for its separation from the melt (50·3–53·3 per cent) were the main reasons for the previous confusion as to the precise nature of the central region of the diagram. Extrapolations of data taken by others at 30 and 40 moles per cent Li_2CO_3 and at 60 and 70 moles per cent Li_2CO_3 with a repeatability of ± 2° C. could yield results showing behaviour anywhere between that of a simple eutectic and a gap of 5 moles per cent, indicating congruently melting compound formation.

The occurrence of $LiNaCO_3$, which melts incongruently, then shows that the X-ray[1,5] and index of refraction[1] data are not inconsistent with the conclusions which can be drawn from properly gathered thermal analysis data.

This work shows that it is possible to perform high-sensitivity (± 0·003° C.) thermal analysis experiments at elevated temperatures, allowing previously unattainable certainty in the determination of phase diagrams.

We acknowledge the helpful discussions of Dr. M. R. Lorenz.

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EXCIMER FLUORESCENCE OF AROMATIC HYDROCARBONS IN SOLUTION

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THE fluorescence spectrum of pyrene in solution consists of two distinct components, a violet emission band *M* with vibrational structure, and a blue emission band *D* which is broad and structureless[1]. At low concentrations only *M* is observed, but as the concentration is increased *D* appears and increases in intensity, while the intensity of *M* decreases. At high concentrations and in the crystalline state[2], the fluorescence is almost exclusively *D*.

The effect was first observed by Förster and Kasper[1], who attributed *M* to the normal fluorescence of unassociated (monomer) molecules and *D* to the fluorescence of excited dimers (excimers), formed by the association of excited and unexcited molecules. This interpretation has been confirmed by Birks and Munro[3], who examined the dependence on time of the two emission components *M* and *D* following excitation by a pulsed light source. Typical results for a

5×10^{-3} *M* solution of pyrene in deoxygenated cyclohexane, showing the decay of *M* and the growth and decay of *D*, are plotted in Fig. 1. Detailed investigation[4] of the dependence on concentration, viscosity and temperature of the growth and decay times and fluorescence intensities of the two emission components have provided data about the kinetics of the diffusion-controlled photo-dimerization process. Stevens[5] has introduced the term 'excimers' to describe the photodimers in order to distinguish them from the excited states of dimers which are stable in the ground state. He has cited delayed fluorescence, self-quenching and sensitized delayed fluorescence as alternative evidence to the *D* emission for excimer formation in aromatic hydrocarbons.

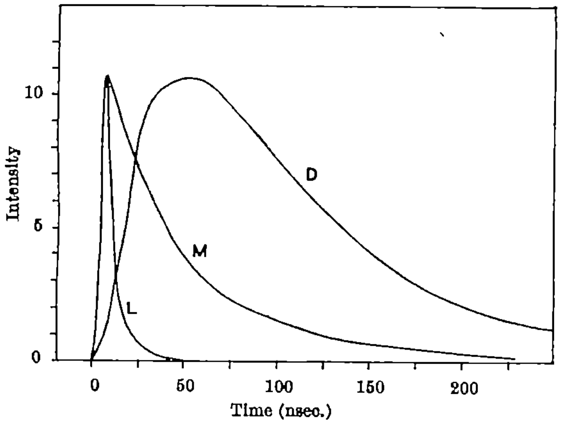

Fig. 1. 5×10^{-3} *M* solution of pyrene in deoxygenated cyclohexane (ref. 3). Growth and decay curves *M*, monomer emission ; *D*, excimer emission ; *L*, light pulse

In the present investigation studies have been made of the fluorescence and absorption spectra of a number of aromatic hydrocarbons in solution in cyclohexane or benzene, and of their dependence on concentration. In 10 of the 18 compounds studied so far a broad structureless emission band *D* is observed in the fluorescence spectrum at high concentrations. It is absent from the structured fluorescence spectrum *M* observed in dilute solutions. *D* lies at longer wave-lengths than *M*, and it increases in intensity relative to *M* as the concentration is increased. Typical fluorescence spectra observed for one of the compounds, 5-methyl 1 : 2-benzanthracene in cyclohexane, are plotted in Fig. 2. No change is observed in the absorption spectrum at high concentrations, showing that the molecular species responsible for the *D* emission are not present in the ground state. These results are consistent with excimer formation and emission. The absence of structure in the *D* emission is due to the dissociation which accompanies the emission.

The observations on the 10 compounds which exhibit excimer emission are summarized in Table 1. The wave-lengths of the first vibrational peak in the monomer emission M_1 and of the peak of the excimer emission D_1 are given. The energy difference ν' $(M_1 - D_1)$ in cm.$^{-1}$ between M_1 and D_1 corresponds to the energy of photo-association.

Two properties have proved useful in selecting compounds likely to exhibit excimer emission: (1) the molar extinction coefficient ε of the first absorption band[6]; and (2) the crystal fluorescence spectrum[2,7]. ε is approximately inversely proportional to τ_0, the natural lifetime of the first excited π-singlet state.

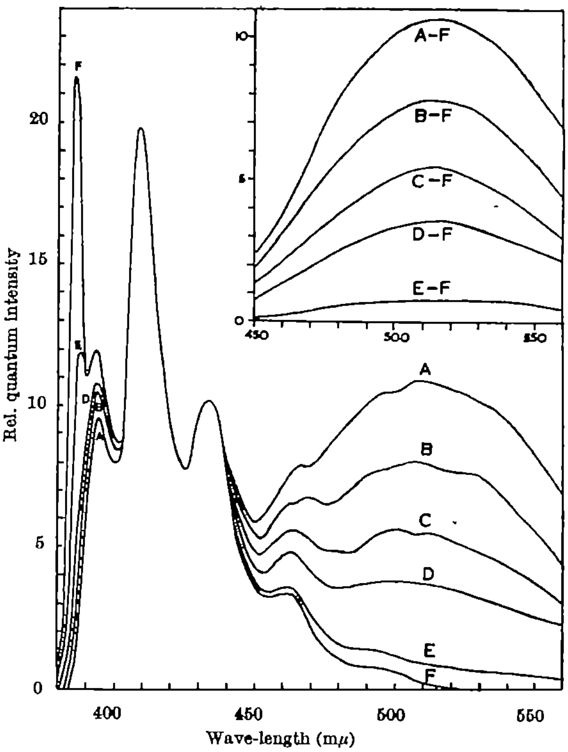

Fig. 2. Fluorescence spectra of 5-methyl 1 2-benzanthracene in cyclohexane at various concentrations. *A*, 10^{-2} *M* ; *B*, 7 5×10^{-3} *M* ; *C*, 5×10^{-3} *M* ; *D*, 3×10^{-3} *M* ; *E*, 5×10^{-4} *M* ; *F*, 5×10^{-5} *M*. Relative quantum intensities are normalized at $\lambda = 430$ mμ

Since a high value of τ_0 should favour excimer formation, the latter should be associated with a low value of ε. In all the compounds (Table 1) in which excimer emission has been observed $\log_{10} \varepsilon < 3{\cdot}5$. The radiative lifetime τ_M of *M* is reduced below τ_0 by internal quenching, excimer formation and oxygen quenching. These effects have been studied in pyrene solutions[3,4], and it is found that oxygen quenching reduces τ_M by a factor of ~ 20. This indicates that excimer emission might be more readily observed in solutions of other compounds if dissolved oxygen were removed. This has not been done in these preliminary studies.

The crystal fluorescence spectra of many of the compounds examined have been observed previously by Birks and Cameron[2]. Changes relative to the dilute solution fluorescence spectra, corresponding to a decrease in intensity of the structured emission *M* and the appearance of a structureless emission *D* at longer wave-lengths, were classified and attributed to molecular interaction. It is clear now that this interaction corresponds to excimer formation. The compounds the crystalline spectra of which are structureless and shifted to longer wave-lengths (pyrene, 3 : 4-benzpyrene, 5-methyl and 8-methyl 1 : 2-

Table 1. COMPOUNDS FORMING EXCIMERS

Compound	M_1 (mμ)	D_1 (mμ)	$\nu'(M_1 - D_1)$ (cm.$^{-1}$)
1 : 2-Benzanthracene	386	513	6,420
2′-Methyl 1 : 2-benzanthracene	388	514	6,310
3′-Methyl 1 : 2-benzanthracene	387	508	6,150
5-Methyl 1 : 2-benzanthracene	386	512	6,380
6-Methyl 1 : 2-benzanthracene	387	511	6,270
8-Methyl 1 : 2-benzanthracene	387	513	6,350
2′,6-Dimethyl 1 : 2-benzanthracene	386	513	6,420
Pyrene	372	487	6,350
1 : 2-Benzpyrene	389	467	4,300
3 : 4-Benzpyrene	403	480	3,980

benzanthracene) all exhibit efficient excimer emission in solution. However, other compounds the crystal spectra of which include much less of the component D (for example, 1 : 2-benzanthracene and its 2′-methyl and 3′-methyl derivatives) also show excimer emission in solution. These results indicate that partial or total excimer emission can occur from crystals which do not possess the 'dimeric' crystal structure of pyrene. It is possible that excimer formation occurs in crystals when excitons are trapped at dislocations.

Two of the other compounds studied, anthanthrene and perylene, also exhibit broad emission bands D at high solution concentrations, which are absent from the emission spectra M of dilute solutions. They are, however, accompanied by corresponding changes in the absorption spectra. At high concentrations a new absorption band D_0 appears at longer wave-lengths than the normal first absorption peak M_0. D_0 is approximately the mirror image of D, and in the case of perylene both D_0 and D show vibrational structure. It is clear that these two compounds form dimers in the ground state and that the D emission occurs from excited dimers. Excimer formation is excluded by the high value of $\log_{10} \varepsilon \sim 4\cdot5$ of the first absorption band, and the consequent low value of τ_0. Spectral data on the peaks M_1, D, M_0 and D_0 are summarized in Table 2. Both compounds exhibit broad structureless emission in the crystalline state[7,8].

Table 2. COMPOUNDS FORMING FLUORESCENT DIMERS

Compound	Emission M_1 (mμ)	Emission D (mμ)	Absorption M_0 (mμ)	Absorption D_0 (mμ)
Perylene	444	568, 620	438	558, 510
Anthanthrene	436	574	433	548

No dimer emission has been observed from concentrated solutions, containing dissolved oxygen, of the following compounds: anthracene, 9,10-dimethyl 1 : 2-benzanthracene, triphenylene, picene, coronene and fluoranthene. Crystalline fluoranthene and 9,10-dimethyl 1 : 2-benzanthracene have broad, featureless emission spectra, but so also do dilute solutions of these compounds, showing that the crystal spectrum is not an infallible guide.

A fuller account of these and related studies on other aromatic hydrocarbons will be published later. The fluorescence spectra were measured with a modified Unicam *SP*500 spectrophotometer. The absorption spectra were measured with a Unicam *SP*700 recording spectrophotometer, purchased with a grant from the British Empire Cancer Campaign.

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ON THE VALIDITY OF THE EVIDENCE FOR SUB-UNITS OF HIGH MOLECULAR WEIGHT RIBONUCLEIC ACIDS

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ALTHOUGH it is generally agreed that ribosomal ribonucleic acid (RNA) when first isolated consists of two components having sedimentation constants of approximately 16 and 23 *S*, several investigators have reported that these high-molecular-weight components represent aggregates of low molecular weight sub-units held together by magnesium ion interactions, hydrogen bonds or peptide links[1–3]. To understand both the structure and formation of ribosomes, it is clearly necessary to accept or reject the sub-unit hypothesis. For this reason we decided to study the methods which had been used to produce sub-units. We examined the effect of dialysis, interaction with bovine γ-globulin, reaction with trypsin, and the effect of heat on *E. coli* ribosomal RNA, tobacco mosaic virus (TMV) RNA and polyuridylic acid. The results summarized here cast serious doubt on the validity of the evidence which has been put forth in support of sub-units in RNA.

Dialysis experiments were carried out under the conditions used by Aronson and McCarthy[1], 2° and 0·01 M *tris*(hydroxymethyl)aminomethane, 0·004 M succinic acid, pH 7·6 (0·01 M *tris* buffer), and sedimentation constants were measured to determine changes in molecular weight. Both TMV RNA and *E. coli* ribosomal RNA were found to degrade after several days of dialysis, but the results were erratic and the degradation was less extensive than that observed by McCarthy and Aronson. The variability in these results suggested that the observed fall in molecular weight might be the result of ribonuclease contamination. We found that the addition of a ribonuclease inhibitor, polyvinyl sulphate[4], at a concentration of 1 per cent of the RNA concentration, stabilized RNA preparations so that the sedimentation constant remained unchanged even after one week of dialysis. Since such prolonged dialysis removes divalent cations, we conclude that RNA does not dissociate into sub-units in the absence of divalent ions provided the ribonuclease activity is suppressed.

Next we examined the interaction of RNA with a globular protein. Bovine γ-globulin was chosen because this was the protein which Brown, Ellem and Colter had reported to be most effective in dissociating RNA (ref. 2). In agreement with the results obtained by Brown *et al.*, we observed a significant drop in $S_{20\,w}$ when TMV RNA was incubated with protein at 5° in 0·15 M sodium chloride at pH 7. However, further studies revealed that the observed drop in sedimentation constant was dependent on time and the rate of degradation was proportional to the protein : RNA ratio. No limiting value of the sedimentation was found, when $(S^0_{20\,w}/S^t_{20\,w})^2$, a quantity roughly proportional to M_0/M_t, was plotted as a function of time. These results therefore suggest that the 'dissociation' reported by Brown *et al.* might be the result of nuclease contamination of the protein. To test the possibility that bovine γ-globulin contains trace amounts of ribonuclease, RNA was incubated with protein under conditions known to inhibit ribonuclease action. The results of incubating 100 γ/ml. bovine γ-globulin with 40 γ/ml. *E. coli* ribosomal RNA at neutral *p*H and low (10^{-4} *M*) magnesium ion concentration were compared with those obtained: (1) under identical conditions but in the presence of polyvinyl sulphate; (2) at *p*H 4·5, and (3) in 2 × 10^{-3} *M* magnesium. 12 hr. after RNA and protein were mixed at a ratio of 1 : 2, the sedimentation constant of both the mixture and a control were measured in 0·15 *M* sodium chloride. No change was found in the sedimentation constant or in the relative amount of the 16 and 23 *S* component under conditions which inhibit ribonuclease, but S_{20w} had fallen to 11 *S* at neutral *p*H and low magnesium ion concentration.

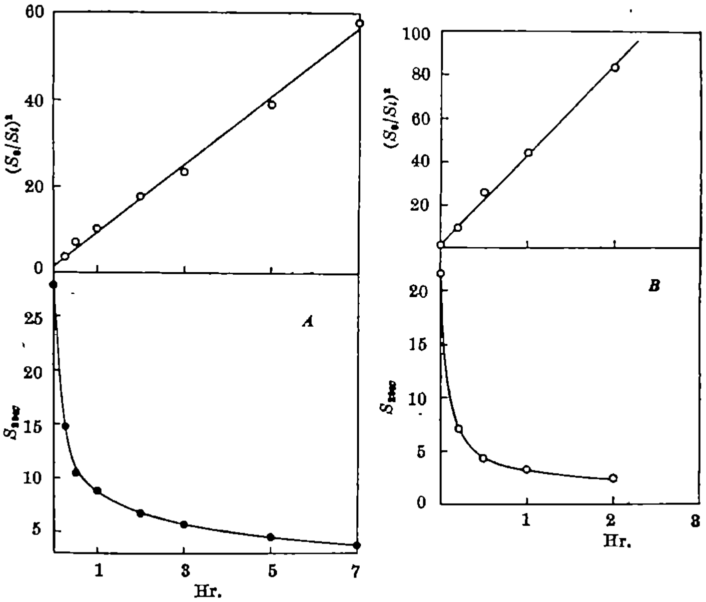

Fig. 1. *A*, hydrolysis of TMV RNA at 90° C. 0·15 *M* sodium chloride + 0·015 *M* sodium citrate; *B*, hydrolysis of *E. coli* ribosomal RNA at 90°. 0·01 *M tris* buffer, *p*H 7·6, plus 0·01 *M* magnesium chloride

Additional evidence for ribonuclease contamination of bovine γ-globulin was obtained by testing the effect of adding protein to both polyuridylic and polyadenylic acid. Ribonuclease readily degrades the former but not the latter. As expected, bovine γ-globulin had no effect on the sedimentation constant of polyadenylic acid but caused a marked decrease in S_{20w} of polyuridylic acid when the two were incubated at 5° for 24 hr. at a protein to RNA ratio of 3 : 1, S_{20w} decreasing from 5·8 to 3·3 *S*.

Our results with bovine γ-globulin made us suspect that the action of trypsin on yeast ribosomal RNA reported by Otaka, Oota and Osawa[3] might also be the result of ribonuclease contamination. For this reason we tested the 'activity' of native and alkaline denatured trypsin on polyuridylic acid. When 0·4 mgm./ml. trypsin was incubated with 10 × vol. of 11·3 mgm./ml. polyuridylic acid in 0·15 *M* sodium chloride, 0·01 *M* potassium phosphate, *p*H 6·3 at 2° for 12 hr. the protein–polyuridylic acid mixture sedimented with S_{20w} of 1·7 *S* while the control (measured simultaneously in a Spinco model *E* ultracentrifuge equipped with double optics) sedimented at 6·6 *S*. When the parallel experiment was carried out with trypsin which had been pre-incubated at *p*H 10 for 18 hr. at 25° (in the absence of divalent cations), the sedimentation constant of the trypsin–polyuridylic mixture was 1·0 *S* while that of the control was 5·2 *S*. As pure trypsin would not be expected to degrade polyuridylic acid and alkali-denatured trypsin should certainly be inactive, the results clearly indicate that an alkali-resistant contaminant is responsible for the observed degradation. Once again the most likely explanation was that trypsin was contaminated with ribonuclease. Naturally we cannot prove from these experiments that the trypsin used by Otaka *et al.* was similarly contaminated, but the fact that it was obtained from the same source (Worthington Biochemical) makes it likely.

Having found that the degradation of RNA as a result of dialysis, interaction with globular proteins and reaction with trypsin could all be attributed to ribonuclease action, it remained of interest to determine if exposure to high temperature, where enzymatic degradation could not be expected to play a major part, would result in the formation of sub-units. Several investigators, including Hall and Doty[5], Takanami[6], Osawa[7] and McCarthy[1], have reported that heating ribosomal RNA from widely differing sources results in a limiting value of S_{20w}. Unfortunately, with the exception of Hall and Doty, none of the reports included evidence that successive heating to the same high temperature would not further degrade the RNA. To clarify this point, several aliquots of both TMV and *E. coli* ribosomal RNA were heated to 90° for varying periods of time, whereupon the samples were quickly cooled and the sedimentation constant measured at 20°. The results obtained on TMV RNA and *E. coli* ribosomal RNA are shown in Fig. 1. It is clear that although a plot of S_{20w} against time appears to level off at about 4 *S*, a plot of $(S^0_{20w}/S^t_{20w})^2$ against time does not indicate a limiting value. The linear dependence observed in the latter case indicates that the molecular weight decay is proportional to time of heating to 90° and is characteristic of first order random degradation kinetics. Moreover, rate constants calculated from these data on TMV RNA are comparable with those reported previously[8] and hence of the same order of magnitude as those obtained for denatured single stranded DNA and for such model compounds as diethyl and dibenzyl phosphate. Thus, these data fail to provide any evidence for the existence of sub-units, for a non-random cleavage of phosphate diester bonds of RNA or for any exceptional instability of the RNA chain.

In conclusion, we find that the sub-unit hypothesis has not been successfully established by any of the evidence reported so far. Detailed time studies, properly interpreted, show that if degradation occurs, it is continuous and does not lead to a limiting value for the molecular weight. Furthermore, neither viral nor *E. coli* ribosomal RNA form low molecular weight sub-units if ribonuclease activity is absent or inhibited.

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LOW CYST(E)INE CONTENT OF BACTERIAL EXTRACELLULAR PROTEINS: ITS POSSIBLE PHYSIOLOGICAL SIGNIFICANCE

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A SCRUTINY of the published figures on amino-acid content of the five different bacterial extracellular enzymes[1–5] which have been successfully isolated during the past few years reveals that they all apparently lack cyst(e)ine. In order to discover whether this might be a general property of bacterial exo-proteins, we have determined the cyst(e)ine content of a number of other purified bacterial exo-enzymes that became available to us during the past eighteen months, and have repeated cyst(e)ine determinations on some of the exo-enzymes already analysed in other laboratories. The results, together with those (published and unpublished) from other laboratories, are summarized in Table 1, where they are expressed as the weight in grams of the exo-protein containing the equivalent of 1 gm.-molecule of cysteine. On comparing these values with the known molecular weight of the proteins, it is clear that, with the exception of the two toxins which appear to have four or more half-cystine residues per molecule, the maximum cyst(e)ine content never exceeds one half-cystine residue per molecule, and in many instances must be zero.

It is somewhat doubtful whether the toxin of *Clostridium tetani* should be included as a true exo-protein, since it alone, of all the proteins tabulated, is found attached to the cells in substantial proportions during the logarithmic phase of growth[27,28], and no critical tests have been done to determine the extent to which it might be liberated by cell autolysis. However, the evidence in favour of diphtheria toxin being a 'true' (that is, physiologically liberated) exo-protein is much better[28]. It is not yet known what number of disulphide bonds, if any, is present in the molecule of either of these two toxins. The thermo-stable α-amylase of the thermophilic organism *Bacillus stearothermophilus* (not included in Table 1), recently shown to contain four half-cystine residues and two disulphide bonds per molecule of mol.wt. 15,600 [29], is quite possibly extracellular, although it was isolated from the filtrate of a relatively old (36 hr.) culture and might therefore have been released by cell autolysis. Bearing in mind these three possible exceptions, the aforementioned generalization is supported by further work to the extent that ten different bacterial extracellular proteins (including five different functional types from six different species of bacterium) are now known to have a cyst(e)ine content so low that in no instance can there be a single disulphide bond present in the molecule, and in eight cases no cysteine is present at all.

The question arises, therefore, of the possible significance of the absence of —S—S— bonds in these bacterial extracellular proteins. The main possibilities appear to be as follows.

Table 1. CYST(E)INE CONTENT OF PURIFIED PREPARATIONS OF EXTRACELLULAR PROTEINS

Organism	Protein	Gm. of protein in preparation containing 1 gm. molecule of cyst(e)ine*	Known m w. of protein	Maximum apparent No. of ½-cystine residues/molecule
Pseudomonas myxogenes	Proteinase	∞[1]	77,000[1]	0
Pseudomonas saccharophila	α-Amylase	400,000[4,7]	40,000[4]	0
Streptococcus haemolyticus	Proteinase	120,000[8]	—	0
Bacillus subtilis	Proteinase ('subtilisin')	∞[9]	27,000[10,11]	0
Bacillus subtilis	Ribonuclease	∞[12] 20,000[8]	10,000–20,000[13] (S_{20} = 1 4)	0 1
Bacillus subtilis	Proteinase	100,000[8,14] 89,000[8]	30,000	0
,, ,,	α-Amylase	∞[8,15] 46,000[8]	49,000[16]	0 1
Bacillus cereus, strain 5/*B*	Penicillinase (constitutive)	∞[5]	35,000[17]	0
Bacillus cereus, strain 569	Penicillinase (induced)	92,000[8]	31,000[17]	0
Vibrio cholerae	Neuraminidase	77,000[8]	90,000[18] 10,000–20,000[19] (S_{20} = 1 3)	1 0
Corynebacterium diphtheriae PW 8	Toxin	18,000[20]	72,000[21]	4
Clostridium tetani	Toxin	approx. 5,000[22] 8,000–11,000[23]	67,000[22] 66,000–74,000[21]	14 9

The sources of the proteins analysed by us were as follows: *Streptococcus haemolyticus* proteinase: Dr. S. D. Elliott, Department of Animal Pathology, Cambridge University; *B. subtilis* ribonuclease: Dr. S. Nishimura, Cancer Institute, Tokyo; *B. subtilis* proteinase and α-amylase: Nagase Co. Ltd., Ohama, Amagasaki; *B. subtilis* α-amylase (recrystallized): Dr. Kakiuchi, Osaka University; *V. cholerae* neuraminidase: Dr. G. Ada, Walter and Eliza Hall Institute, Melbourne; *B. cereus*, strains 5/*B* and 569 penicillinases: crystalline specimens from this laboratory[25,26].

* Where ∞ is given as a value, the only information available was that no cyst(e)ine was detected by the method used, limits of sensitivity not being stated.

(1) *It may be characteristic of a particular catalytic type of enzyme.* However, in at least three instances catalytically similar enzymes from fungal, plant or animal sources are known to contain disulphide linkages in the molecule: (*a*) the α-amylases both of mammalian pancreas and *Aspergillus* have 4–8 half-cystine residues (all probably in the disulphide linkage) in each molecule, the size of which is identical with that of *B. subtilis* α-amylase[30]; (*b*) bovine pancreatic ribonuclease, having a molecular weight very similar to, and specific activity identical with, that of the *B. subtilis* enzyme[13] has four disulphide bonds[31]; (*c*) several animal proteases (for example, pepsin, trypsin and chymotrypsin) have two or more disulphide bonds per molecule[32].

(2) *It may be characteristic of bacterial proteins in general.* Only very few cell-bound bacterial proteins have been purified or analysed for cyst(e)ine; and in most cases they have been isolated from *Escherichia coli*, which does not liberate significant quantities of extracellular protein; they cannot thus be considered very satisfactory examples for comparison in this respect.

We have therefore determined the cyst(e)ine content of total cell protein in all the bacterial species from which individual exo-proteins have been previously analysed, and the results are summarized in Table 2.

Table 2. CYST(E)INE CONTENT OF BACTERIAL CELL PROTEIN

Organism	Weight of protein in gm. containing 1 gm. molecule of cysteine
1. *Escherichia coli*	7,600*
2. *Pseudomonas fluorescens KV* 2	14,200
3. *Pseudomonas saccharophila*	13,100
4. *Staphylococcus aureus* 524	7,000
5. *Bacillus subtilis* 6346	16,300
6. ,, ,, *H*	9,350
7. *Vibrio cholerae* 4 *Z*	11,300
8. *Streptococcus haemolyticus C* 748	8,250
9. ,, ,, *D* 58	9,500
10. *Corynebacterium diphtheriae PW* 8	9,150
11. *Bacillus cereus* 569	12,700
12. ,, ,, 5/*B*	10,100

Sources of organisms: No. 3: Dr. M. Doudoroff, Dept. of Bacteriology, University of California, Berkeley; Nos. 8 and 9: Dr. M. McCarty, Rockefeller Institute, New York; No. 6: Dr. Iizuka, Applied Microbiology Institute, Tokyo; No. 10: Dr. G. C. Pope, Wellcome Research Laboratories, Beckenham; Nos. 2, 4, 5, 7, 11 and 12: lab. strains.

Media. For Nos. 2, 5, 11 and 12: casein hydrolysate medium + citrate (CH/C)[26]; for No. 3: a maltose + NH_3 + salts medium[33]; for No. 4: CH/C + 0·001 per cent nicotinic acid and 0·001 per cent thiamine; for No. 7: 0·05 *M tris* maleate buffer, *p*H 8·0 + 0·5 per cent L-glutamic acid, 0·9 per cent glycerol, 0·004 per cent adenine and 0·35 per cent KH_2PO_4; for Nos. 8 and 9: Hartley beef broth[34]; for No. 10: papain digest of beef + 0·08 per cent $MgSO_4.7H_2O$ and 0·25 per cent maltose.

The two species of *Pseudomonas* were grown at 30° in stationary culture. All other cultures were grown at 35° and aerated by shaking. Organisms were inoculated at a density corresponding to 0·02 to 0·05 mgm. dry wt./ml. and cropped after 16–18 hr. incubation.

* This figure, relating to *E. coli*, was calculated from results obtained by Roberts *et al.*[35].

So far as this analysis goes, the values agree well with those obtained by Mondolfo and Hounie[36] using a different (microbiological) technique. It seems clear that the mean cyst(e)ine content of cell-bound bacterial protein is very considerably higher than that of the extracellular proteins isolated from the same organisms. There does not, therefore, seem to be any good reason for supposing that bacteria in general, and exo-protein-forming bacteria in particular, produce protein which, regardless of whether it is cell-bound or liberated into the medium, is exceptionally low in cyst(e)ine. It would, of course, be interesting to know the cyst(e)ine content of the corresponding total extracellular protein produced by these organisms. But the proportion thus liberated rarely exceeds 3 per cent of the total cell protein, and is frequently much lower. Thus contamination with cell protein liberated by a very minor degree of cell autolysis (which is difficult both to estimate and to prevent) would invalidate any such analysis.

(3) *It may be characteristic of their extracellular location.* Little or nothing is yet known concerning the mechanism by which bacteria liberate enzymes and other proteins into the surrounding medium from apparently intact cells. But it seems almost certain that it will prove to be fundamentally different from processes occurring in mammalian tissues, such as the pancreas or salivary glands, where digestive enzymes appear first inside the cell, within zymogen granules which later move to the cell surface and discharge their contents outside[37,38]. The site of formation of bacterial exo-enzymes is not yet known; they may or may not have to pass through the cytoplasmic membrane in the process of liberation, though there is some evidence to suggest that the usually very small portion found still attached to the cell may be on the outside of the permeability barrier, and that the site of synthesis is the membrane itself[38,39]. However this may be, it seems certain that these enzymes have to pass through the rigid mucopeptide mesh and possibly other structures of the bacterial cell wall before they are finally liberated. The extent to which this cell-wall lattice acts as a barrier to the passage of protein molecules is not known; but there are suggestions that it may be far from negligible[40]. It is almost certain that no such rigid obstruction exists to the escape of pancreatic enzymes from the cell as soon as they have emerged beyond the limits of the cytoplasmic membrane. The presence, therefore, of disulphide bonds in pancreatic enzymes such as ribonuclease and α-amylase, which are later liberated into the duct lumen, does not mean that the absence of disulphide bonds in similar types of exo-enzyme of bacterial origin is unconnected with their property of 'extracellularity'. Indeed, this very fact may reasonably be taken as supporting the hypothesis that the absence of disulphide bonds might be directly related to the peculiar conditions associated with the passage of bacterial enzymes through a rigid cell-wall barrier.

It may be relevant to note in this connexion that other bacterial proteins of a partially extracellular nature appear also to lack disulphide bonds. Kobayashi, Rinker and Koffler[41] report that purified samples of flagella protein ('flagellin') from one *Proteus* and three *Bacillus* species in all probability contain only one half-cystine residue per molecule. The protein extractable with formic acid from cell-wall preparations of *E. coli*[42] gave completely negative tests for cysteic acid after oxidation and hydrolysis[43], which would indicate a cyst(e)ine content of not more than 0·2 per cent by weight. The extent to which such proteins might be regarded as 'extracellular' is obviously a matter of definition; more important, perhaps, is the degree to which they might possibly have certain characters in common with proteins that are released from the cell in a soluble state, by reason, perhaps, of peculiarities associated with their surface location. But even less is known about possible barriers to their penetration of surface structures than is the case with free extracellular proteins.

If the relationship between extracellularity and absence of disulphide bonds in bacterial proteins is a real one, it is tempting to speculate on its possible significance.

Disulphide linkages in a protein are the most important, if not the only, covalent (and, as such, the strongest and most important) bonds contributing to the stability and rigidity of the tertiary structure of the molecule[44]. A protein molecule lacking disulphide bonds would have to rely mainly on the weaker and more easily ruptured H-bonds and metal- and salt-linkages, for preserving its folded state. Bacterial exo-enzymes might therefore be expected to have more than the usual degree of flexibility and to be capable of more easily unfolding and refolding than proteins which contain —S—S— bonds. The possibility that these bacterial enzymes might have become adapted to pass more freely through a rigid cell-wall meshwork by evolving a degree of flexibility through elimination of disulphide bonds, seems at least worthy of consideration.

The value of such a speculation must depend in the first place on further information both on the cyst(e)ine and —S—S— bond content of a wider variety of bacterial proteins of all kinds and on the extent to which some of them may legitimately be classed as 'truly' (that is, physiologically) extracellular, and not liberated by cell damage. Without this, even the generalization on which this speculation is based can scarcely be considered as sufficiently well established.

At the same time it is important to know more about: (*a*) the detailed structure of the bacterial cell-wall and its permeability properties for large molecules; (*b*) the extent to which it is possible for the polypeptide chains of bacterial exo-enzymes to exist in an unfolded state, and subsequently to fold up without loss of biological activity, into the specific tertiary structures which they undoubtedly possess after liberation into the medium; (*c*) the factors which could stimulate or inhibit reversible unfolding of these enzymes (or maintenance of the unfolded state) under conditions existing in the cell, and their effect on exo-enzyme liberation; (*d*) alterations in the ability of proteins to penetrate through artificial and natural membranes of graded porosity, after treatment known to cause a partial or complete unfolding of the molecule. So far, however, the only work directly relevant to this possibility concerns the penicillinase of *B. cereus*, which Citri *et al.*[45] have shown to be capable of undergoing a limited degree of reversible unfolding; though there is no direct evidence relating this to the mechanism of its liberation from the cells. Further information would be valuable and interesting enough, quite apart from its relevance to a hypothesis which, though perhaps somewhat speculative, is nevertheless one which involves some fairly precise predictions, capable of experimental verification.

Cyst(e)ine Estimation

In view of the very low cyst(e)ine contents of the proteins being studied, the very small amounts (2–5 mgm.) that sometimes were available, and the well-known difficulty of preventing destruction of the amino-acid on hydrolysis, it was essential to devise a micro-method of maximal sensitivity, and to take special precautions to prevent loss, by prior oxidation to cysteic acid, and careful removal of excess acid.

For total bacterial protein, 1·0 ml. of 50 per cent (w/v) trichloroacetic acid (TCA) was added to 9 ml. of a washed cell suspension, from a log-phase culture containing about 50 mgm. dry bacterial wt. After standing 15 min. at room temperature, the mixture was heated to 95° for 30 min. and then left at 2° for 16 hr. The precipitate was washed twice with 5 per cent TCA, re-suspended in 10 ml. water, about 1 per cent of the total dissolved in *N* sodium hydroxide for estimation of protein[46], and the remainder evaporated to dryness and dissolved at a concentration of about 20 mgm./ml. in 98–100 per cent formic acid. With purified proteins, a known quantity was dried *in vacuo* and similarly dissolved in formic acid. These protein solutions were then oxidized by adding 2·5 ml. performic acid per mgm. protein and leaving for 4 hr. at room temperature. Samples were then evaporated to 5 ml. at 35°, redissolved in 50 ml. water, dried down, and the oxidized protein hydrolysed with 4 ml. 6 *N* hydrochloric acid in sealed ampoules at 105° for 16 hr. Most of the hydrochloric acid was then removed at 100° in a stream of air, and the remaining traces by repeated addition and evaporation of 3·0 ml. lots of water. The technique is based on that described by Schram *et al.*[47], with minor modifications.

The method used for separation of cysteic acid (CA) from other amino-acids present in the hydrolysates depended on whether or not diaminopimelic acid (DAP) was present in the preparation. DAP, which occurs in the cell walls of certain species of bacterium[48] and is, therefore, precipitated, by the technique used here, along with the amino-acids of true cell protein, does not easily separate from cysteic acid chromatographically. In its absence (that is, in all purified protein preparations, and in total cell protein from *Staphylococcus aureus* and *Streptococcus haemolyticus*), CA was isolated by loading up to 100 μl. of the hydrolysate, corresponding to 0·5–1·0 mgm. of protein, together with CA markers, on to Whatman No. 3 paper as a 3 cm. band, and allowing descending chromatography in *n*-butanol/acetic acid/water (63 : 10 : 27, by vol.) for three days. In the presence of DAP (that is, with all other cell protein preparations), preliminary separation of CA was achieved by ionophoresis. About 10 μl. of the hydrolysate (corresponding to 0·5–1·0 mgm. of protein) was loaded on to similar paper at the centre of a line drawn 10 cm. from the long side, after dipping into 2 *M* acetic acid (*p*H 2·4) and blotting off excess liquid. A potential gradient of 20 V./cm. was applied for 1¼ hr., the paper dried, and CA markers applied to the line so as not to overlie the partially separated amino-acids. Chromatography, similar to that already described, but for 18–24 hr. only, was then applied at right angles to the direction of the preceding ionophoresis. Since CA is the only amino-acid to move towards the anode on electrophoresis at *p*H 2·4, adequate separation from DAP and other amino-acids is thus achieved.

The CA thus isolated was estimated with ninhydrin according to the method used by Mandelstam and Rogers[49], by comparison with known 'marker' quantities of CA (1–20 μgm.) which were always run on each paper. The standard curve thus obtained gave extinction values at 570 mμ which were proportional to quantities of CA down to 2 μgm.

Protein hydrolysates tended to 'smear' slightly over the chromatography paper, so that weakly positive spots (equivalent, in ninhydrin colour, to up to 2 μgm. of cysteic acid out of a total load of 700 μgm. or more) were sometimes obtained with proteins (for example, α-amylase) that were known not to contain cyst(e)ine. Thus the recorded cyst(e)ine contents of 1 gm.-mole in 50,000 gm. or more of protein are consistent with complete absence.

Recovery of CA from oxidized and hydrolysed samples of insulin (World Health Organization standard) at loads of 100–150 μgm. gave yields of 75–80 per cent of theoretical. An artificially reconstituted low-cyst(e)ine protein made by oxidizing and hydrolysing a mixture of 2·9 mgm. of *B. subtilis* α-amylase + 250 µgm. insulin (that is, final cyst(e)ine content of 1·0 per cent) at a total load of 643 µgm. (having a theoretical CA content of 8·6 µgm.) gave a yield of 85 per cent. Smaller proportions of insulin gave somewhat lower yields (for example, with 44 µgm. insulin + 944 µgm. α-amylase, that is, a cyst(e)ine content of 0·51 per cent, the yield was only 60 per cent). Assuming that it might have been possible to 'lose' up to 50 per cent of about 4 µgm. of CA in a total load of 1 mgm. protein hydrolysate, the presence of a single half-cystine residue might have been missed in a protein with a molecular weight of 85,000 or more, and the possible presence of one disulphide bond might have been missed in proteins with molecular weight of 170,000 upwards. It seems, from the known molecular weights of the proteins investigated, that this eventuality is out of the question.

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LIQUEFACTION OF GELATIN BY SOME *STREPTOMYCES*

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THE cultural media used for the liquefaction of gelatin in taxonomic investigation of *Streptomyces* are often of different composition. In fact, Waksman and Curtis[1], Waksman[2], Millard and Burr[3], Frommer[4], Shinohu[5], use plain gelatin. Lindenbein[6], Corbaz *et al.*[7], Baldacci *et al.*[8], Kurtzner[9], Gause *et al.*[10], Hoffmann[11], Frommer[4], use gelatin with peptone and glucose. Shinohu[5] uses gelatin with peptone, beef extract and sodium chloride. Kzainsky[12], Jensen[13] and Taber[14] use gelatin with mineral salts and Gordon *et al.*[15] gelatin with agar, beef extract and peptone.

During a systematic study of several *Streptomyces* isolated from the soil and from other materials, I have performed many experiments in order to compare the media used by different authors on the production of melanoid-soluble pigments, and the liquefaction of the gelatin, and assimilation of carbohydrate and nitrogen compounds. The results obtained on the production of melanoid-soluble pigments have already been published[16].

This article reports the results on the gelatin liquefaction assay. The strains of *Streptomyces* were the same as those used in the investigation of soluble melanoid pigments[16]. The assay of gelatin liquefaction was performed by the stab method in test tubes of 150 × 15 mm., containing 20 ml. of gelatin (Difco) base medium, sterilized at 120° C. for 20 min. The inocula were made with a sterile needle using sporulate cultures obtained in tubes

Table 1. LIQUEFACTION IN MEDIA WITH GELATIN, PEPTONE AND GLUCOSE

Gelatin			15			15			15			15			15			15	
Glucose			—			2			2			—			2			2	
Peptone			—			—			0 5			0·5			—			0·5	
Dist. water			100 S.T.			100 S.T.			100 S.T.			100 S.T.			100 S.S.			100 S.S.	
Days from inoculum		14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50
Systematic position*																			
Strept. sp. *A*	511	2	10	20	—	—	3	2	6	10	2	7	16	2	6	14	2	7	18
Strept. sp. *A*	512	2	9	20	1	4	10	·	·	8	2	7	20	·	2	18	2	5	10
Strept. chartreusis	254	2	5	15	—	—	—	—	3	18	—	5	12	—	3	18	2	6	12
Strept. pyridomiceticus	328	—	3	16	·	4	18	·	6	17	2	7	13	·	5	15	1	5	17
Strept. roseus	399	—	—	8	—	3	7	—	2	7	—	—	4	—	—	—	—	1	5
Strept. sp. *B*	1	1	6	13	1	6	15	—	6	14	1	7	16	1	5	17	1	7	19
Strept. lavendulae	B171	—	—	1	—	—	3	—	—	1	—		3	—	—	3	—	—	1
Strept. viridochromogenes	64	—	3	12	—	—	—	—	6	25	1	6	13	—	4	15	1	6	13
Strept. violaceus	135	2	4	10	·	2	5	—	—	4	—	—	1	·	·	9	—	·	9
Strept. griseolus	361	2	4	15	·	5	15	1	7	17	1	6	12	·	6	16	2	7	20

Strept. sp. *A* and *Strept.* sp. *B* are probably new species.

* Waksman key: Waksman, A. S., *The Actinomyces* (Williams and Wilkins Co., Baltimore, 2, 1961).

S.T., sterilized together; S.S., sterilized separately; —, negative liquefaction; ·, beginning of liquefaction; figures, mm. of liquefied gelatin.

Table 2. LIQUEFACTION IN MEDIA WITH GELATIN AND GLUCOSE

Gelatin		20			20			20			20			20			20			20			20			20	
Glucose		—			1			2			5			10			1			2			5			10	
Dist. water		100 S.T.			100 S.T.			100 S.T.			100 S.T.			100 S.T.			100 S.S.			100 S.S.			100 S.S.			100 S.S.	
Days	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50
Strains																											
511	—	1	10	2	10	11	1	2	12	—	2	10	N	N	N	1	9	12	—	9	13	·	9	13	1	9	10
512	2	8	11	1	3	16	2	10	15	—	8	10	N	N	N	2	12	15	2	8	10	3	10	12	2	10	10
254	·	10	10	—	10	16	—	·	11	—	—	—	N	N	N	2	12	15	·	13	17	3	15	18	2	10	17
328	·	2	5	1	7	9	·	10	10	—	—	·	N	N	N	—	4	7	·	·	6	·	2	6	1	5	10
399	—	6	15	—	—	5	—	2	6	—	—	·	N	N	N	—	3	7	—	·	9	2	9	10	2	12	14
1	2	7	11	2	10	14	—	—	10	—	—	·	N	N	N	·	9	13	—	7	15	—	—	3	—	—	—
*B*171	—	·	3	—	·	5	—	·	6	—	—	—	N	N	N	—	·	3	1	5	6	1	6	7	2	8	10
64	1	6	15	—	·	6	—	—	10	—	—	7	N	N	N	—	2	5	—	2	5	·	3	5	·	1	5
135	2	12	12	2	10	12	2	9	10	·	6	6	N	N	N	3	10	11	1	7	10	—	—	1	—	—	—
361	2	9	12	2	10	13	1	11	15	3	11	15	—	—	—	3	11	13	3	11	13	3	12	14	3		16

N, no growth; —, growth but negative liquefaction; ·, beginning of liquefaction; figures, mm. of liquefied gelatin.

Table 3. LIQUEFACTION IN MEDIA WITH GELATIN, DIFFERENT TYPES OF SUGARS, ETC.

				Plain gelatin and																										
	Plain Gelatin			Glucose 2 per cent S.T.			D-xylose 2 per cent S.T.			Maltose 2 per cent S.T.			Lactose 2 per cent S.T.			Sucrose 2 per cent S.T.			Raffinose 2 per cent S.T.			$NaNO_3$ 1 per cent S.T.			$NaNO_2$ 1 per cent S.T.			$NaNO_2$ 1 per cent Glucose 2 per cent S.T.		
Days	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50	14	30	50
Strains																														
511	2	10	11	—	8	11	N	N	N	3	10	11	3	10	11	2	10	12	2	10	11	2	10	10	N	N	N	3	11	11
512	3	10	10	—	7	11	N	N	N	3	10	12	2	10	12	—	6	10	·	7	11	2	7	9	N	N	N	—	—	2
254	—	7	10	—	—	—	N	N	N	—	5	8	—	5	8	—	6	8	—	5	8	·	6	8	N	N	N	—	—	—
328	·	5	6	·	5	6	N	N	N	—	2	3	—	2	3	—	2	4	—	1	5	·	2	5	N	N	N	2	5	7
399	—	2	6	—	3	6	N	N	N	—	3	7	—	3	7	—	3	6	—	5	7	—	2	6	N	N	N	·	5	6
1	2	7	10	—	—	5	N	N	N	2	7	10	1	7	10	·	10	12	2	7	9	2	6	9	·	6	7	—	1	2
171	·	3	5	1	3	6	N	N	N	1	4	5	1	4	5	·	3	5	1	4	5	2	4	5	—	6	6	1	4	5
*B*64	1	3	7	—	—	1	N	N	N		1	1	·	1	1	1	2	5	·	2	3	2	10	12	N	N	N	2	6	7
135	·	3	5	2	10	10	N	N	N	·	2	5	·	2	5	·	3	6		4	7	2	10	12	N	N	N	—	—	5
361	2	10	11	3	11	14	3	12	15	3	11	13	1	11	13	2	10	12	3	10	12	3	11	17	2	7	7	2	10	12

N, no growth; —, growth but negative liquefaction; ·, beginning of liquefaction; S.T., sterilized together; figures, mm. of liquefied gelatin; plain gelatin, gelatin 15 gm. dist. water 100 ml.

with yeast extract malt agar[17]. After inoculation, the tubes were maintained at 20° C. and the observations were carried on each second day for a period of two months.

Table 1 shows the results obtained on 6 gelatin media with and without glucose and peptone, sterilized together or separately. The best results, regarding the rate of liquefaction, were obtained on plain gelatin and on gelatin with peptone and glucose. No liquefaction was observed for Strain 399 on gelatin and glucose sterilized separately and for the Strain 254 on gelatin and glucose sterilized together.

Table 2 records the results on gelatin with increasing doses of glucose. It may be deduced that the presence of glucose in the media influences the liquefaction especially if it is sterilized together with gelatin. Strains 399, 254, 328, 1 and *B*171, in fact, showed themselves incapable of producing liquefaction in gelatin 20 per cent and glucose 5 per cent. Strains 135 and 1 did not produce liquefaction in gelatin 20 per cent and glucose 10 per cent sterilized separately. On gelatin 20 per cent and glucose 10 per cent sterilized together 9 strains of the 10 studied did not grow. On this medium the growth of Strain 361 was only observed, but it did not liquefy.

As regards the rate of liquefaction, taking into account the technique used for the inoculum, I might add that it is very variable and inconstant. In plain gelatin some strains began even a month after the inoculum while when glucose was added to the gelatin some other strains liquefied even more slowly.

Table 3 shows the results in media with gelatin and different types of sugars and other compounds. It may be deduced that all the strains tested showed themselves capable of liquefying plain gelatin 15 per cent after 20 days from the inoculum. In gelatin 15 per cent and glucose 2 per cent sterilized together, liquefaction did not occur in Strains 64 and 254 even after 50–60 days from inoculum. Furthermore, Strain 64 liquefied very poorly gelatin with maltose and gelatin with lactose. On gelatin 15 per cent with D-xylose 2 per cent growth and liquefaction were observed with Strain 361 only. On gelatin 15 per cent with sodium nitrite 1 per cent sterilized together, Strains 135, 511, 512, and 64 did not grow at all; and Strains 135, 254 and 328 grew after 50 days from inoculation. On gelatin 15 per cent with sodium nitrate 1 per cent the results were like plain gelatin 15 per cent; but on gelatin 15 per cent with sodium nitrate 1 per cent and glucose 2 per cent, no liquefaction was observed with Strain 254 and a delay of liquefaction with Strains 512, 135 and 1.

In order to interpret the facts observed, some determinations of *p*H and redox-level were carried out before and after sterilization of the media prepared with three different types of gelatin (Difco, C. Erba and D.G.F.).

Redox potentials (by potentiometer and colorimetric methods) did not reveal appreciable variations in all media used, while the values of *p*H tended to become lower during sterilization.

This fact is in relation to the types of gelatin and to the types of sugars (greater with D- and L-xylose and with glucose than with sucrose, maltose, lactose, raffinose). On plain Difco gelatin 15 per cent the *p*H was 6·68 before the sterilization and 6·46 after the sterilization. On Difco gelatin 15 per cent and glucose 2 per cent, 6·59 and 6·02; on Difco gelatin 15 per cent and glucose 10 per cent, 6·52 and 5·54; on Difco gelatin 15 per cent and D-xylose (or L-xylose) 2 per cent, 6·67 and 5·62; on Difco gelatin 15 per cent and sodium nitrite, 1 per cent, 6·79 and 7·22. Similar results were also observed with C. Erba gelatin and with D.G.F. gelatin used as control. Moreover, gelatin sterilized together with D-xylose, or L-xylose, or glucose, become brown as a result of condensation of amino-acids and reducing sugars (Maillard theory) or because of a decomposition of sugars to furfuraldehydes or similar compounds (active-aldehyde theory)[18]. To these compounds and to the consequent reduced values of *p*H can also be attributed the lack of growth on gelatin 15 per cent sterilized together with glucose 10 per cent or with D-xylose 2 per cent.

No growth of some strains on gelatin 15 per cent plus sodium nitrite 1 per cent can be found in the real inhibitory effect of this salt[19]. The *p*H was not reduced after sterilization and the change of colour to brown-greenish in the medium reveals the reaction from amino-acids and sodium nitrite.

The facts observed lead to the conclusion that the presence of sugars in media may give completely negative results or may influence the rate of liquefaction. The rate of liquefaction depends on the technique of inoculation, the temperature of cultivation, the quantity of inoculum and also on the gelatin. Gelatin is a very complex organic substance, and its constituents change with materials and techniques used to obtain and to purify it.

With regard to the necessary uniformity of the methods of study of the Actinomycetes, the results obtained lead me to prefer the use of plain gelatin for a criterion of the identification. It must be added that this may give results which are not always constant because of the variable composition of gelatin, particularly in regard to the rate of liquefaction.

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CARRAGEENIN AS A PROTEIN PRECIPITANT

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SINCE the observation of Oncley[1] that dextran sulphate, under specific conditions, can be used as a precipitant for β-lipoproteins, increasing attention has been paid to reactions involving sulphated polysaccharides with serum proteins. After considering a range of polyanions, Bernfeld[2,3] concluded that sulphated amylopectin, with three sulphate groups per repeating unit, was the most satisfactory precipitant for β-lipoprotein. While the mechanism of the reaction is unknown, these lipoprotein–polysaccharide complexes may be broken by centrifugation in a saline gradient and the β-lipoprotein liberated from polysaccharide contaminant. Lipoproteins have successfully been assayed by this method and isolated for chemical characterization. Alternatively, direct nephelometric measurements of the turbidity of these complexes in dilute suspensions provides a measure of the β-lipoprotein concentration.

Serum proteins readily complex with certain sulphated polysaccharides, and the complexes so formed can normally be dissociated by high concentrations of salt. However, the subsequent removal of the polysaccharide from proteins of average density is difficult. Miller[4] precipitated an abnormal serum macroglobulin with heparin and dissociated the complex in concentrated saline. The macroglobulin was not chemically characterized presumably due to the incomplete removal of the liberated heparin.

The purpose of this article is to report the use of another sulphated polysaccharide, carrageenin, which, in addition to being an effective precipitant is readily removed from the protein carrageenin complex.

Carrageenin was extracted from the red alga *Chondrus crispus* by the method of Smith *et al.*[5]. Separation into the κ- and λ-fractions was accomplished by precipitation with 0·25 *M* potassium chloride, the former being insoluble, the latter soluble in this solvent. Three sub-fractions of κ- and five sub-fractions of λ-carrageenin were obtained by gradient addition of 95 per cent ethanol. The conditions of fractionation are given in Table 1, together with the intrinsic viscosities of the sub-fractions (labelled $\lambda 1$–$\lambda 5$ and κ_1–κ_3) measured in phosphate buffer μ 0·15 *p*H 6·8.

Table 1. FRACTIONATION OF CARRAGHEENIN

Fraction	Alcohol conc. vol. per cent	Amount precipitated per cent of total	$[\eta]$ dl./gm.
λ1	0–12	8·6	6·5
λ2	12–24	4·4	19·9
λ3	24–36	70·0	13·0
λ4	36–48	21 0	8·8
λ5	48–60	1 0	2·7
κ_1	0–30	34 0	8·8
κ_2	30–45	26·0	6·4
κ_3	45–75	40 0	9·7

Fetuin, isolated by ammonium sulphate precipitation of fœtal calf sera, has been shown to contain two α_1-glycoproteins[6,7], one of which is a macroglobulin[8]. The major glycoprotein ($S^0_{20w} = 3·2$) was separated from the macroglobulin ($S^0_{20w} = 20·2$) by high-speed centrifugation[8]. Physical characterization of these compounds[9] showed the major component to be isoelectric at pH 4·1 and the macroglobulin at pH 4·7. This difference suggested that the two components might be fractionated by precipitation with carrageenin over a pH range. Fetuin is readily soluble in 0·25 M potassium chloride whereas κ-carrageenin is not; hence κ-carrageenin–fetuin complex when dispersed in 0·25 M potassium chloride could yield a protein solution and a carrageenin precipitate.

1 ml. of a 0·5 per cent solution of κ_2-carrageenin was slowly added with stirring to 40 ml. of a 1 per cent solution of fetuin buffered at various pH values between 4·0 and 5·5. The buffers were mixtures of sodium acetate, acetic acid and sodium chloride at a total ionic strength of 0·1 and the carrageenin was dissolved in the appropriate buffer prior to addition. The precipitate, which formed after standing for 3 hr. at 0° C., was removed by centrifugation and dissolved in a minimal volume of pH 7·0μ = 0·1 buffer. The soluble complex and the original supernatants were then dialysed overnight with stirring against 0·25 M potassium chloride at 0° C. and the resulting precipitates were removed by centrifugation. The clear supernatants were examined in the Spinco model E ultracentrifuge and the composition of the components assayed by area computation corrected for cell dilution.

The results (Table 2) show that while both globulins were precipitated at each pH there was evidence of concentration of the macrocompound at specific pH values. This effect was optimal at pH 4·5 where a six-fold concentration of the macroglobulin was effected. Moreover, the macroglobulin was absent in the supernatant at this pH. Paper electrophoresis followed by selective staining with bromphenol blue and reduced Schiff's reagent showed that the precipitated material was a glycoprotein migrating as an α_1-globulin. Complete absence of contaminating carrageenin was indicated by negative staining with toluidine blue. Furthermore, the carrageenin removed from the complex with 0·25 M potassium chloride, when hydrolysed with acid, was shown to be ninhydrin negative. Thus, glycoprotein was absent, thereby confirming the efficiency of the potassium chloride partition. We have used κ-carrageenin as above to isolate β-lipoprotein from human serum and have found that 0·25 M potassium chloride is likewise effective in breaking the polysaccharide–lipoprotein complex.

Table 2. EFFECT OF pH ON GLYCOPROTEIN PRECIPITATION BY κ_2-CARRAGEENIN

pH	Percentage $S^0_{20w} = 3·2$ glycoproteins	 $S^0_{20w} = 20·2$ glycoproteins
4·0	75	25
4 5*	63	37
4 7	85	15
5·0	68	34
5·5	87	13
Control fetuin	94	6

* Precipitate formed at pH 4·5 redissolved and reprecipitated to give the composition 66 per cent $S^0_{20w} = 3·2$ and 34 per cent $S^0_{20w} = 20·2$ glycoproteins.

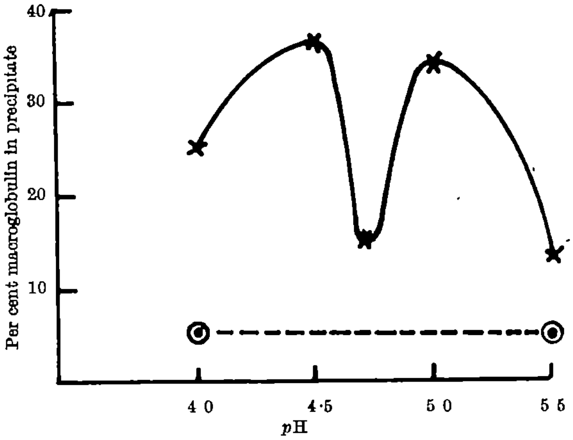

Fig. 1. Percentage of macroglobulin in the κ_2-carrageenin–fetuin precipitate as a function of pH. The dotted line shows the percentage of macroglobulin in the original fetuin sample

If the concentration of the macroglobulin (Table 2) is plotted against the pH of precipitation (Fig. 1) a trough is shown at pH 4·7, the isoelectric pH of the macroglobulin. Were the reaction to be ionic one would expect a rapid decline in the percentage of the macroglobulin precipitated on the alkaline side of the isoelectric pH. Since this did not occur the reaction mechanism is probably first ionic followed by a second stage of more complex nature.

Further work is planned to cover more completely the pH-range in the region of 4·1 the isoelectric pH of the major glycoprotein fraction. Attention will also be paid to the physical characteristics of the polysaccharide used since κ_1- and κ_3-carrageenin, both of which have higher intrinsic viscosities than κ_2, gave precipitates containing proportionally less of the macroglobulin. By inference, λ5-carrageenin of low intrinsic viscosity (2·7 dl./gm.) might be a more satisfactory fraction to use as a protein precipitant, but λ-carrageenin is soluble in 0·25 M potassium chloride. This limitation could be overcome by the use of the enzyme carrageenase[10]. The application of these observations to a study of both normal and abnormal serum macroglobulins is apparent since it may provide a mechanism for the isolation of these important proteins.

We thank Mr. G. Wilson for the ultracentrifuge analyses, Mr. C. Guthrie for the preparation of the carrageenin fractions, Miss C. M. Gilbo for the paper electrophoresis studies, and Dr. J. F. O'Dea for advice.

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COLCHICINE-INDUCED POLYPLOIDY IN *MUSA*

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PREVIOUS work on bananas has shown that: (*a*) in Nature the polyploid seedlings are formed by the union of a haploid pollen gamete ($n = 11$) with a restituted polyploid egg cell[1]; (*b*) the edible bananas of commerce are triploid plants which have descended from parthenocarpic diploids and seeded species by means of hybridization, mutation, and selection by man throughout centuries past[2]. A method of obtaining tetraploids from diploids could have practical advantages in a banana-breeding programme involving polyploid plants, and where there are problems of male and female sterility in some of the diploid parents[3]. This article describes a method of colchicine application to diploid *Musa* seedlings and the effect of the colchicine treatment on the growth of the resulting polyploid seedlings.

Seeds were obtained from an open-pollinated bunch of *M. balbisiana* Colla and from a hand-pollinated cross between *M. balbisiana* × *M. acuminata* Colla sub-sp. undetermined. After harvesting, seeds were dried and stored in polyethylene bags. Stored seeds were soaked in water for 5 days at 35° C. before being placed between layers of cheese cloth buried in a mixture of equal parts of clay loam, sand and vermiculite. Germination, as indicated by the displacement of the micropilar plug, commenced 9–10 days after planting. Soaking and planting dates of three groups of seeds were distributed over a period of three days so that seedlings of varying age could be selected for colchicine treatment.

The seedling stages used in this work were: (I) intact seed; (II) seeds with dislodged micropilar plugs, the first visible stage of seed germination; (III) seeds with emerged seedlings showing epicotyl and the beginning of a primary root; (IV) seedlings with coleoptile-like first leaves 1–2 cm. long[4] and with 3–5 adventitious seminal roots 1½–3 cm. long (Fig. 1).

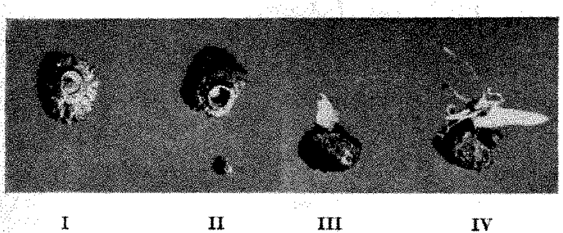

Fig. 1. Stages of seedling development in *M. balbisiana* Colla: (I) intact seed; (II) first visible stage of seed germination with dislodged micropilar plug; (III) seedling showing epicotyl and the beginning of a primary root; (IV) seedling with coleoptile-like first leaf and adventitious seminal roots

The treatments consisted of submerging the seeds or seedlings, at different stages of growth, in various concentrations of aqueous colchicine solution for given times. After treatment, seedlings were washed with water and transplanted to flats. Each treatment included 100 seeds or seedlings which were planted in four replicates in a randomized block design. Observations on the emergence of the first leaves above the soil-level were made at 2–3-day intervals. After two months, the seedlings were transplanted into black plastic pots, and chromosome counts were made by root-tip smear in aceto-orcein stain. Chromosome counts were made sporadically until seedlings were 10 months old and before transplanting to the field for further observations. (I thank Dr. M. O. Thomas for help in counting the chromosomes.)

Colchicine treatment increased mortality, retarded growth and induced doubling of the chromosome complement in *M. balbisiana*. These responses depended on the developmental stage of the seedling, the concentration of the colchicine solution and the duration of exposure of the seedlings to the solution (Tables 1 and 2). At emergence, the tetraploid and the octaploid seedlings were easily distinguished from the diploids. The coleoptile-like first leaf-sheath of the polyploid seedlings was short and thick, giving the emerging seedlings a much greater diameter than the diploids. The lamina of the second, third and sometimes the fourth leaf was either absent or was greatly reduced in size. The treated seedlings were slow to emerge; but after emergence the unaffected diploid seedlings resumed their normal growth while those which had been induced to polyploidy remained retarded. The length and the abundance of roots also declined as ploidy was increased. The reduction in, or the absence of, a root system seemed to be one of the factors responsible for the death of the octaploid-appearing seedlings.

The colchicine-induced tetraploids resembled tetraploid seedlings obtained by hybridization. The tetra-

Table 1. Effect of Colchicine Concentration on Growth-rate, Mortality and Production of Tetraploid Seedlings of *M. balbisiana*

Seedling* response after 10 hr. treatment	Concentration in aqueous solution (per cent) None	0·5	1·0	1·5
Growth-rate in days†	6·9	22·3	24·6	21·9
Mortality (per cent)	8·5	60·0	74·5	83·5
Tetraploid at 10 months	0·0	7·5	7·5	4·0

* Seedlings with epicotyl 1–2 cm. long and with 3–5 adventitious seminal roots.

† Weighted averaged number of days required by each seedling to re-emerge.

Table 2. Effect of Exposure Time to 0·5 per cent Colchicine on Four Stages of Seedlings Development of *M. balbisiana* × *M. acuminata*

Growth stage	Exposure time (hr.)	Ploidy-level (per cent)* Diploid	Tetraploid	Octaploid	Mortality (per cent)
(I)†	2	49·3	6·5	6·5	37·7
	4	50·0	6·7	5·0	38·3
	8	33·3	5·0	1·1	60·6
	20	14·7	4·4	1·5	79·4
(II)	2	2·2	4·4	19·8	73·6
	4	0·0	0·0	4·1	95·9
	8	0·0	2·0	4·2	93·8
	20	0·0	0·0	1·0	99·0
(III)	2	4·3	31·2	20·4	44·1
	4	1·1	20·4	21·5	57·0
	8	3·2	7·5	20·4	69·9
	20	0·0	3·4	6·7	89·9
(IV)	2	11·7	39·4	9·5	39·4
	4	5·4	32·2	25·8	36·6
	8	4·2	34·4	15·6	45·8
	20	1·1	21·5	6·1	61·3

* Two months after emergence.

† See text and Fig. 1 for seedling stages.

ploids had drooping thick leaves with short petioles, but appeared sturdier than the diploids (Fig. 2). The surviving octaploids either retained their higher ploidy, with characteristic brittle, deformed and thick leaves (Fig. 2) or reverted to tetraploids. The reversion from tetraploid to diploid was easily recognized by the changes in shape and thickness of leaves, length and thickness of petioles, and the degree of bending of petioles. Root-tip smears of some of the tetraploid-appearing seedlings showed a diploid chromosome number suggesting a chimera of tetraploid aerial parts with diploid root system; however, after a month or two the newer leaves of these plants displayed diploid characteristics (Fig. 3). This fact indicated that the root-tip chromosome counts had shown reversion before it could be detected in the aerial parts of the plants.

The unsoaked intact seeds were less affected by colchicine treatment than seeds in early stages of germination (stages II and III in Table 2). This was most likely due to the presence of the thick seed coat and the quiescent state of the embryos. Seeds at their earliest visible stage of germination (stage II, Fig. 1) were the most susceptible to the effects of colchicine. Furthermore, treatment at this stage produced comparatively more octaploid- than tetraploid-appearing seedlings. As seedlings grew through stages III and IV (Fig. 1), mortality decreased while the percentage of tetraploids formed increased. When seedlings slightly older than those of stage IV were treated, the effect of the colchicine was superficial and the ploidy of the growing point was unchanged. The great majority of these seedlings remained diploid.

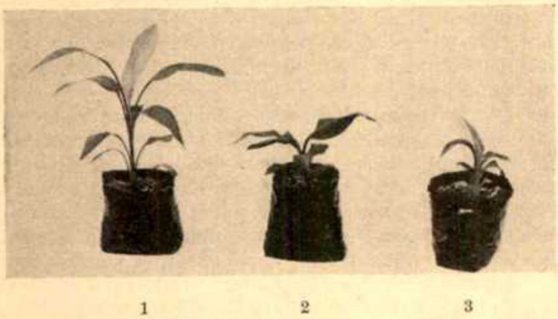

Fig. 2. Four-month-old seedling from a cross between *M. balbisiana* × *M. acuminata* : (1) diploid ($2n = 22$); (2) tetraploid; (3) octaploid

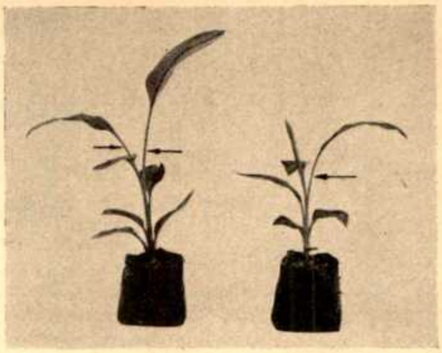

Fig. 3. Reversion from tetraploid characteristics to that of diploid in *M. balbisiana* seedlings. Arrows indicate leaves displaying diploid features

These investigations show that colchicine treatment of diploid *Musa* seedlings at certain stages of development results in the formation of a high percentage of tetraploids having thick leaves, short petioles and a drooping habit. The growth-rate of the tetraploid seedlings was much slower than that of the diploids. Many of the octaploid and tetraploid seedlings reverted to tetraploids and diploids respectively as the plants grew. This reversion could be detected in the appearance of the aerial parts of the plants and was substantiated by the root-tip chromosome counts.

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GENETIC HETEROGENEITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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THE inherited deficiency of glucose-6-phosphate dehydrogenase among human subjects is widespread in occurrence and varies markedly in frequency among different population groups[1-6]. There is evidence to suggest that the enzyme defect may not reflect the same genetic alteration in all affected persons (the term 'affected persons' refers here to those with glucose-6-*P* dehydrogenase deficiency). The activity of the enzyme in red cells and leucocytes of affected males of Greek and Southern Italian extraction has been compared with American Negro males, and found to be significantly lower among the Caucasians[7]. The decreased thermostability of glucose-6-*P* dehydrogenase activity in lysates of erythrocytes and leucocytes prepared from the affected Caucasian males is more marked than that from affected Negro males[8]. Studies of the properties of the dehydrogenase purified from red cells of normal subjects and affected Negro males failed to reveal any differences in the characteristics of the catalytic site, electrophoretic mobility or thermostability[9,10]. Kirkman[11] has reported that glucose-6-*P* dehydrogenase purified from red cells of a Caucasian subject with chronic non-spherocytic hæmolytic anæmia differed in the properties of its catalytic site from the enzyme of normal subjects. We report in this

Table 1. COMPARISON OF THE PROPERTIES OF GLUCOSE-6-*P* DEHYDROGENASE OF NORMAL SUBJECTS AND AFFECTED MEMBERS OF THE B. FAMILY

Subjects	Enzyme-level*		Stability of G6*PD* in hæmolysates†	Specific activities*	Properties of the purified enzyme‡				
					Yield	Michaelis constants			Inhibitor constant for nicotinamide
	RBC	*WBC*				TPN	G6*P*	2DG6*P*	
	Units	Units	Initial activity (per cent)	Units	Per cent	$M \times 10^{-6}$	$M \times 10^{-5}$	$M \times 10^{-3}$	$M \times 10^{-2}$
Control subjects §	16·2 ± 2·4	44·4 ± 19·3	74 ± 12	3·2	41	3·2	3·5	3·0	2·1
B. Family ‖									
A. B. Propositus	7·0	41·0	66	1·3	37	36·0	9·0	5·1	2·0
Q. G. Maternal uncle	6·4	48·0	72	0·9	38	24·0	7·0	4·1	2·4
G. B. Sister	7·8	46·2	68	1·7	35	28·0	12·0	3·9	2·2
M. G. Mother	8·1	—	—	—	—	—	—	—	—
A. G. Maternal aunt	6·7	—	—	—	—	—	—	—	—

* A unit of enzyme activity is defined as the change of 1·00 optical density unit/min./gm. of hæmoglobin for erythrocytes (*RBC*) or per 10^9 cells for leucocytes (*WBC*) or per mgm. protein for purified enzyme.

† Stability of glucose-6-*P* dehydrogenase (G6*PD*) during incubation of hæmolysates was determined according to previously described procedures (ref. 8) and is expressed in terms of the per cent of the initial activity remaining after incubation for 1 hr. at 37°.

‡ These properties refer to the fraction prepared by the first technique. For detailed description of the procedures used to obtain these data see ref. 10. 2DG6*P* is 2-deoxyglucose-6-phosphate.

§ Values for enzyme-levels in red cells and white cells of control subjects represent the mean ± one standard deviation of 85 determinations. All other values are the mean of 7 determinations (ref. 7). For comparison with values indicated in the table, the following represent the average ± one standard deviation for red cell enzyme-levels in various groups of affected subjects: Negro males, 2·7 ± 1·5 units; Negro females, 6·9 ± 2·3 units; Caucasian males, 0·4 ± 0·4 units, and Caucasian females, 6·8 ± 3·2 units; and for white cell enzyme-levels: Negro males, 38·1 ± 11·6 units; Caucasian males, 14·0 ± 5·9 units; values for Caucasian and Negro females are within the normal range (ref. 7).

‖ For the B. family each value represents the average of at least two separate determinations.

article our investigations on the occurrence of glucose-6-*P* dehydrogenase deficiency in a Caucasian family of northern Italian origin in which the expression of the defect among males differs from previously observed affected male subjects. Furthermore, the enzyme purified from erythrocytes of three members of this family has characteristics of its catalytic site and electrophoretic mobility which are different from those of the enzymes of normal and other affected subjects studied.

In the course of studying the incidence of glucose-6-*P* dehydrogenase deficiency in northern Italy, a male, A. B., was found to have a moderate decrease in the level of the red cell enzyme comparable with that generally encountered among affected females and higher than that characteristic of affected males. Sex chromatin studies on leucocytes and buccal mucosa of this subject indicated a normal male pattern. In seven members of the family of A. B., blood was obtained for determination of red cell glucose-6-*P* dehydrogenase activity. The defect appeared to be inherited from the mother. The father and a paternal uncle had normal enzyme-levels. Moderately deficient levels of red-cell glucose-6-*P* dehydrogenase were present in the mother (M. G.), a maternal uncle (Q. G.), a maternal aunt (A. G.) and the sister (G. B.) of the propositus (Table 1). Another maternal aunt had normal enzyme-levels and there were no other siblings. While the findings in the B. family are compatible with sex-linkage as the mode inheritance of glucose-6-*P* dehydrogenase deficiency[1-6], the data are too meagre to permit any conclusions on this point. All subjects tested had normal values for hæmoglobin, red and white cells and reticulocytes and no history of anæmia.

The leucocyte glucose-6-*P* dehydrogenase activity was tested in the two affected males (A. B. and Q. G.) and one affected female (G. B.) and were within the normal range (Table 1). Previous investigations of leucocyte glucose-6-*P* dehydrogenase-levels have revealed a marked reduction among affected Caucasian males, slightly reduced to normal values among affected Negro males, and normal levels among affected females of both Negro and Caucasian origin[7].

The stability of glucose-6-*P* dehydrogenase activity during incubation of hæmolysates prepared from subjects A. B., G. B. and Q. G., was found to be comparable with that of subjects with normal levels of the enzyme (Table 1). No evidence was found for an activation of the enzyme on addition of stroma prepared from normal red cells. The methods used to test for stromal activation of glucose-6-*P* dehydrogenase were similar to those described by Ramot *et al.*[12].

Sufficient blood for purification of red cell glucose-6-*P* dehydrogenase was obtained from A. B., G. B. and Q. G. In addition, preparations of the enzyme purified from erythrocytes of normal and various other affected subjects were studied concomitantly. The method used in purification of glucose-6-*P* dehydrogenase has been described in detail[10], and yields an ammonium sulphate fraction which is a 300- to 500-fold purified preparation of the enzyme in a yield of 35–45 per cent of the initial activity (first technique). A further purification was achieved by treating this ammonium sulphate fraction with 'Sephadex', diethylaminoethyl cellulose (DEAE) column chromatography, and precipitating the enzyme from the eluate of the DEAE column with ammonium sulphate. These additional steps provide a preparation 3,000- to 5,000-fold purified in a yield of 15–20 per cent of the original enzyme activity (second technique). All the studies with purified glucose-6-*P* dehydrogenase were performed with the original ammonium sulphate preparation unless otherwise indicated. In the course of the preparation of glucose-6-*P* dehydrogenase from erythrocytes of A. B., G. B. and Q. G., no apparent differences from the enzyme of normal subjects were noted with respect to the fractionation of the enzyme at various stages in the purification procedure[10] (Table 1).

The enzyme preparations from A. B., G. B. and Q. G., have Michaelis constants for triphosphopyridine nucleotide (TPN), and for glucose-6-*P* (measured at *p*H 7·6 and *p*H 8·4) which are higher than normal (Table 1). The decreased affinity for TPN is more striking than that for glucose-6-*P*. The enzyme preparations from these subjects did not appear to be significantly altered in affinity for nicotinamide, a competitive inhibitor of TPN, or 2-deoxyglucose-6-*P*, which can serve as a substrate for the enzyme (Table 1). No differences from the normal enzyme were noted in the *p*H optima curves.

The electrophoretic mobility of the purified preparations of glucose-6-*P* dehydrogenase in starch gel, in 0·025 *M tris* buffer, *p*H 7·6 were studied using the methods described in detail elsewhere[10]. The electrophoretic mobility of the A. B. and Q. G. preparations were indistinguishable, and both migrated

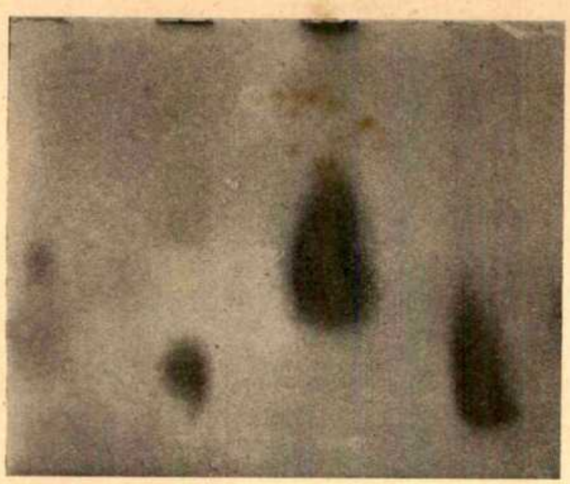

Fig. 1. Difference in electrophoretic mobility in starch-gel of glucose-6-*P* dehydrogenase of normal subjects (Nos. 1 and 5), and O.G. (No. 3) and A.B. (No. 7). The origin is at the top of the figure and migration proceeded towards the anode. The location of the dehydrogenase was identified by specifically staining for the enzyme (ref. 10). The larger size of spots Nos. 5 and 7 reflect a greater amount of enzyme applied

at a faster rate than the enzyme from normal subjects (Fig. 1). (The G. B. preparation of glucose-6-*P* dehydrogenase was not available in sufficient amount for the electrophoretic studies, and only the A. B. preparation was obtained in sufficient quantity to permit purification through to the second technique.) The rate of migration of the fraction from A. B., prepared by the second technique, was slower than that prepared by the first technique and approached, but was still faster than, that of the normal enzyme. The fractions of normal enzymes prepared by the two techniques had identical rates of electrophoretic mobility. Michaelis constants for TPN and glucose-6-*P* of the fractions of A. B. prepared by the two techniques were similar. There are several possibilities that could account for the faster rate of electrophoretic migration of the fractions of A. B. and Q. G., prepared by the first technique, including a difference in molecular weight or an alteration in net charge resulting from either a difference in amino-acid composition or an associated small charged molecule. No results have been obtained that permit conclusions on this point.

This study provides evidence for a variant of glucose-6-*P* dehydrogenase deficiency which differs from that observed in other subjects in respect to the level of the enzyme in affected males, and to the properties of the catalytic site and the electrophoretic mobility of the enzyme. These results suggest that glucose-6-*P* dehydrogenase deficiency in man is genetically heterogeneous and can be explained by one of several genetic mechanisms. Glucose-6-*P* dehydrogenase deficiency in the B. family might be due to a non-sex-linked gene and all the affected subjects studied are heterozygotes. Alternatively, the difference in the enzyme defect in the B. family and that of other affected subjects might reflect different mutations on the *X*-chromosome locus for glucose-6-*P* dehydrogenase or differences in genes which affect various quantitative aspects of the formation and function of this enzyme.

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AN ASSOCIATION BETWEEN SOIL CONDITIONS AND DENTAL CARIES IN RATS

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REPORTS have previously been given of a variation in prevalence of dental caries which occurred between children resident in the adjacent towns of Hastings and Napier in Hawke's Bay, New Zealand[1,2]. It was suggested that the lower caries in Napier children may have been related to the consumption of vegetables grown on the recent marine soils on which the city is largely situated. Detailed analyses have shown that notable differences occur in the mineral content, and particularly in the molybdenum content, of vegetables grown on Napier and Hastings soils, and these differences are such that it seems possible they could influence the caries susceptibility of local children.

To test this hypothesis, an experiment has been undertaken to determine the effect on prevalence of

Table 1. CARIES PREVALENCE IN RATS FED A CARIOGENIC OR CARIOGENIC ASH-SUPPLEMENTED DIET

Group	Diet fed	No. of animals	Mean body-weight (gm. per animal*)	Mean No. of carious lesions per animal	Mean caries severity score per animal
1	Control	17	265·1 (± 34·7)†	6·4 (± 4·5)†	16·6 (± 12·1)†
2	Control + Napier ash	20	293·7 (± 27·5)	3·5 (± 2·2)	7·4 (± 3·8)
3	Control + Hastings ash	20	308·0 (± 22·0)	5·3 (± 3·9)	13·9 (± 11·9)
4	Control + Hastings ash + molybdenum	20	303·2 (± 28·0)	4·5 (± 3·7)	9·5 (± 11·1)

* Age of animals, 124 days.
† Standard deviation.

Table 2. ANALYSES OF DIETS

Group	Diet	Ca	Mg	K	Na	P	Mo	Zn	Mn	B	Al	Ti	Cu	Ba	Sr	F
		(per cent)					(p.p.m.)									
1	Control	0·31	0·03	0·4	0·04	0·27	0·05	9	1·3	0·7	4	0·1	0·8	0·1	0·3	0·3
2	Control + Napier ash	0·37	0·06	0·7	0·04	0·34	1·60	14	2·2	2·5	25	1·0	2·0	0·4	1·6	0·3
3	Control + Hastings ash	0·37	0·06	0·7	0·04	0·33	0·20	14	3·5	2·5	18	0·6	2·5	0·4	1·9	0·2
4	Control + Hastings ash + molybdenum	0·38	0·07	0·7	0·04	0·34	1·35	14	3·9	2·6	18	0·6	2·0	0·4	1·8	0·2

Results on oven-dry basis

caries of the ash of vegetables grown on soils of Napier and Hastings when added as supplements to a cariogenic control diet fed to albino rats. The vegetable chosen was the French bean (*Phaseolus vulgaris*, var. The Prince) since this best reflected the characteristic differences between Napier and Hastings vegetables. Approximately a half-ton of French beans was grown on market gardens in Napier, and a similar quantity in Hastings. The beans (green pods with seeds) were dried at 100° C. and then ashed as required at 500° C.

Analysis of these beans confirmed the mineral differences previously found[2]. The molybdenum content of Napier beans was 12·3 p.p.m. (180 p.p.m. on ash basis) and of Hastings beans 1·0 p.p.m. (15 p.p.m. on ash basis).

The present experiment involved the comparison of prevalence of caries in four groups of albino rats (Wistar strain), each group initially containing 20 male animals. Diets were as follows:

Group 1: Control diet.
Group 2: Control diet 99 per cent, plus Napier bean ash 1 per cent.
Group 3: Control diet 99 per cent, plus Hastings bean ash 1 per cent.
Group 4: Control diet 99 per cent, plus Hastings bean ash 1 per cent, the molybdenum content of this ash having been increased to a concentration equivalent to that found in Napier bean ash.

The cariogenic control diet consisted of: sucrose, 68 per cent; casein, 25 per cent; salt mixture, 185 (ref. 3), 3 per cent; soybean oil, 3 per cent; and vitamin mixture, 1 per cent. The type of salt mixture used in the diet was selected because it contained no additions of minor elements.

The addition of molybdenum to the Hastings ash in Group 4 was made because this element gave the most pronounced difference in the Napier and Hastings ashes, and there is evidence which suggests that it may have a caries-reducing effect[4-6]. This additional molybdenum was added to the Hastings ash in the form of an aqueous solution of ammonium molybdate, the ash afterwards being re-dried.

The experimental animals in the four groups were born to mothers fed since mating the same diet afterwards fed to their offspring, because it was considered important that the groups of experimental animals should be exposed both pre- and post-natally to any mineral influences from the diet. Allocation of animals to groups had therefore to be made on the basis of the mothers rather than of the offspring. This was done by taking 40 virgin does and dividing them by the use of random numbers into four equal groups. Each group was placed in a galvanized cage with two bucks of known fertility and fed from the day of mating the appropriate experimental diet. For the second half of pregnancy each doe was isolated in a stainless steel breeding box with shredded paper added for nesting. Throughout the experiment the animals were given distilled water for drinking via stainless steel drinking tubes. Reproductive performance was good in all groups. The litters were weaned at 27 days, and at 31 days twenty male offspring were drawn from each of the four groups of mothers to form the various experimental groups. Twenty male offspring drawn from mothers fed the control diet only formed Group 1; twenty male offspring drawn from mothers fed the control diet plus the Napier bean ash formed Group 2, and so on. The selected offspring were transferred to individual polyethylene cages with stainless steel grids (Malthus, R. S., unpublished results) and each group was maintained on the appropriate diet for a further period of 12 weeks. Animals were weighed at weekly intervals. At 8 and 12 weeks after weaning examinations of the teeth of the animals were made using a mouth prop with ether anæsthesia. At 8 weeks no carious lesions were seen, but at 12 weeks all groups had developed lesions and the experiment was terminated.

The animals were killed with coal gas, the heads removed, autoclaved and cleaned of soft tissue. The jaws and teeth were stained by Keyes's method[7] and scored for caries by Davies's method[8]. The teeth were afterwards ground and caries scoring verified by a method corresponding to Carr's[9].

At the termination of the experiment it was found that the body-weights of animals in Group 1 receiving the control diet only were significantly lower than those in the other three groups receiving the ash-supplemented diets. This may have been due to the marginal levels of calcium and phosphorus in the control diet. During the experiment two animals in Group 1 died under ether anæsthesia, and one was found dead, the cause of death being unknown.

Table 1 shows the caries scores for the four groups of animals. It was found that animals receiving the control diet only (Group 1) had more caries than those in the other three groups receiving the ash-supplemented diets. The most interesting finding was that animals receiving the control diet supplemented with the Napier ash (Group 2) had significantly less caries than those receiving the control diet supplemented with Hastings ash (Group 3). It was found also that animals receiving the control diet supplemented with Hastings ash (Group 4), the molybdenum content of which had been increased, had less caries than those receiving the Hastings ash supplement alone (Group 3).

It will be noted from Table 1 that while caries prevalence was reduced in the animals receiving the supplement of Hastings ash plus molybdenum (Group 4), the caries score for these animals was somewhat higher than for those receiving the supplement of Napier ash (Group 2). The reason for this difference is not clear and may involve a number of factors including interactions between molybdenum and other elements.

Analyses of diets are shown in Table 2. As would be expected, the levels of various elements in the ash-supplemented diets are higher than in the control diet. No differences occur in the levels of major elements in diets supplemented with Napier or Hastings ash. Some differences occur in the levels of aluminium, titanium and manganese; but the most striking difference in micro-elements is still in the molybdenum content. As the molybdenum in the control diet is low, the molybdenum content of the diets supplemented with Napier or Hastings ash closely reflects the molybdenum differences originally shown for the beans.

The experiment has shown that the mineral ash of a vegetable grown on the marine soil at Napier reduces caries prevalence in rats, and that this effect is likely to be related to its relatively high content of molybdenum possibly in association with other elements. This result supports our hypothesis that the lower caries prevalence in Napier children as compared with Hastings children is the result of soil differences in these areas which are reflected in the mineral composition of vegetables.

This investigation was supported in part by a U.S. Public Health Service Grant (*D*965-C2). We also thank Glaxo Laboratories and Roche Products, Ltd., for their support.

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CROP YIELDS IN EXPERIMENTS AND ON FARMS

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WHEN attempts are made to assess the agricultural potential of new areas of agricultural land, or when a new crop is introduced into a region where agriculture is already practised, the results of experiments are often used to establish the general yield-level of the crop.

In any strict comparison between farm yields and experimental yields the following features must be common to both the experiment and the farm crop: (1) they must be grown in the same climatic region on similar soils in the same season; (2) the species and varieties of the crop must be the same; (3) cultivation and fertilizer practices should be similar.

As a preliminary investigation of the relationship between farm yields and experimental yields it was decided to select two dissimilar crops for which the relevant information was available, the extensively grown Victorian wheat crop and Queensland sugar cane, which is cultivated intensively.

In Victoria, wheat variety trials have been carried out for a number of years at Dookie and Longeronong Colleges on 1/40 of an acre plots, using standard farm cultural practices and quantities of phosphate fertilizers (Sims, H., private communication). The area of each variety of wheat grown on farms in the counties (Moria and Lowan) in which the trials are located can be obtained, and the results of the variety trials, weighted for variety, can be compared with the average yields of the farm crops in these counties over a period of eighteen years (Arnold, V. H., private communication).

In Queensland, the Bureau of Sugar Experiment Stations has carried out a large number of trials of different varieties of sugar cane for many years in plots located in farmers' fields in all major sugar-growing areas[1]. As the area of each variety and of plant and ratoon crop for each mill district are known, the experimental yields weighted for variety, and plant and ratoon crop areas can be compared with the average farm crop yield for the mill district in which the trial is situated over a period of six years for eight districts (Young, H. E., private communication).

When farm yields were plotted against experimental yields it was found that the data for both crops lay along a Mitscherlich curve of the formula :

$$y = A(1 - e^{-bx})$$

where y = farm yield, x = experimental yield, A = the maximum farm yield obtainable with infinitely high experimental yields.

The curves and formula for each crop are shown in Figs. 1 and 2.

The most striking feature displayed by this relationship is that in years of low yield, farm yields approach experimental yields more closely than in years when yields are high. This relationship applies to both crops, although wheat is a temperate annual and in Australia is cultivated on an extensive basis, while sugar cane is a tropical perennial, and in Queensland is intensively cultivated on relatively small farm allotments.

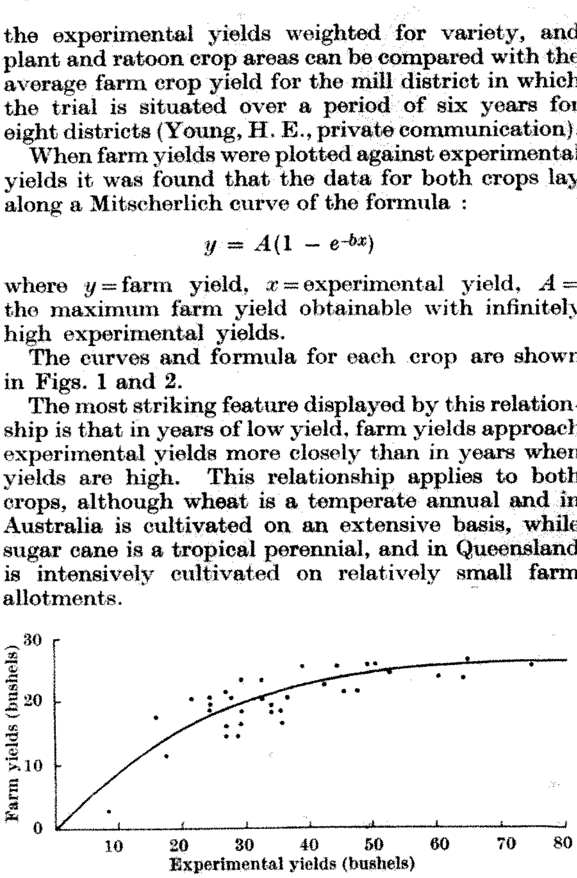

Fig. 1. Victorian wheat, 1940–59. $y = 26{\cdot}93\,(1-e^{-0.0444x}) \pm 2{\cdot}8$ bushels

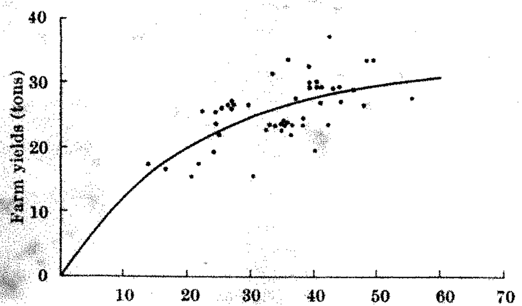

Fig. 2. Queensland sugar cane, 1954–59. $y = 32{\cdot}976\,(1-e^{-0{\cdot}0461x}) \pm 3{\cdot}8$ tons

Mean weighted farm yields as a percentage of experimental yields for the two crops were :

Victorian wheat	57
Queensland sugar cane	76

As climatic conditions, soil, fertilizer and cultural practices are the same in the experimental plots and on farms, the lower farm yield must be associated with the larger scale of farming operations or with the managerial ability of farmers. In an experiment, cultivation, sowing and harvesting are normally carried out at the correct time.

The managerial skill of farmers will vary over a wide range in any area; but even a skilled farmer, working a large area of land, is unable to carry out cultural operations over the whole area at the right time or with optimum efficiency. In favourable years the optimum timing and efficiency of operations for the experiment give exceedingly high yields which are not reflected on farms. In unfavourable years the correct timing of operations is less beneficial to the experiment because yield is largely determined by climatic conditions and experimental yields approximate to farm yields.

The exact proportion which mean farm yields form of the mean of experimental yields over a period of years will depend on the number of favourable and unfavourable years included in the period examined. The ratio will be high if there are many unfavourable years and low if there are many favourable years. In addition, the proportion is probably affected by the intensity of cropping. The smaller the area of a farm crop, the more closely experimental conditions and experimental yields are approached.

From the evidence obtained from these two crops it would seem unwise to assume that commercial crops will produce more than two-thirds of the mean yield of experiments using the same varieties, cultural practices and levels of fertilizer. Further work covering a wide range of crops and climatic conditions is necessary before a definite relationship between farm and experimental yields can be established.

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EFFECTS OF X-RAYS ON THE AGEING OF SEEDS

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It has long been known that ageing, storage at high temperatures or exposure to X-rays decreases the viability of seeds and increases the frequency of chromosome aberrations and mutations in the resulting plants[1–3]. Storage of seeds following irradiation augments the radiation effect[4–7]. The radiation effect is reduced if the seeds are stored at low temperatures[8]. Old seeds are more sensitive to X-ray than are fresh ones[9,10]. The following experiments were carried out to determine the nature of the ageing of seeds induced by irradiation.

The onion seeds used in the first tests were provided by the Charter Seed Co., from their 1960 crop. The seeds were divided into two lots. One lot was exposed to 1,000 r. of X-rays on February 15, 1961. The other lot was used as the control. Both lots were stored in an oven at 42° C. to hasten the ageing process. At intervals samples of 100 seeds of each lot were germinated on moist filter paper in a Petri dish. Root tips of the first seeds which germinated were fixed as soon as they reached the stage of the first nuclear divisions, since subsequent divisions have a lower frequency of chromosome aberrations due to the retardation or prevention of divisions in many of the progeny of cells with a high frequency of aberrations[11]. In most cases root tips were also taken from the seeds, which germinated the second day, to see if delay in germination is associated with a greater frequency of chromosome aberrations.

The root tips were fixed in alcohol acetic acid and stained in aceto-orcein. Anaphase figures were scored for chromosome bridges and acentric fragments. The early tests, based on an analysis of about 100 cells in each of four slides, gave rather uniform results as indicated by the standard errors. But as the seeds aged the variability increased with increasing aberration frequencies. The variance was also higher in the root tips of seeds which germinated the second day, due to the smaller number of division figures available —about 50 per slide. The results are presented in Table 1.

The artificial ageing did not increase the aberration frequency during the first three weeks, but with subsequent ageing the aberration frequency increased rapidly and at an accelerated rate in the control seeds. The aberration frequency in the X-rayed seeds seemed to increase earlier with ageing, but not so much as would be expected if the irradiation had simply aged the seeds to the level that the controls had reached

Table 1. CHROMOSOME ABERRATIONS PER 100 CELLS IN FIRST DIVISION OF ONION ROOT TIPS AT SUCCESSIVE DATES OF GERMINATION FOLLOWING IRRADIATION WITH 1,000 R. OF X-RAYS ON 2/15

*C*1 denotes control seeds which germinated first; *C*2 the control seeds which germinated the following day. *X*1 and *X*2 denote corresponding series of the X-rayed seeds

	Date of germination					
Series	2/20	3/6	3/14	4/4	4/11	4/27
*X*1	49 ± 6	56 ± 4	69 ± 18	104 ± 9	149 ± 12	200 ± 12
*C*1	10 ± 1	12 ± 2	10 ± 1	43 ± 3	85 ± 9	141 ± 11
D	39 ± 6	44 ± 4	59 ± 18	69 ± 9	64 ± 15	59 ± 17
*X*2		107 ± 25	100 ± 6	141 ± 14	190 ± 35	282 ± 27
*C*2		31 ± 16	46 ± 10	75 ± 5	85 ± 17	190 ± 30
D		76 ± 30	54 ± 12	66 ± 15	105 ± 38	92 ± 40

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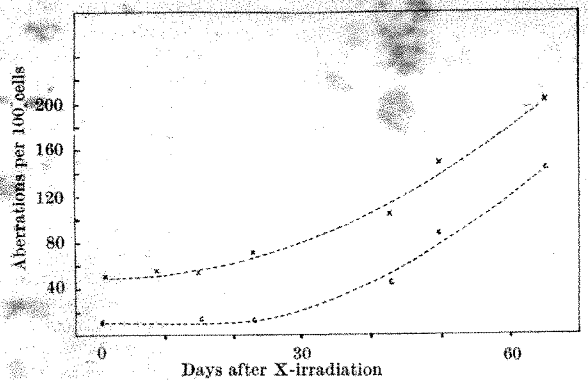

Fig. 1. The frequency of chromosome aberrations in root tips of control and X-rayed seeds germinated at intervals after storage in an oven at 42° C. to hasten ageing

after about six weeks of ageing. Although the differences between the aberration frequencies of the X-rayed seed and the control seed increases with ageing, the relative increase on the X-rayed seed with ageing is less than that of the controls. But when the aberration frequencies of the X-rayed and the control seeds are plotted against the duration of ageing the two curves are essentially parallel after the initiation of the increase in aberration frequency (Fig. 1). The same trends were found for the root tips fixed on the first day of germination ($X1$–$C1$) and those which germinated the following day ($X2$–$C2$), although variance in the latter was higher due to fewer division figures and higher aberration frequencies.

Although the individual differences are often of doubtful significance there is a consistent difference in the aberration frequencies of the root tips from seeds which germinated the first day and those which germinated the second day. This was true for the control ($C2$–$C1$) and the X-rayed ($X2$–$X1$) seeds. For each of the two series a t test showed that the differences are significant. Subsequent tests showed that further delay in germination was associated with an increasing aberration frequency. In another lot of seed tested the aberration frequency per 100 cells was 36 for the seeds which germinated the first day and 54, 59, 77 and 85 respectively for each of the following days. After the fifth day very few seeds germinated and the root tips had few division figures. Delayed germination of onion seed is associated with a higher frequency of chromosome aberrations.

Seed germination shows little correlation with the frequency of chromosome aberrations. There was no significant difference in the germination of the control and X-rayed seeds. During the 10 weeks of artificial ageing the aberration frequency of the control seeds increased from 10 to 141. Germination was 94 per cent at the start of the experiment and had dropped to 74 per cent at the end of the test. As Gustafsson[4] has shown, barley seeds exposed to a very high dose of radiation (75,000 r.) will still germinate even though every cell will contain many chromosome aberrations. Such seedlings soon die due to chromosome damage. At lower doses of irradiation the seedlings may continue to grow, but many are retarded in their development.

A second experiment was done with seeds of the onion variety Ailsa Craig, obtained from a local source. It was apparently older than the variety Silver Skin used in the first experiment, since the spontaneous chromosome aberration frequency exceeded 40 per 100 cells before the artificial ageing began. Although the aberration frequency was high and there were fewer division figures per slide, the results were very similar to those obtained in the first experiment. There was, however, no lag-time in the increase of aberrations with ageing. The control seeds had an aberration frequency of 35 per 100 cells at the beginning of the experiment, and had apparently already reached the stage of accelerated ageing when the test was started.

These results indicate that ageing of seed, as measured by the frequency of chromosome aberrations, is not a uniform process, but accelerates with time. The irradiation only increases the level of ageing. Lindop and Rotblat[12] find similar results with mice. Irradiation shortens the life-span in proportion to X-ray dose, but the survival curves of irradiated and control mice are parallel. The irradiated mice behave like the controls at an older age. Radiation simply adds a deleterious effect, but does not accelerate the subsequent rate of ageing.

Ageing of seeds, however, does appear to make seeds more sensitive to X-rays[9,10]. Similar differences have been found in mice by Sacher[13]. He found little difference in the sensitivity of mice to X-rays with increasing age between 100 and 600 days, but after the mice pass the age of 600 days the sensitivity to irradiation increased rapidly. Natural ageing also increases more rapidly towards the end of the normal life-span.

The differences between natural and X-ray induced ageing have been beautifully demonstrated by Clark and Rubin[14]. They found no difference in the life-span of diploid and haploid male wasps. When the adult wasps were given an X-ray dose of 50,000 r. the life-span was greatly reduced, but the diploids lived much longer than the haploids. Since somatic mitosis does not occur in the adult wasps the radiation damage cannot be attributed to chromosome loss at mitosis. The shorter life of the irradiated wasps is attributed to the induced deleterious mutations, which would be expected to be more lethal in the haploid than in the diploid. Since there is no difference in the life-span of the non-irradiated diploid and haploid wasps, natural ageing cannot be attributed to the accumulation of deleterious mutations or gross chromosome aberrations. Radiation induced ageing differs from natural ageing.

Since irradiation does not significantly decrease seed germination the ageing effect of X-rays differs from natural ageing. Chromosome aberration frequency is not highly correlated with seed germination, indicating that death of the dormant seed is not due to chromosome aberrations. Nor does the ageing of X-rayed seed increase the 'rate' of aberration any faster than ageing of non-irradiated seeds. These results would seem to support the conclusions of Harrison and McLeish[15] that chromosome aberrations are the result, not the cause, of ageing,

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LETTERS TO THE EDITOR

GEOPHYSICS

Dependence of the Ionospheric *F* Region on the Solar Cycle

THE general increase of ionospheric critical frequencies with sunspot number is well known, but the corresponding changes in ionospheric structure are not obvious from the critical frequency data alone and have not been extensively discussed in the literature. It is therefore interesting and instructive to examine a sample of ionospheric electron density distributions from different latitudes, seasons, and phases of the solar cycle.

Three stations—Huancayo, Peru; Washington, D.C. (Belvoir); and Anchorage, Alaska—were selected to represent low, medium, and high latitudes, respectively. Each of these stations has operated for more than one solar cycle. Winter and summer months were then selected, for which the mean Zurich sunspot numbers were, as nearly as possible, 0, 100 and 200. Within these months magnetically quiet days were selected for which noon f_0F-2 was as nearly as possible equal to median noon f_0F-2. Thus, it is hoped, these noon observations are representative of the solar, seasonal, diurnal, and latitudinal conditions from which they were selected.

The ionograms for these dates and times were converted to electron density profiles, using Budden's method[1]. Each profile was extrapolated above $h_{\max}$ *F*-2 by the process described by Wright[2], but with one important difference, as discussed in the following section.

Several workers (for example, Hirono[3]) have demonstrated that an ionospheric layer approaches an equilibrium shape under the combined influences of diffusion and attachment-like loss which is formally identical with the well-known Chapman distribution. Although electron production cannot be ignored in the daytime, it is still reasonable to expect that the *F*-2 peak and higher regions are strongly influenced by these processes, and that at the *F*-2 peak and above the quiet ionosphere approaches the Chapman form. To this extent, then, the characteristic thickness at the *F*-2 peak becomes a measure of the scale height and is usable for extrapolation of the profile above $h_{\max}$ *F*-2. Each of the profiles discussed here has accordingly been extrapolated above $h_{\max}$ *F*-2, using their individual thickness parameters. This differs from the constant scale height suggested earlier[2] as being appropriate to sunspot maximum conditions.

The result of this analysis is shown in Figs. 1–3. The most striking characteristic of all these profiles is that an increase in sunspot number adds additional ionization at greater heights, leaving the electron densities at lower heights comparatively unchanged. While the electron densities above the *F*-2 peak are the result of the extrapolation described here, it is important to note that they are more probably under-estimates than over-estimates, since the latter would imply a negative scale height gradient near the *F*-2 peak.

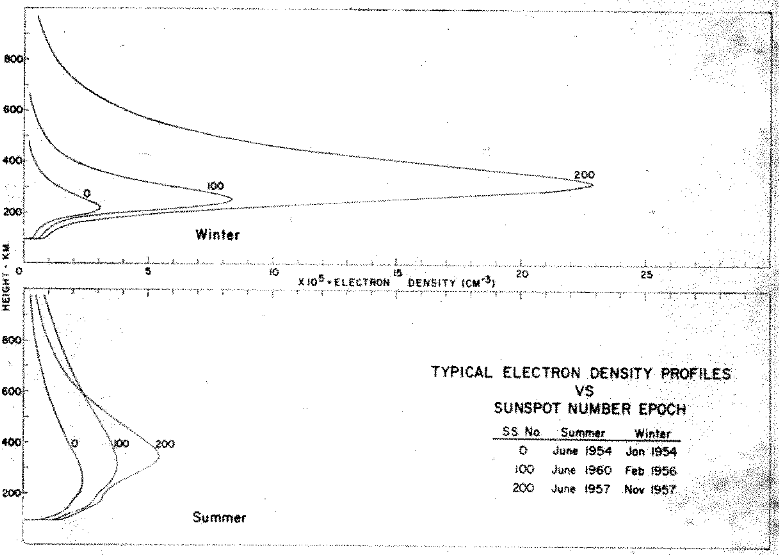

Fig. 1. Typical electron density profiles at three sunspot epochs for winter and summer noon conditions at Anchorage

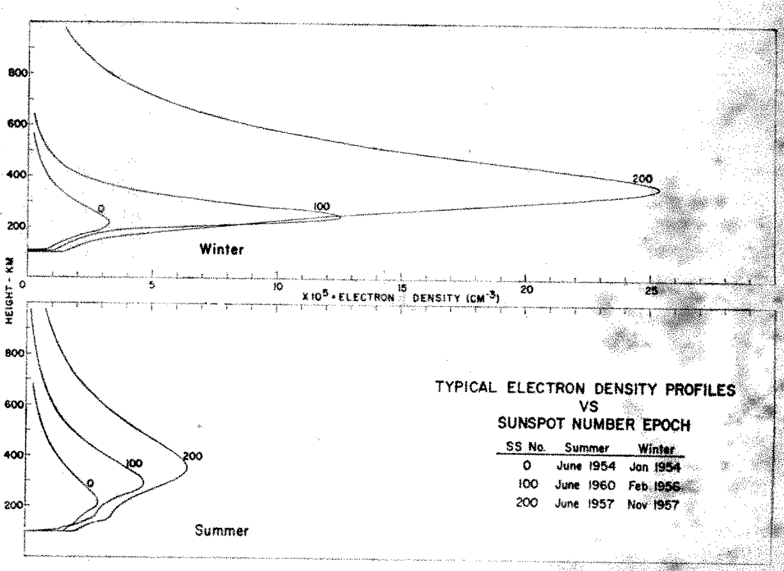

Fig. 2. Typical electron density profiles at three sunspot epochs for winter and summer noon conditions at Washington

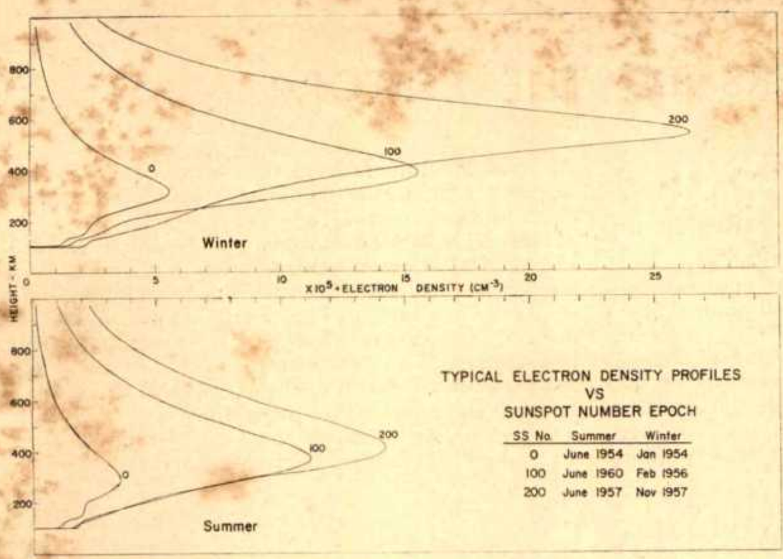

Fig. 3. Typical electron density profiles at three sunspot epochs for winter and summer noon conditions at Huancayo

The seasonal anomaly, by which the winter electron densities greatly exceed those in the summer, is very prominent at high sunspot number but is scarcely discernible during the solar minimum period. Elsewhere[4], it is shown that for sunspot maximum conditions and mid-latitudes, this seasonal anomaly is in fact a summer anomaly in the sense that the diurnal variation of summer electron content is anomalously low while the winter electron content follows a consistent 'Chapman' law over a wide range of latitudes. At Huancayo, the seasonal variation is doubly curious: since this station is slightly south of the equator, the 'winter' anomaly is more properly termed the 'December anomaly'[5]. It is present even at sunspot minimum.

A more extensive analysis of the sunspot cycle dependence of the ionosphere is being investigated in this laboratory.

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Direct Observation of Correlation between Auroræ and Hiss in Greenland

AT the Ionosphere Station in Godhavn, located at 69° 16′ N., 53° 30′ W. geog. and 79·9° N., 32·6° E. geomagnetic, routine recordings of very-low-frequency-phenomena for 2 min. every hour were made since the beginning of the International Geophysical Year in July 1957. In order to undertake a more detailed study of hiss, a selective amplifier and a pen recorder were added to the existing equipment in October 1960.

It is now possible to record noise continuously at selected frequencies. 825 c./s. and 8 kc./s. were chosen as measuring frequencies. The 8 kc./s. records were used for carrying out an investigation of the correlation between hiss and auroræ similar to that reported by Martin *et al.*[1].

Contrary to the findings from the 825 c./s. recordings, the 8 kc./s. results indicate a close correlation between strong noise and the occurrence of auroræ.

In order to examine this correlation more closely the apparatus was so constructed that one could listen to hiss through a loud-speaker and at the same time observe auroræ visually.

Observations carried out during January and February 1961 gave the following results: the 8 kc./s. hiss read on the pen recorder can be regarded as a steady background-hiss without greater diurnal variations. It has a shallow minimum at local noon and a maximum in the early night hours. Superimposed on this background noise there appear strong outbreaks lasting from a few minutes to about 1 hr. The hiss outbreaks are of an intermittent character,

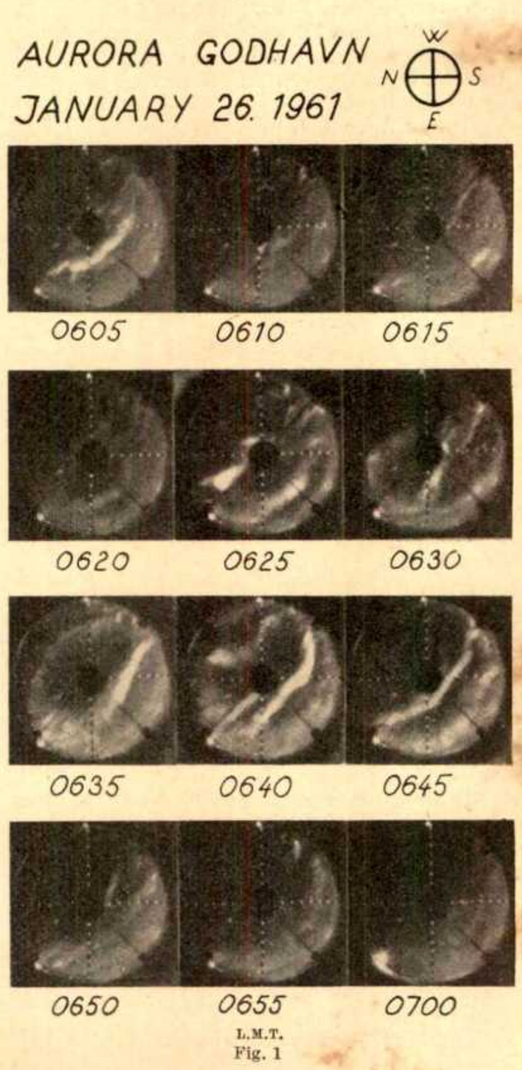

Fig. 1

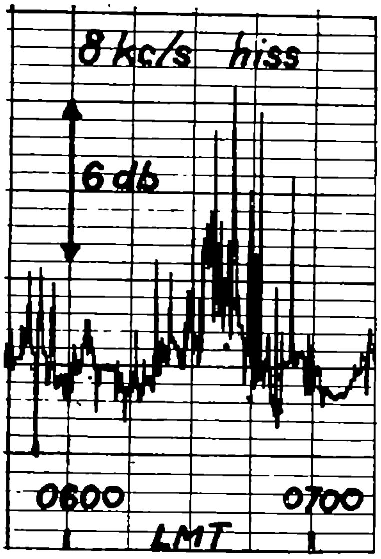

Fig. 2

sounding in a loudspeaker almost as the noise of a shower hitting a metal roof.

In the 30 cases of simultaneous hiss-listening and observations of auroræ there has been a clear correlation between the two phenomena. The strength of the hiss has shown second-to-second and minute-to-minute variations similar to those of the intensity of light in, and the activity of, the auroræ. Great activity of auroræ has never been observed without recording a strong hiss, and a strong hiss has never occurred when auroræ were missing.

Hiss was recorded both when the auroræ were low and high in the heavens, while ionospheric absorption and magnetic disturbance were recorded only when auroræ were present, near the zenith.

As an illustrative example, an 8 kc./s. hiss recording and all-sky-camera recordings of auroræ on January 26, 1961, are shown (Figs. 1 and 2).

We thank Mr. Knud Lassen for advice and inspiring discussions.

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PHYSICS

Law for the Composition of de Broglie's Velocities

The application of the special theory of relativity in physics, and especially in mechanics, is still very incomplete. The treatment of the various laws associated with different kinds of pendulums in different circumstances is of great importance, because—from a purely kinematic view—all kinds of clocks and watches are, theoretically speaking, equally affected 'through their movement'. However, from a physical point of view this is not proved. I am at the moment working on this problem and my full results will be published elsewhere. However, to test the point other relativistic collision laws were required. This has been my main field of investigation and has been outlined elsewhere[1]. So far when dealing with de Broglie's velocities, I have taken no consideration of any oscillators. In this communication I shall consider these enormous velocities.

Let us first consider three co-ordinate systems $S_1(x_1, t_1)$, $S_2(x_2, t_2)$ and $S_0(x_0, t_0)$, origins of which 0_1, 0_2 and 0_0 move with uniform velocities along a straight line. The system S_1 moves, with respect to the system S_2, with uniform velocity v_1 and the system S_2 moves, with respect to the system S_0, with uniform velocity v_2. Then the system S_1 will move, with respect to the system S_0, with uniform velocity v_0, and if we take Poincaré's fundamental law for the starting point, we have:

$$v_0 = \frac{v_1 + v_2}{1 + \frac{v_1 v_2}{c^2}} \tag{1}$$

where c is the uniform velocity of light[2]. If we suppose that at 0_1 there is an oscillator (particle) with a frequency ν_1, then it will be—considered from system S_2—accompanied by a plane series of waves. This series of waves vibrates with a phase-velocity u_1 (ref. 3). Similarly we find an oscillator (particle) with a frequency ν_2 at 0_2 which, considered from system S_0, is accompanied by a plane series of waves with a phase-velocity u_2. On the other hand, the oscillator (particle) with frequency ν_1 at 0_1—considered from system S_0—also appears accompanied by a plane series of waves with a phase-velocity u_0. It is known that:

$$u_i = \frac{c^2}{v_i}, \quad (i = 0, 1, 2) \tag{2}$$

Therefore from equation (1):

$$u_0 = \frac{c^2}{v_0} = c^2 \Bigg/ \frac{v_1 + v_2}{1 + \frac{v_1 v_2}{c^2}} = \frac{c^2 + v_1 v_2}{v_1 + v_2} \tag{3}$$

But

$$\frac{c^2 + v_1 v_2}{v_1 + v_2} = \frac{\frac{c^2 + v_1 v_2}{v_1 v_2}}{\frac{v_1 + v_2}{v_1 v_2}} = \frac{\frac{c^2}{v_1 v_2} + 1}{\frac{1}{v_2} + \frac{1}{v_1}} = \tag{4}$$

$$\frac{\frac{c^4}{v_1 v_2} + c^2}{\frac{c^2}{v_1} + \frac{c^2}{v_2}} = \frac{c^2 + u_1 u_2}{u_1 + u_2}$$

and from equation (3) we have:

$$u_0 = \frac{c^2 + u_1 u_2}{u_1 + u_2} = c^2 \Bigg/ \frac{u_1 + u_2}{1 + \frac{u_1 u_2}{c^2}} \tag{5a}$$

The relation (5*a*) is a new law for the 'composition' of de Broglie's velocities. This can be rewritten:

$$u_0 = \frac{u_1 + v_2}{1 + \frac{u_1 v_2}{c^2}} = \frac{\frac{c^2}{v_1} + v_2}{1 + \frac{v_2}{v_1}} = \tag{5b}$$

$$\frac{c^2 + v_1 v_2}{v_1 + v_2} = c^2 \Bigg/ \frac{v_1 + v_2}{1 + \frac{v_1 v_2}{c^2}} = \frac{c^2}{v_0}$$

On the other hand we have from equations (4) and (5):

$$\frac{u_1 + u_2}{1 + \frac{u_1 u_2}{c^2}} = \frac{v_1 + v_2}{1 + \frac{v_1 v_2}{c^2}} = v_0 \qquad (6)$$

but such an addition of velocities u_i ($i = 1,2$) would be nonsensical from the physical point of view—unlike the equations 5*a* and *b*.

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Does Matter have a Half-Life?

IN a recent book Prof. R. O. Kapp[1] has suggested that atoms of matter disappear spontaneously in a statistically random manner with a half-life of about 4×10^8 years and that it is this extinction of matter which causes gravitational attraction. It is the purpose of this communication to point out that there is no reason why the confirmation or disproof of this suggestion should not be established by means of existing techniques.

The gravitational constant G is usually reported[2] as $(6{\cdot}670 \pm 0{\cdot}005) \times 10^{-8}$ dyne. cm.^{2}gm.$^{-2}$ and it is not unreasonable to assume that the sensitivity of the apparatus used for the determination of G can be made somewhat better than the stated error of 5 parts in 6,000 and may well be 0·04 per cent or even less. It is difficult to estimate the speed with which such an apparatus would respond to a change of this magnitude, but it is not unlikely that a change of half-sec. duration or possibly even less would be detectable.

Assuming that G is determined by a Cavendish–Boys type of apparatus and that the weight of the attracting lead spheres is about 1,000 gm., the number of lead atoms involved would be $(1{,}000 \times 6{\cdot}023 \times 10^{23})/207{\cdot}2$ or about 3×10^{24}. Now, if the half-life of matter is taken as 4×10^8 years, the number of lead atoms which would cease to exist in every half-sec. interval must be about $(3 \times 10^{24} \times 0{\cdot}693)/(4 \times 10^8 \times 6 \times 10^7)$ or 1×10^8.

Assuming a normal Gaussian distribution it follows that there is 1 chance in every $1{\cdot}6 \times 10^4$ that the actual number of atoms which disappear in any given half-sec. interval will differ from this value by a detectable 0·04 per cent (which is 4 times the standard deviation)[3].

In other words, provided the deflexion of the beam is monitored continuously for a month at a time, the apparatus should spontaneously record a value of G which is either high or low for about 300 of the half-sec. intervals during such a one month period (which involves 5×10^6 half-sec. intervals). A control experiment would, of course, have to be carried out with an otherwise identical beam in the absence of lead weights.

The feasibility of monitoring the apparatus for a month on end may seem somewhat far-fetched, but R. H. Dicke *et al.* have successfully monitored their apparatus in a repeat of the Eotvos experiment for a period of several months[4].

Moreover, only a slight increase in the assumed sensitivity or the speed of response of the apparatus would produce a marked increase in the frequency of detectable deviations of G from the average value. It is even conceivable that the half-life of matter may be somewhat greater than the value suggested by Kapp and that the uncertainty in the currently accepted value of G may be partly due to statistical variations in the rate of disappearance of matter and not wholly the result of extraneous causes.

Provided an apparatus with sufficient sensitivity and speed of response can be constructed, it should thus be possible, by an analysis of the frequency and magnitude of such apparent variations in G, not only to verify that matter does have a half-life (if it in fact does disappear spontaneously) but to estimate the magnitude of such a half-life as well. The importance of a positive result for such an experiment on our understanding of gravitational attraction can scarcely be over-emphasized.

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DR. SWART makes an interesting suggestion which is theoretically sound. He shows himself aware of the three main practical difficulties, and it may be worth while to emphasize these for the benefit of anyone who might wish to translate the proposal into an experiment.

The first difficulty is that the Cavendish–Boys type of apparatus is at present not quite sensitive enough. One could perhaps hope to increase its sensitivity by the requisite factor of about 3; but this would only add to the second difficulty, which is that of securing a sufficiently quick response. Apparatus of this type measures in effect the time-integral of power; hence the well-known inverse ratio between sensitivity and speed of response. However, the technique of measurement has improved enormously since the year when Boys determined the value of G. It is not unreasonable to hope that Dr. Swart's proposal may soon become feasible, if it is not so already. Perhaps a technique using a differential method would be more promising than that used by Cavendish and Boys.

The third difficulty is the effect of Earth tremors which would shake the apparatus. Adequate isolation from these may not be possible; but Dr. Swart's proposed control experiment with an otherwise identical beam in the absence of lead weights might provide means of distinguishing between the effect of shaking the apparatus (noise) and the effect of fluctuations in the gravitational field.

The suggested test mass, namely, 1,000 gm. serves to illustrate the magnitudes entering into the problem. For an actual experiment, however, the optimum mass would have to be determined. This would, of course, be a function of the constants of the experimental apparatus.

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METALLURGY

An Anti-ferromagnetic Heusler Alloy, Cu_2MnSb

In the course of a comprehensive examination of the Heusler alloys it has been found that the alloy Cu_2MnSb, previously reported as ferromagnetic[1], shows some of the properties of an antiferromagnet. The alloy was prepared by melting at 1,000° C., homogenizing at 650° C. for 50 hr., and quenching in water. The composition of the specimen was $Cu_{2\cdot000}Mn_{0\cdot998}Sb_{1\cdot026} \pm 0\cdot001$. X-ray diffraction analysis showed the presence of Heusler structure similar to that of the alloy Cu_2MnAl, with a lattice parameter 6·097 ± 0·004 Å., which is intermediate between the values of 5·95 Å., and 6·20 Å. for Cu_2MnAl and Cu_2MnIn respectively, both of which are ferromagnetic.

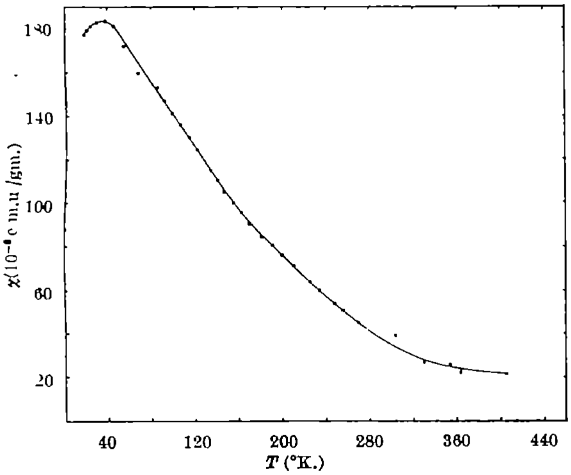

Fig. 1. Variation in magnetic susceptibility (χ) with temperature of Cu_2MnSb

No lines other than those attributable to the ordered Heusler phase were observed. The variation in magnetic susceptibility with temperature is shown in Fig. 1. Below 38° K., the susceptibility χ decreases with decreasing temperature, indicating the presence of antiferromagnetic order; above this (Néel) temperature the alloy is paramagnetic in that χ decreases with increasing temperature, but there is no indication that a Curie–Weiss law is followed within the range of temperature investigated. A specimen with slightly different composition showed the same characteristics.

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Crystal Structures of $ThRh_2$, ThRu and ThIr

An investigation of the constitution of alloys of thorium and the platinum metals is in progress, and the structures of five intermetallic compounds of the type Th_7X_3 have been reported previously[1]: the structure of Th_7Rh_3 has since been confirmed by Ferro and Rambaldi[2]. Dwight, Downey and Conner[3] have established that the compound $ThRh_3$ has the Cu_3Au structure ($L1_2$). This communication presents the structure of three new compounds $ThRh_2$, ThRu and ThIr.

Alloys were prepared by arc-melting 1-gm. samples of the component metals in a zirconium-gettered argon atmosphere. The alloys were brittle and powders for X-ray studies were prepared by crushing in air; powder patterns were obtained with a Guinier-type focusing camera using mono-chromatized copper radiation.

Phase relationships in thorium–rhodium alloys of 60–75 at. per cent rhodium are complex, compounds having been identified by metallographic and X-ray methods at approximately 63, 65, 66·6 and 75 at. per cent rhodium. The structure of $ThRh_3$ is given in par. 1 and X-ray patterns of the compounds at 63 and 66·6 at. per cent rhodium were complex and have not been indexed, but the film of the compound at 65 at. per cent rhodium was indexed on the basis of a hexagonal lattice $a = 4\cdot629 \pm 0\cdot002$, $c = 5\cdot849 \pm 0\cdot003$ Å. $c/a = 1\cdot264$. Systematic extinction of the $000l$ and $hh2\bar{h}l$ reflexions were noted when l was odd and of $hkil$ lines when $h-k = 3n$ and l was odd. The observed intensities showed excellent agreement with those for the Ni_2In structure ($B8_2$) space-group D^4_{6h} P $6_3/mmc$.

The $B8_2$ structure proposed for $ThRh_2$ is related to the $B8_1$ NiAs lattice by filling up the trigonal positions in the latter structure, the completely filled-up version having the composition A_2B. If the trigonal positions are incompletely filled it is possible for this structure to occur over a range of compositions or at the non-stoichiometric composition. The effect of temperature on the relationship between the complex structure in alloys of 66·6 at. per cent rhodium and the comparatively simple Ni_2In structure of 65 at. per cent rhodium has not yet been established.

ThRu and ThIr are formed directly from the melt: their X-ray patterns were indexed as orthorhombic with the following lattice parameters:

	'*a*' (Å.)	'*b*' (Å.)	'*c*' (Å.)
ThRu	3·878 ± 0·002	11·29 ± 0·01	4 071 ± 0·002
ThIr	3·894 ± 0·003	11·18 ± 0 01	4·266 ± 0·003

No hkl reflexions were observed unless $h+k$ was even and $h0l$ lines were present only when l was even. The lattice parameters and systematic extinctions suggested that ThRu and ThIr might be isostructural with ThCo (ref. 4) space group D^{17}_{2h} Cmcm. Line intensities calculated on the basis of this structure with $y_{Th} = 0\cdot14$ and $y_X = 0\cdot41$ where X is Ru or Ir gave good agreement with the observed intensities; other compounds reported to have this structure include ThAl (ref. 5), CeNi (ref. 6), PuNi (ref. 7) and DyGa (ref. 8).

I thank Prof. J. G. Ball under whose supervision this work was carried out; also the Atomic Energy Research Establishment, Harwell, for financial support and for allowing me to make use of some of their experimental facilities.

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RADIATION CHEMISTRY

Estimation of the Levels of Cæsium-137 in Sea-water by the Analysis of Marine Organisms

It is necessary to know the levels of cæsium-137 in sea-water which has been released by nuclear explosions or waste disposal, although the sampling of a large volume of sea-water, usually 100 litres or more, is not always easy or convenient.

Since the weight of marine organisms equivalent to the sea-water with respect to potassium or natural cæsium content is only one-thirtieth or less, the analysis of the former in place of the latter might supply convenient means of estimating the oceanic level of cæsium-137 if an equilibrium is assumed between them.

We have analysed various marine organisms collected in the vicinity of Japan for both natural cæsium-133 and radioactive cæsium-137; the former being analysed by a neutron activation method (to be described elsewhere) and the latter by a radiochemical separation followed by low-background β counting[1]. The results obtained are shown in Table 1.

Table 1. Estimates of Concentration of Cæsium-137 in Sea-water

Time of sampling	Locality	Organisms	^{137}Cs/^{133}Cs (× 10^8)	Estimate of ^{137}Cs in sea-water* (μμc./l.)
Dec. 1958	Suruga Bay	*Sergestes lucens* (shrimp)	2·5	1·3
March 1959	Sagami Bay	Diatoms	3 7	1 9
June 1959	Tokyo Bay	*Neomysis japonica* (zooplankton)	1·2	0 6
Aug. 1959	Sagami Bay	*Eisenia bicyclis* (brown seaweed)	1 6	0 8
Aug. 1959	Tokyo Bay	*Tapes japonica* (littleneck clam)	2·0	1 0
March 1960	W. Pacific	*Sardinops melanostica* (Japanese sardine)	0·8	0 4
March 1960	Japan Sea	*Euphausia pacifica* (zooplankton)	1·4	0·7
Oct. 1960	W. Pacific	*Cololabis saira* (Pacific saury)	3·3	1 7
Oct. 1960	W. Pacific	*Sphyraena* sp. (barracuda)	3·3	1 7
Oct 1960	W. Pacific	*Lateolabrax japonicus* (Japanese sea bass)	1·6	0 8
Oct. 1960	W. Pacific	*Trachurus japonicus* (jack mackerel)	1 5	0 8
Oct. 1961	W. Pacific	*Cololabis saira* (Pacific saury)	0·8	0·4
Oct. 1961	W. Pacific	*Sphyraena* sp. (barracuda)	1 0	0 5
Oct. 1961	W. Pacific	*Lateolabrax japonicus* (Japanese sea bass)	2·3	1 2
Oct. 1961	W. Pacific	*Trachurus japonicus* (jack mackerel)	0·7	0 4

* On the basis of the concentration of cæsium-133 in sea-water being 0 5 μgm./l.

When an equilibrium has been attained between sea-water and marine organisms, the ratio of cæsium-137/cæsium-133 in the former should be equal to that in the latter. On the basis of the concentration of cæsium-133 in sea-water being 0·5 μgm./l., as given by Smales and Salmon for the Atlantic[2], and assuming this value is also valid for the Pacific and coastal waters, we have estimated the levels of cæsium-137 in sea-waters as given in the last column of Table 1.

The estimate indicates somewhat higher values for coastal waters in 1959 than those given by one of us previously for 14 samples of surface waters, namely, 0·38 μμc./kgm. on the average[3]. The estimate in the Western Pacific in October 1961 from 4 species of fish, 0·6 μμc./l. on the average, is approximately half that in the same month of the preceding year, 1·3 μμc./l. These values are much higher than those in the Atlantic or the Eastern Pacific, as already mentioned by Miyake *et al.*[4].

It is interesting to note that the concentration of cæsium-133 is fairly constant, as shown in Table 2, in several fish species which are popularly consumed in Japan. Presumably, this will be caused by the homogeneous distribution of cæsium-133 in the ocean and the biological insignificance of the chemical element. The standard deviation of the cæsium-133 content in different species around the mean value of 21 μgm./kgm. is ± 12 per cent. Accordingly, the analysis of cæsium-133 will not be necessary in the rough estimation of the levels of cæsium-137 in sea-water so far as those fishes are used as monitor.

Table 2. Cæsium-133 Content of Some Fishes

Fish	Cæsium-133 μgm./fresh kgm. (whole body)
Cololabis saira (Pacific saury)	19
Sphyraena sp. (barracuda)	22
Lateolabrax japonicus (Japanese sea bass)	19
Trachurus japonicus (jack mackerel)	25
A kind of flatfish*	21
Average	21 ± 2·5

* Japanese name, 'karei'.

Thus, it may be concluded that the estimation of the levels of cæsium-137 in sea-water can conveniently be made by analysing approximately 100 gm. fresh weight of the whole body of some kind of fish for cæsium-137, although it should be kept in mind that the estimate does represent the concentration of cæsium-137 in sea-water of not a fixed point but an average in space, depth and time, reflecting the life-history of an organism.

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'Hot'-Atom Chemistry of Radiocobalt in Hexamminecobalt(III) Hexacyanocobaltate(III) and Hexamminecobalt(III) Hexacyanoferrate(III)

Behaviour of cobalt-60 recoils arising from thermal neutron capture and isomeric transition on cobalt atoms in either hexamminecobalt(III) complex salts or hexacyanocobaltate(III) complex salts has already been investigated[1-6]. However, the system which contains both NH_3 and CN groups together has not yet been investigated. In order to elucidate the role of CN groups in recoil reactions, we selected, as such a system, two complex salts of the formula $[Co(NH_3)_6][M(CN)_6]$, where M = Co or Fe, since no hexamminecobalt(III) cyanide is known at present.

Solid complex salts $[Co(NH_3)_6][Co(CN)_6]$ and $[Co(NH_3)_6][Fe(CN)_6]$, were prepared by the methods described in literature and were subjected to neutron irradiation in an experimental hole in the *JRR*-1 reactor for 5–6 hr. at pile temperature. Thermal neutron flux was approximately 3 × 10^{11} n./cm^2. sec. After varied storage time following the irradiation the irradiated salts were dissolved in 0·3 M hydrochloric acid and species labelled with cobalt-60 were separated by procedures involving ion exchange, paper electrophoresis, recrystallization, or a combination of these. Essentially the same distribution of

Table 1. PERCENTAGE DISTRIBUTION OF ^{60}Co-LABELLED SPECIES AFTER THE DISSOLUTION OF IRRADIATED $[Co(NH_3)_6][Co(CN)_6]$ AND $[Co(NH_3)_6][Fe(CN)_6]$

Target	Time of storage	^{60}Co-labelled species (per cent)*							
		CoA_6^{3+}	CoA_5X^{2+}	$CoA_4X_2^+$	Neutral species	$CoA_2X_4^-$	$CoAX_5^{2-}$	CoX_6^{3-}	Co^{2+}
$[Co(NH_3)_6][Co(CN)_6]$	1 day	13·4	2·9	2·4	3·5	2·8	3·9	7·1	63·5
	3 months	20·2	2·3	0·6	2·0	1·4	4·1	8·9	58·8
	4·5 months	25·1	3·1	0·8	1·5	2·1	5·1	8·3	52·6
$[Co(NH_3)_6][Fe(CN)_6]$†	1 day	8·1	10·4	2·6	7·7	4·1	4·7	8·7	50·9
	2·5 months	20·3	3·8	1·0	6·8	4·0	5·1	8·7	41·3
	4 months	22·7	5·3	1·7	8·5	4·1	3·2	9·2	38·1

* A denotes an NH_3 group and X a CN group in most cases. However, it is also possible that some of A's may be water and some of X's may be chlorine or other anions present in the solution.

† Summation of percentage does not amount to 100 because of the presence of unidentified species (3–6 per cent)

labelled species was obtained even if different separation procedures were used.

The results are shown in Table 1. Major components labelled with cobalt-60 in the solution of the irradiated salt were CoA_6^{3+}, Co^{2+}, and CoX_6^{3-} (A = mostly NH_3, X = mostly CN). On the storage of the irradiated salts at room temperature, CoA_6^{3+} was increased, Co^{2+} was decreased and CoX_6^{3-} remained almost unchanged. It is worth while to mention that appreciable amounts of ^{60}Co-labelled cyanocobaltate ions were found in the solution of irradiated $[Co(NH_3)_6][Fe(CN)_6]$. The distribution was not altered at all when the irradiated salt was dissolved in 0·3 M hydrochloric acid containing macroscopic quantity of Co^{2+} beforehand. It should also be mentioned that the distribution of ^{60}Co-labelled species in $[Co(NH_3)_6][Fe(CN)_6]$ was essentially similar to that in $[Co(NH_3)_6][Co(CN)_6]$. The latter salt contains cobalt atoms in both cationic and anionic components, whereas the former salt contains cobalt only in cationic component. Therefore, comparison of distributions of species labelled with cobalt-60 in these salts might afford clues for elucidation of the nature of recoil reactions, especially, for estimation of the size of 'hot' zone involved in such reactions.

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CHEMISTRY

Distribution Patterns of Isotopic Peaks in the Mass Spectrum of Stannic Bromide

ONE of the problems in high-molecular-weight mass spectrometry is to establish unambiguously the definite mass to be assigned to the fragmentation peaks in the higher mass (200–800) range. A suitable calibrating material is stannic bromide. It has a vapour pressure at 25° C. of a little less than 1 torr, and from its elementary isotopic constitution the relative peak heights can be calculated for the molecular parent ion and its fragments.

For simplicity the abundance fraction of bromine-79 and bromine-81 can be taken each as 0·50, and the lower abundance isotopes tin-112, tin-114 and tin-115 omitted from the calculation. The fractional abundance of the tin isotopes 116, 117, 118, 119, 120, 122 and 124 were taken as: 0·143, 0·077, 0·238, 0·087, 0·328, 0·047 and 0·060, respectively[1].

In the mass spectrometer there appear the ions $SnBr_4$, $SnBr_3$, $SnBr_2$, SnBr, Sn and Br, in the approximate relative intensities 42, 100, 24, 68, 24 and 29 respectively. These relative intensities cannot be calculated, but in a given ion, the complex isotopic peaks which constitute it can be.

This is done as has been shown for other materials[2,3]. If the fractional bromine-79 and bromine-81 abundances are taken as a and b, the ion group peak heights for a single tin isotope of abundance S is given by the distribution equation $S(a + b)^n$, where n takes the values 4–1 for the various bromine-containing ions. This is calculated and weighted for each tin isotope

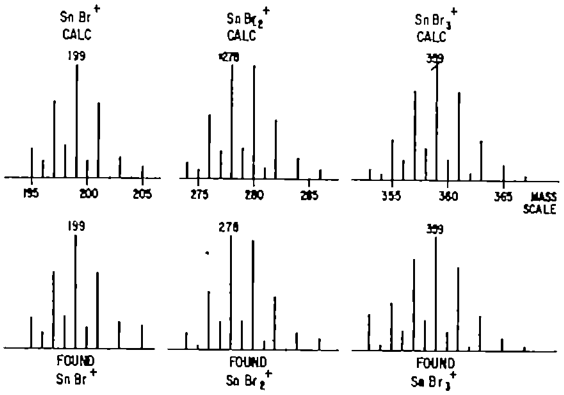

Fig. 1. Isotopic peak distribution for ion fragments of stannic bromide

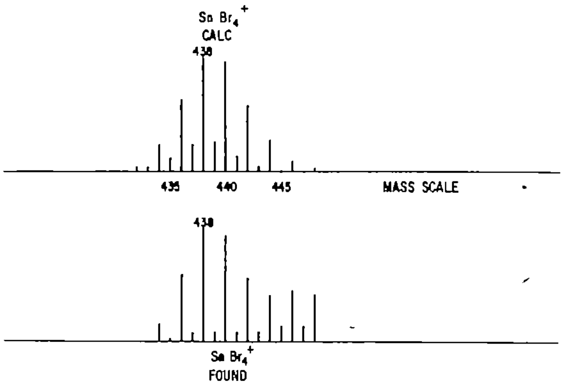

Fig. 2. Isotopic peak distribution for molecular ion $SnBr_4^+$

and the totals summed for each mass in the group. The distributions calculated and found are shown in the graphs of Figs. 1 and 2. Particularly useful are the maximum peak heights at 440, 438, 359, 280, 278. The peak 199 appears among the mercury isotopes. The main discrepancy between the distributions calculated and found just above mass 444 is probably due to an impurity.

Some of the peaks are quite complex. For example, the highest peak of mass 359 in the $SnBr_3$ ion is made up of: $^{116}Sn^{81}Br_3$, $^{118}SnBr_2{}^{79}Br$, $^{120}Sn^{81}Br^{79}Br_2$ and $^{122}Sn^{79}Br_3$.

If pure, stannic iodide, even though less volatile, would furnish excellent mass markers. Since iodine is monoisotopic, all the ions would exhibit the tin isotopic distribution pattern, with masses of the highest peak in each group at 628, 501, 374 and 247. These are for the iodine containing ions formed with tin-120. However, any slight bromine contamination in the iodide yields a great number of mixed iodide-bromide peaks, due to the large number of such combinations possible.

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Absorption Spectra of the D-State Ions $TiCl_6^{-3}$, $TiCl_4^-$, $CrCl_4^{-2}$, $FeCl_4^{-2}$ and $CuCl_4^{-2}$ in Chloride Melts

THE absorption spectra of dilute solutions of the ions $TiCl_6^{-3}$, $TiCl_4^-$, $CrCl_4^{-2}$, $FeCl_4^{-2}$ and $CuCl_4^{-2}$ in chloride melts have been obtained. Of particular interest is the magnitude of *Dq*, the crystal field splitting parameter, deduced from the locations of the near infra-red absorption bands. In the present communication, a detailed discussion of the spectra and their interpretation in terms of distortions of the complexes will not be given. The salient results of the investigation are, however, listed in Table 1, this constituting the first report of the tetrahedral spectra of a number of these ions.

Table 1. ABSORPTION SPECTRA OF *D*-STATE IONS IN CHLORIDE MELTS*

Ion	Electronic configuration	Energy at absorption maxima (cm.⁻¹)	Width at half-maxima (cm.⁻¹)	Transition	*Dq* (cm.⁻¹)
$TiCl_6^{-3}$	d^1 ^2D	10,000 13,000	4,000 3,500	T → E	~1,150
$TiCl_4^-$	d^1 ^2D	8,000 6,000	3,500 3,000	E → T	~700
$CrCl_4^{-2}$	d^4 ^5D	9,800	4,600	T → E	980
$FeCl_4^{-2}$	d^6 ^5D	5,100	3,100	E → T	510
$CuCl_4^{-2}$	d^9 ^2D	9,500	3,400	T → E	950

* The solvent was LiCl–KCl eutectic and the temperature 400° C. in all cases except $TiCl_4^-$ where the solvent was $CsGaCl_4$ and the temperature 750° C.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

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Absorption and Afterglow Spectra of Selenium Dioxide

IN a report now in the press[1] we have attempted to interpret the continuous emission and absorption spectra of sulphur dioxide by assuming a photo-dissociation and recombination process.

In this report, we extend the examination to the selenium dioxide molecule, which has an electronic configuration similar to that of sulphur dioxide (and also tellurium dioxide). As with the other diatomic oxides, the energy of the excited levels decreases as one goes to heavier molecules: that is to say, from sulphur dioxide to selenium dioxide and then to tellurium dioxide. One would expect a corresponding shift in the respective continuous and discrete band spectra.

In studying this molecule, we used the same photometric method as for sulphur dioxide. To stabilize the concentration of selenium dioxide, we connected two furnaces (F_1 and F_2) so that F_1 had the solid oxide contained in a side tube while the other, F_2, operating at a higher temperature, contained the absorption vessel connected to the small tube in F_1

The range of temperature in the F_1 furnace was quite limited because at less than 250° C. the vapour pressure was too low for adequate absorption measurements, while, near 300° C., the absorption was almost complete in the region between 2600–3000 Å. This use of two furnaces allowed the investigation of the influence of the temperature on the absorption spectra.

The absorption band spectrum has been examined by Evans[2], Choong-Shin-Piaw[3] and Duchesne and Rosen[4]. Most of the bands have been classified in three distinct groups, corresponding to the spectral regions: (*A*) 2070–2210 Å.; (*B*) 2210–3300 Å.; (*C*) 3400–3800 Å.

As in the case of sulphur dioxide, raising the temperature has a double effect: (1) the rotation structure of the individual bands is made to disappear progressively into a continuous absorption spectrum; (2) a new continuous spectrum appears which is associated with the increase of the total absorption.

It is this last absorption which was measured. For a given temperature in the F_1 furnace—that is to say, for a given value of pressure—we measured the optical density $d(T_1)$ and $d(T_2)$ corresponding to the two different temperatures of the furnaces F_1 and F_2.

In the regions where there was no new absorption, $\frac{d(T_2)}{d(T_1)} \approx 1$. This was indeed the case for the bands located in the 2500–3000 Å. spectral region. In other spectral regions, the excess density:

$$d_c = d(T_2) - d(T_1) \quad T_2 > T_1$$

was arbitrarily assumed to be equal to that of the continuous absorption.

Fig. 1 shows our results for $T_1 = 250°$ C. and $T_2 = 370°$ C. With rising temperature, two regions of continuous absorption appear: one between 3000–3350 Å. and the other less than 2600 Å. The maximum absorption cross-section was of 8×10^{-21} cm.² at 250° C.

As is well known, for diatomic molecules, the absorption spectrum may be continuous in one spectral region and discrete in another when both the electronic states of the transition are stable. The absorption is entirely continuous, or shows broad intensity fluctuations, when one of the potential curves is of a repulsive type. In the first case, the

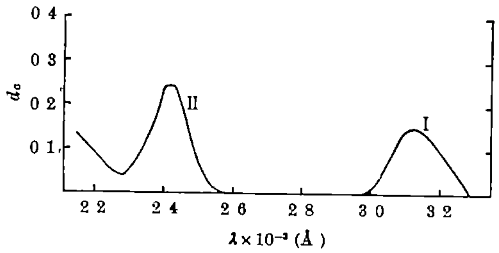

Fig. 1. Optical density d_c of the continuous spectrum obtained by the subtraction of the optical density at 250° C. from that at 370° C.

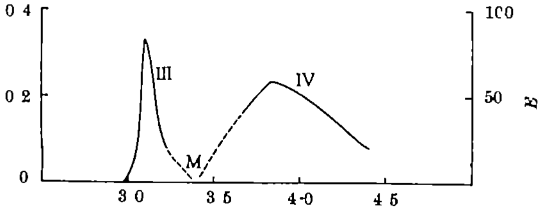

Fig. 2. Comparison between the intensity distribution E of the continuous afterglow spectrum and the optical density d_c of the continuous absorption spectrum. The wave-length of the point M, obtained by extrapolation, corresponds to the dissociation energy of selenium dioxide into selenium monoxide and oxygen. Curve III: The scale on the left represents the optical density of selenium dioxide at 360° C. (pressure = 250 mm. mercury). Curve IV. The scale on the right gives the distribution of relative intensity of the new afterglow spectrum of selenium dioxide

continuous absorption is located on the shorter wave-length side of the band spectrum. One can wonder whether the continuous absorptions shown in Fig. 1 are associated with the band systems (A, B, C) mentioned above. If that were the case, the continuous band I of Fig. 1 would correspond to C system and the continuous band II to B system.

If the upper electronic state of the transition giving the continuous absorption were unstable and the lower state stable (fundamental level), one would expect to have a continuous emission located on the longer wave-lengths side of the continuous absorption. This would be the result of a recombination process transforming selenium and oxygen into selenium dioxide with radiation.

Continuous Spectra in the Afterglow. To choose between the two possible interpretations, we sought to excite and photograph the afterglow spectrum of selenium dioxide. The excitation was produced by a 50-cycle high-frequency pulse generator and the observation made with the aid of a synchronous rotating disk. Under these conditions, we observed a continuous afterglow spectrum between 3400 Å. and 4900 Å. The intensity is considerably weaker and less extensive than the continuous afterglow spectrum of sulphur dioxide (under similar conditions). Fig. 2 shows curve III of continuous absorption at 360° C. and 250 mm. mercury pressure, and alongside curve IV, which gives, in arbitrary units, the relative intensity distribution, E, of the continuous afterglow spectrum. It is seen that the relative position of these two curves, III and IV, is much like those of sulphur dioxide, with the exception that the spectral region is much more restricted. The common spectral limit of the two curves near 3400 Å. corresponds to the dissociation energy of 29,400 cm.$^{-1}$ = 3·64 eV. This value seems likely as the dissociation energy of selenium dioxide into selenium monoxide and oxygen.

It seems that we have to conclude that the continuous absorption and emission of Fig. 2 are due to the two inverse processes of molecular dissociation and recombination:

$$SeO_2 + h\nu \longrightarrow SeO + O \text{ and}$$
$$SeO + O \longrightarrow SeO_2 + h\nu$$

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Silver/Silver Oxide Electrodes for Fuel Cells

Many laboratories active in the field of fuel cell research have adopted the silver/silver oxide electrode as the oxygen electrode in conjunction with strong alkali (generally 20–40 per cent potassium hydroxide) electrolyte. There have been many investigations of the fundamental operating characteristics of such an electrode under current drain conditions, and various theories have been advanced to account for its behaviour. Although various polarization mechanisms have been considered, as in the work of Dirkse[1], it is remarkable that the significant solubility of silver in strong potassium hydroxide has been systematically ignored. This solubility is adequately recorded in the work of Mathur and Dahr[2], and of Johnston, Cuta and Garrett[3] for room-temperature operation under quiescent conditions and a synopsis of these authors' results is given in Table 1.

Table 1. Solubility of Silver/Silver Oxide in Potassium Hydroxide at 25° C.

Normality	Mathur and Dahr gm. Ag/l		gm. equiv. Ag_2O/l. × 10^{-4}	Johnston Cuta and Garrett gm equiv. Ag_2O/l × 10^{-4}
0·002	0·0093	—	0·87	—
0·001	0·0016	—	0·15	—
0·0012	—	0·00355	—	0·329
0·01	0·0010	—	0·10	0·076
0·02	—	0·00067	—	0·062
0·05	—	0·0012	—	0·110
0·10	0·0032	—	0·30	—
0·117	—	0·0028	—	0·26
0·40	—	0·0216	—	2·00
1·03	0·0162	—	1·50	—
2·26	0·0309	—	2·86	—
2·33	—	0·040	—	3·70
5·20	0·0457	—	4·23	—
6·60	—	0·0583	—	5·40

Although the results of Johnston *et al.* are somewhat higher, they are in reasonable agreement and confirm a minimum solubility at about 0·02 normal with solubility rising sharply in strong potassium hydroxide solutions such as used in typical fuel cells.

No direct information is available for higher temperatures in strong caustic, but a factor of four obtains for dilute solutions. Using this factor, a solubility in the range 0·18–0·25 gm./l. of silver as a complex anion can be anticipated for fuel cells operating in the temperature-range 60–80° C.

This solubility of silver is fully adequate to account for the polarization effects observed, and, in experiments in this laboratory on hydrox and methanol cells, a silver transport as high as 20 mgm./cm./hr. for a fuel cell under load has been confirmed by spectrographic, X-ray fluorescent and chemical analyses. It is, however, very probable that the quiescent solubility of silver in potassium hydroxide will be greatly exceeded in fuel cells operating at high current density. All previous data should, therefore, be reviewed in the light of this information, which should afford a better understanding of the electrochemistry of fuel cells.

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Intermolecular Energy of Benzene-Cyclohexane

Bottomley and Coopes[1] have recently measured the second virial coefficients of mixtures of benzene and cyclohexane vapours. They derive the values of B_{12}, the virial coefficient for the interaction of one molecule of benzene (1) with one of cyclohexane (2), at 35, 50 and 70° C. They say that these values do not confirm the deduction from measurements of the critical temperatures of these mixtures[2] that the characteristic energy of interaction ε_{12} is smaller than both ε_{11} and ε_{22}. This conclusion appears to be unwarranted and disguises the fact that their measurements of B_{12} are in much better accord with the measurements of the critical temperature than were either of the two earlier measurements of B_{12}.

It is impossible to deduce accurately even the relative sizes of the energies from the virial coefficients since benzene and cyclohexane do not conform exactly to the same reduced equation of state. This is shown in Fig. 1, where B/V^c is plotted as a function of T/T^c (benzene, V_1^c = 260 cm.^{3} mole^{-1}, T_1^c = 561·9° K., cyclohexane, V_2^c = 308 cm.^{3} mole^{-1}, T_2^c = 553·4° K.)[2,3]. The two curves are not coincident. An estimate of the hypothetical critical temperature T_{12}^c (and so of the relative size of ε_{12}) can be made from B_{12}, as follows. Choose a value of V_{12}^c from the familiar equation:

$$(V_{12}^c)^{1/3} = \tfrac{1}{2}(V_1^c)^{1/3} + \tfrac{1}{2}(V_2^c)^{1/3}$$

calculate (B_{12}/V_{12}^c) at each point, and find what value of T_{12}^c is needed for the graph of (B_{12}/V_{12}^c) as a function of (T/T_{12}^c) to fall between the lines for benzene and cyclohexane. This temperature is 550° K., as is shown in Fig. 1. The variation permitted in this value of T_{12}^c is ± 4° K. (subject to the correctness of B_{12} and V_{12}^c) if the points for (B_{12}/V_{12}^c) are not to lie outside those for benzene and cyclohexane. This mean value of T_{12}^c is well below the arithmetic means of T_1^c and T_2^c (557·7° K.), and, in fact, even below T_2^c. It compares very well with the value determined directly from the critical temperatures[2], namely, 548·9° K.

The extent of the agreement between our results and the new values of B_{12}, and the extent of the disagreement with earlier values of B_{12} is shown by tabulating the difference $(T_{12}^c - \frac{1}{2}T_1^c - \frac{1}{2}T_2^c)$, which is a measure of the amount by which ε_{12} falls below the arithmetic mean of ε_{11} and ε_{22} (Table 1).

Table 1

Measurements	$(T_{12}^c - \frac{1}{2}T_1^c - \frac{1}{2}T_2^c)$ deg.
Critical temperatures (ref. 2)	−8·9
B_{12} (Waelbroeck, ref. 4)	−0 5
B_{12} (Cox and Stubley, ref. 2)	>0
B_{12} (Bottomley and Coopes, ref 1)	−8 ± 4

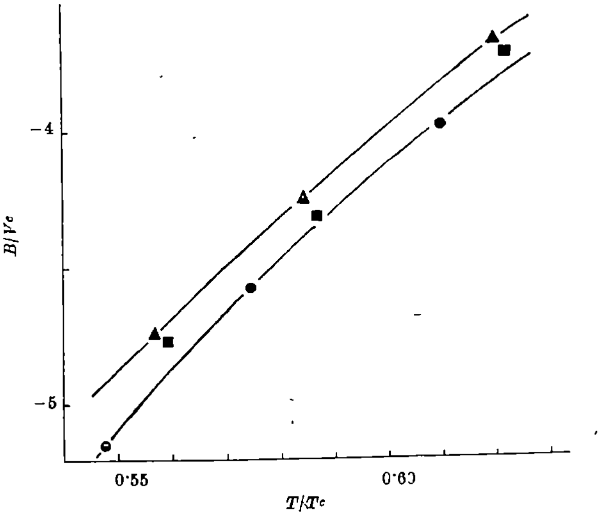

Fig 1. Reduced second virial coefficients for benzene (circles) and cyclohexane (triangles). The squares are (B_{12}/V_{12}^c) for a reduced temperature determined by T_{12}^c = 550° K.

Confirmation of the relative weakness of ε_{12} for aliphatic-aromatic interactions is provided both by the excess thermodynamic functions of the liquid mixtures[5] and by measurements of the solubility of vapours of volatile hydrocarbons in solvents of high molecular weight[6]. However, none of these results can show whether the relative weakness of ε_{12} is caused by a real weakness of the 1–2 forces or by an unusual strength of the 1–1 forces.

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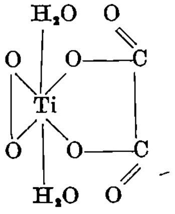
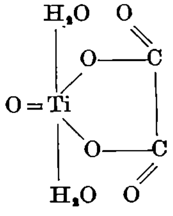
Infra-red Absorption Bands of Co-ordinated Water in Titanium Complexes

ORANGE red and amorphous peroxy-titanium complexes of oxalic, malonic and maleic acids[1–3], when vacuum-dried, have co-ordinated water molecules firmly bonded to the central titanium atom as shown in formula (I).



(I)



(II)

The peroxy-oxygen from these compounds is slowly lost even at room temperature because of the strained peroxy-group[3,4]. The compounds, when kept at 95°–100° C. for about three days, give deperoxygenated compounds of the type (II). However, a sample of peroxy-titanium oxalate sealed in a glass tube lost all its peroxy-oxygen in about four years and gave a white crystalline basic oxalate (II). The amorphous nature of the compounds may be due to random hydrogen bonding in the complexes. The crystallinity observed in one of the deperoxygenated titanyl oxalates may be due to the rearrangement of the molecules during ageing for more than four years. The infra-red absorption of these compounds was studied to find out the effect of co-ordination and hydrogen bonding on the infra-red bands of the free water.

Very little is known about the stretching and bending frequencies of co-ordinated water of transition metal complexes[5]. Fujita *et al.*[6] assigned the co-ordinated water bands in the region 800–1,000 cm.$^{-1}$; but these bands are only due to the rocking motion of the entire water molecule[5]. In the free water molecule, the oxygen moves very little during vibration because of its linkage to two light hydrogen atoms. Even on its co-ordination with a metal atom very little effect is expected on its fundamental modes of vibration.

The infra-red absorption spectra of the first two compounds (Table 1) were taken with a Perkin–Elmer single-beam spectrometer, model 112, and of the rest with infra-cord double-beam spectrophotometer, model 137, using sodium chloride prisms. The observed frequencies for the co-ordinated water are listed in Table 1. In all the compounds, the anti-symmetric and symmetric stretching vibrations of water overlapped in the region around 3,200 cm.$^{-1}$ This band was broad and strong in the amorphous compounds, while it was sharp and strong in the crystalline deperoxygenated oxalate. By elimination of the dicarboxylate group bands, the weak band in the region 850–870 cm.$^{-1}$ was attributed to the rocking mode of vibration of co-ordinated water. This assignment is complicated and at times uncertain because of the presence of strong absorption bands due to the ligands of the complexes.

Table 1. INFRA-RED FREQUENCIES OF CO-ORDINATED WATER

Compounds	Frequencies in cm.$^{-1}$ Stretching	Bending	Rocking
Peroxy			
Oxalate	3,250 (*s*, *b*)	1,640 (*s*)	850 (*w*)
Maleate	3,190 (*s*, *b*)	1,658 (*s*)	854 (*w*)
Malonate	3,125 (*s*, *b*)	1,660 (*s*)	865 (*w*)
Deperoxygenated			
Oxalate	3,185 (*s*, *b*)	1,668 (*s*)	860 (*w*)
Maleate	3,175 (*s*, *b*)	1,664 (*s*)	869 (*w*)
Malonate	3,180 (*s*, *b*)	1,664 (*s*)	870 (*w*)
Oxalate (crystalline)	3.448 (*s*)	1,610 (*s*)	856 (*w*)

s, strong ; *b*, broad ; *w*, weak.

The results in Table 1 show that in the crystalline deperoxygenated oxalate sample, the stretching band for co-ordinated water occurs at 3,448 cm.$^{-1}$ and the bending one at 1,610 cm.$^{-1}$. The stretching band of the co-ordinated water is lowered by a few hundred cm.$^{-1}$ and the bending one is raised by 15 cm.$^{-1}$ from the fundamental vibrations of free water molecule (ν_1 = 3,652, ν_2 = 1,595, ν_3 = 3,756 cm.$^{-1}$). This finding conforms to the theoretical expectations of Sartori *et al.*[7], who concluded that the stretching frequency of co-ordinated water would be lowered by 300–400 cm.$^{-1}$, while the bending would be raised by some tens of cm.$^{-1}$ from the fundamental frequencies of free water. The stretching band of co-ordinated water in the amorphous compounds occurs between 3,175 and 3,250 cm.$^{-1}$, and the bending one around 1,650 cm.$^{-1}$. These frequencies are influenced by the hydrogen bonding in the molecules, showing that hydrogen bonding further lowers the stretching frequency by about 250 cm.$^{-1}$ and the bending is raised by about 40 cm.$^{-1}$ from those of the co-ordinated water of the crystalline oxalate, which may be relatively free from hydrogen bonding.

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Significance of Experimental Rate Constants

THERE are difficulties in defining rate constants for the reactions of solids[1]. Since a rate constant describes a rate it must have the dimension (time)$^{-1}$. Constants with other dimensions with respect to time may exist in an empirical relation between extent of reaction and time, but they should never be wrongly identified as rate constants.

Consider the following relation as defining an experimental rate constant:

$$r = kf(x) \qquad (1)$$

where x is a measure of the extent of the reaction. r is the reaction rate (dx/dt); $f(x)$ is a function of x which is independent of temperature and time; k is the experimental rate constant defined by $f(x)$.

Such a rate constant is defined in terms of a particular $f(x)$ and contains an arbitrary constant, since any function $g(x) = cf(x)$ will satisfy the definition if c is independent of temperature. The experimental activation energy is not affected by this constant factor of the rate constant.

For Gomes's example, $x = a\,t^{1/2}$

$$r = a\,t^{-1/2}/2 = a^2/2x$$

(a^2) is the rate constant for $f(x) = (1/2x)$ and the experimental activation energy is unambiguous.

In order to interpret an experimental rate constant a hypothesis as to the mechanism is needed. A rate constant has a simple significance if the rate is proportional to the concentration of a single activated complex which is formed directly from the reactants If the rate-determining activated complex (or complexes) is not formed directly from the experimental reactants as measured by x, then it may still be possible to interpret the experimental rate constant in terms of a number of simple rate constants.

Experimental rate constants may contain one or more of the following complications. The first three are combinations of simple rate constants and the fourth is due to a temperature-dependent factor (of the rate constant) which can be interpreted as some property of a solid phase in the system.

(*a*) The product of a simple rate constant and one or more equilibrium constants (provided the equilibrium constants can be expressed as a ratio of simple rate constants, otherwise see (*c*) below). Such a compound rate constant will obey the Arrhenius relation, and the experimental activation energy will be the heat of formation from the experimental reactants of the rate-determining activated complex.

(*b*) A compound rate constant containing as a factor the product of several simple rate constants each one to a fractional power so that the compound rate constant has the dimension (time)$^{-1}$, for example, $(k_1 k_2)^{1/2}$. Such a rate constant will obey the Arrhenius relation and the experimental activation energy is the mean heat of formation from the experimental reactants of the several activated complexes.

(*c*) A compound rate constant containing as a factor the sum of several simple rate constants. Such a rate constant will not obey the Arrhenius equation exactly; the apparent activation energy will vary with the temperature. Consider, for example, the first-order consecutive reactions:

$$A \underset{2}{\overset{1}{\rightleftharpoons}} B \xrightarrow{3} C$$

Suppose k_1, k_2 and k_3 are simple rate constants obeying the Arrhenius relation. For steady-state conditions, the concentration of B changing slowly compared with the overall rate, the compound rate constant is $k_1 k_3/(k_2 + k_3)$ and the apparent activation energy is $(E_1 k_3 + E_3^1 k_2)/(k_2 + k_3)$. This is a mean between E_1 and E_3^1 weighted by the relative magnitudes of k_2 and k_3. E_1 is the activation energy of reaction one and $E_3^1 = E_3 + E_1 - E_2$ is the (compound) activation energy for the formation of activated complex three from the experimental reactant A.

(*d*) A rate constant as defined here may contain a temperature-dependent factor which a hypothetical mechanism may ascribe to some property of a solid phase in the system, for example, the permeability of a coherent layer of product. The presence of such a factor may be suspected if the experimental rate constant deviates greatly from the Arrhenius relation but its presence is generally not detected except from knowledge of the mechanism.

On the other hand, similar temperature-dependent constants may appear explicitly in an empirical relation between the extent of reaction and time. In these instances it may be necessary to include the constant as a factor of the measure of the extent of the reaction in order to define a rate constant, for example, the logarithmic law:

$$mx = \ln(kt + 1)$$

(m) is not a rate constant as it does not have the dimension (time)$^{-1}$. If it varies with temperature an experimental rate constant can be defined in terms of $y = mx$ since $r = dy/dt = ke^{-y}$

The major difficulties in defining rate constants for the reactions of solids are:

(*a*) The presence in the experimental rate constants of factors which can be interpreted as the temperature-dependent property of a solid phase but which cannot be expressed in terms of simple rate constants.

(*b*) For powdered solids it is usually difficult to measure particle shapes, sizes and positions sufficiently accurately to test fully the often complicated rate equations predicted by quite simple reaction mechanisms.

(*c*) Rate equations for the reactions of solids rarely approximate to the form needed to define a single experimental rate constant. It would seem better to discard the concept of an experimental rate constant than to generalize equation (1) and define several experimental rate constants although this can be done sometimes by writing the rate equation as

$$\sum_n \sum_m [r/k_m f_m(x)]^n = 1 \qquad (2)$$

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BIOCHEMISTRY

Phenethyl Alcohol in Wines

PHENETHYL alcohol has been found in fusel oils in but a few cases[1,2], and only in trace amounts. Probably for this reason it has been assumed that it is not formed in fermentation of natural substrates[3-5]. However, it is well known in the perfume industry that in steam distillation phenethyl alcohol is mainly left in the pot residue[6]. The low volatility of phenethyl alcohol thus explains its absence in fusel oil fractions.

The recent discovery of phenethyl alcohol in beer[7-9], sometimes in amounts approaching to those of isoamyl alcohol[7,10], raises the question of whether this substance is to be regarded as peculiar to beer, or whether it is a common product of yeast fermentations. I have therefore attempted to estimate the content of this alcohol in several different wines, using a method developed especially for the analysis of this substance in beer[11]. The method involves extraction of wine with ether, removal of interfering substances with alumina, chromatography of the concentrated extract on a silicic acid column, and ultra-violet spectrophotometric estimation of phenethyl alcohol in the appropriate fraction. The method is not ideal for wine, and the values obtained are probably somewhat too low. The application of the method to wines with a high alcohol content is especially difficult. Thus it was necessary to subject the extract of a South African muscatel wine (with 18 per cent alcohol, v/v) to repeated chromatography on silicic acid before the characteristic fine structure spectrum of phenethyl alcohol could be observed. The recovery in such cases is probably far from quantitative.

Table 1. AMOUNT OF PHENETHYL ALCOHOL IN SOME WINES

Origin of wine	Type of wine	Mgm. phenethyl alcohol/l.
French	red, Bordeaux	51
French	red, Burgundy	37
Hungarian	red, sweet	27
French	white, Bordeaux, dry	34
French	white, Bordeaux, sweet	20
South African	muscatel wine, sweet	> 7

The results are shown in Table 1. All the wines contain phenethyl alcohol, and the concentration-range is the same as was found in beers[7], that is. 10–50 mgm./l. In this respect phenethyl alcohol differs from the common fusel alcohols, isoamyl alcohol and isobutanol, which are present in wines in amounts several times higher than in beers.

As some fruits are known to contain phenethyl alcohol, a sample of grape must from fresh Spanish green grapes was subjected to the same analytical procedure, but no traces of phenethyl alcohol could be detected.

According to the classic theory of Ehrlich[12], phenethyl alcohol might be formed from phenylalanine on fermentation. The concentration of phenylalanine in seven different Californian grape musts lies between 30 and 80 mgm./l.[13], and in five different French wines 0–4 mgm./l.[14]. If these figures can be taken as representative for musts and wines in general, the disappearance of phenylalanine and formation of phenethyl alcohol during fermentation is in very good quantitative accord with Ehrlich's theory. On similar grounds, I have expressed the opinion that the Ehrlich mechanism would explain the presence of phenethyl alcohol in beer[7].

However, this accordance with Ehrlich's theory may be only apparent and accidental. Recently, Stevens has found small amounts of phenethyl alcohol (1–2 mgm./l.) in fermented substrates free of amino-acids, together with tyrosol (*p*-hydroxy-β-phenylethanol)[15]. Independently, I have carried out fermentations in substrates free of phenylalanine, but with otherwise widely different sources of nitrogen, using a brewery strain of *Saccharomyces carlsbergensis*. In them I have always found phenethyl alcohol in amounts of the same order of magnitude (14–29 mgm./l.) as formed by the same yeast in the normal fermentation of malt wort (21–23 mgm./l.) (to be published later).

The present view is that fusel alcohols are either overflow products of amino-acid synthesis within the cell or by-products derived from the deamination and decarboxylation of amino-acids found in the fermentation medium[5,15,16]. They are considered to be formed from the corresponding keto acids which constitute a common intermediate step in both processes. Thus the initial precursors of fusel alcohols may be carbohydrates as well as amino-acids, depending on the factors which influence the supply and withdrawal of each particular keto acid pool. Guymon *et al.*[5] suppose that this theory is limited to the metabolism of leucine, isoleucine, and valine; but Stevens's and my results seem to imply that it may be extended at least to tyrosine and phenylalanine metabolism.

I thank Dr. L. Enebo, Stockholm Brewing Co., for advice, and Mr. B. Risberg, director of AB Vin- och Spritcentralen, for the samples of wine.

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Red Proteins from Bovine Milk

SEVERAL workers have reported the isolation of a red coloured protein ('red protein') from bovine[1–3] and human[4–8] milk. The occurrence of a similar red protein in serum, responsible for transport of iron (transferrin), is now well established[9]. It has been suggested[10] but not always accepted[11] that serum transferrin occurs to some extent also in milk. In the work reported here an attempt has been made to isolate the different red proteins occurring in bovine milk and to compare them with one another and with bovine serum transferrin.

During the chromatographic fractionation of the 'albumin fraction'[12] of a pooled bovine whey on diethyl amino-ethyl (DEAE) cellulose, two similar salmon-pink coloured fractions, F_1 and F_2, were obtained[13]. Removal of slow sedimenting impurity from the fractions was achieved by the use of 'Sephadex *G*.75'. Purified bovine serum transferrin was kindly provided by Dr. J. Williams.

Although similar in colour and spectrum, the two fractions, F_1 and F_2, behave quite differently on examination by starch gel electrophoresis in 0·0088 *M* borate − 0·022 *M* sodium hydroxide buffer at *p*H 9·2. Though F_1 gives a single protein band at the origin which spreads slightly (Fig. 1*a*), F_2 gives a distinctive pattern (Fig. 1*b*) comprising several adjacent bands of similar intensity with a trace of more rapidly moving material. Bovine transferrin gives a very similar pattern without the fast impurity. Immunoelectrophoretic comparison[14] of F_2 and bovine serum transferrin against rabbit antisera (anti-bovine colostrum and anti-bovine serum) suggests a close antigenic relationship (Fig. 2*a*). This is confirmed by the combination of gel diffusion and immunoelectrophoresis[15] shown in Fig. 2*b*. Thus with transferrin in one of the troughs, the precipitation line

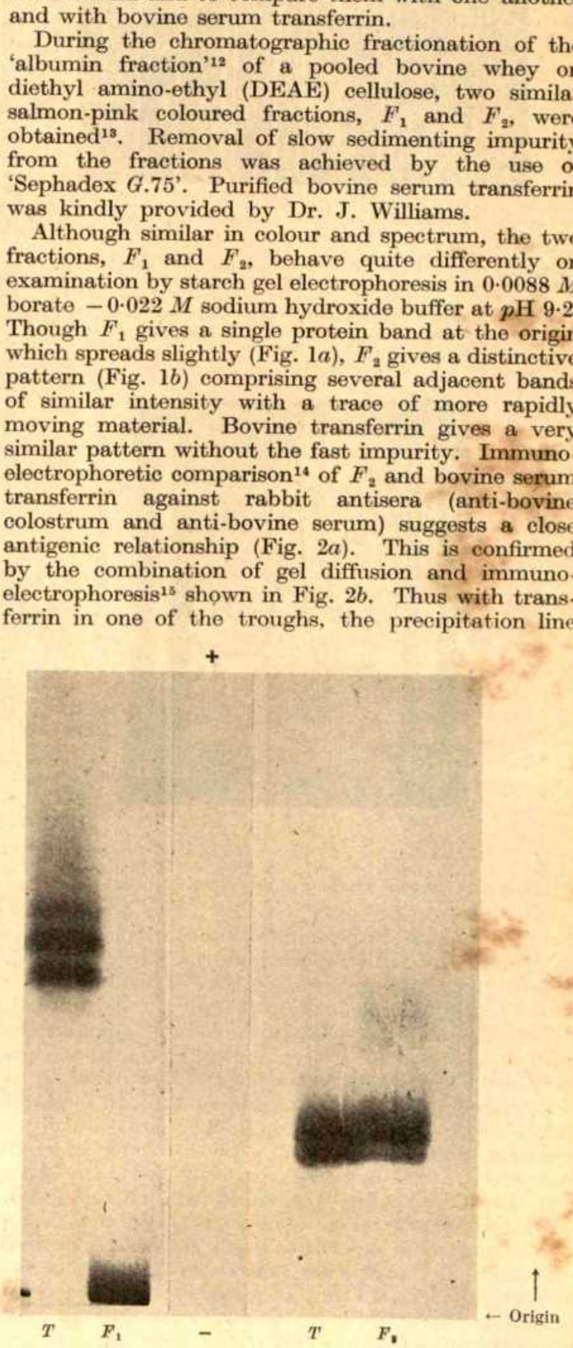

Fig. 1. Starch-gel electrophoretic comparison of *a*, Fraction F_1 with bovine serum transferrin (*T*); 6 V./cm. applied at 18 ± 2° C. for 8½ hr.; *b*, Fraction F_2 with bovine serum transferrin (*T*); 6 V./cm. applied at 18 ± 2° C. for 6 hr.

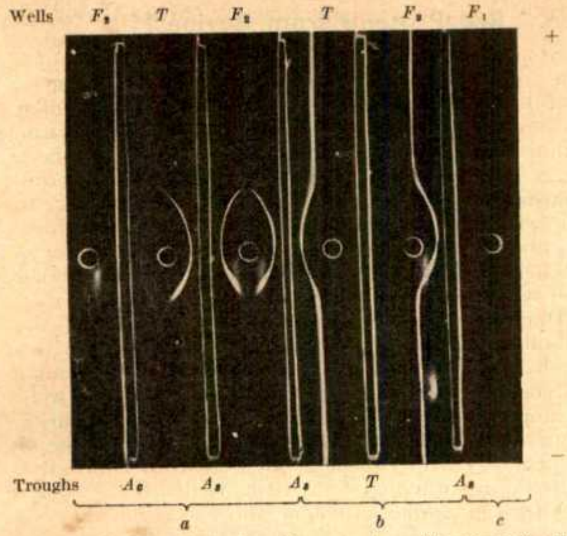

Fig. 2. Immuno-electrophoretic comparison of fractions F_1, F_2 and bovine serum transferrin (T) in 0·05 M veronal buffer at pH 8·6. Wells contain F_1, F_2 and T, as shown. Troughs contain rabbit anti-bovine colostrum (Ac), rabbit anti-bovine serum (As) or T, as indicated. Approximately 10 V./cm. applied parallel to trough direction for 2 hr.

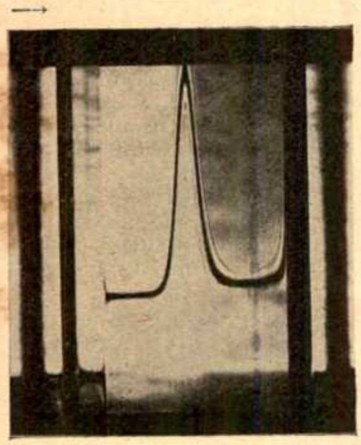

Fig. 3. Sedimentation pattern for fraction F_1 in phosphate–sodium chloride buffer, I = 0·1, pH 7·8 after 80 min. at 59,780 r.p.m. protein concentration = 1·5 gm./100 ml.

formed against anti-bovine serum in an adjacent trough, merges with the precipitation arc due to F_2, against the same antiserum. Hence F_2 and bovine serum transferrin are closely related if not identical.

Solutions of F_1 are salmon-pink in colour and the absorption spectrum is very similar to that described for Groves's red protein[3]. Fig. 2c shows that F_1 is not related to any serum component. It migrates as a single well-defined boundary in the ultracentrifuge (Fig. 3) giving $[S_0]_{c=0} = 5{\cdot}65\,S$ in close agreement with Groves's reported value (5·55 S). The isoelectric pH is established as $8{\cdot}0 \pm 0{\cdot}2$. Although at neutral or acid pH it migrated as a single component with only slight asymmetry in free boundary electrophoresis, at more alkaline or at extremely acid pH's it was resolved into two components. An attempt was made to separate the latter by refractionation on DEAE cellulose. Three, and possibly five, components having different electrophoretic behaviour on starch were obtained. All have the salmon-pink colour of the starting material and a very similar absorption spectrum. Slight differences in the extinction coefficients both at 280 mμ and 460 mμ occur, but these may result from incomplete purification. One of the fractions tends to precipitate on exhaustive dialysis against distilled water. The others are highly soluble. The sedimentation constants of two of the fractions are somewhat higher than for the mixture at comparable concentration.

In view of the multiple character shown to exist for several well-defined proteins emerging from chromatographic columns[16,17] or detected by starch-gel electrophoresis[18] and similar methods, the occurrence of several F_1 fractions is not surprising; but the possibility that the different components are transformation products of a single protein, induced by the method of fractionation, cannot at present be excluded.

We are grateful to Dr. A. E. Pierce for the supply of bovine milk and rabbit anti-bovine sera, and to Mr. A. Feinstein for performing the immuno-electrophoresis experiments.

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Metal Ions as Stabilizers or Destabilizers of the Deoxyribonucleic Acid Structure

RECENTLY there has been considerable interest in the interaction of metal ions with nucleic acids and polynucleotides, primarily as a consequence of the fact that the hydrogen-bonded structure of these macro-molecules collapses in solution in the absence of metallic salts[1–4]. It is perhaps of equal importance that some metal ions are as effective in destroying the native structure of deoxyribonucleic acid (DNA) as others are in maintaining it. Such destabilization has previously remained largely undetected because investigations of metal interactions with DNA have generally been carried out in approximately 0·1 M solution of an electrolyte such as sodium chloride, in which the destabilization effects are masked[5]. However, the double-stranded helical configuration of DNA is stable in solutions containing as low as 10^{-3} M concentration of electrolyte[6]. In the present work, various divalent metal ions have been added in 10^{-4} M concentration to solutions of calf-thymus

DNA (5×10^{-5} *M* in phosphate) in 5×10^{-3} *M* sodium nitrate. Changes in optical density at 260 mμ are then observed in a Beckman *DU* spectrophotometer on heating these solutions at the rate of 1° per min. to 90° and then cooling at the same rate[7]. The conversion of the low optical density solution to the high optical density solution is correlated with the transformation of the double-stranded helix to single strands, and the mean temperature of transition (T_m) is a measure of the stability of the molecule[7].

The various divalent metal ions examined fall into three categories in their effect on the DNA 'melting curves', and these are illustrated in Fig. 1. Curve *A* is obtained in the absence of divalent ions. In the presence of Mg^{+2} (curve *B*), T_m is increased from 63° to 80°, and there is a greater decrease in optical density on cooling. In contrast, in the presence of Cu^{+2} (curve *C*), T_m is decreased to 42°, and there is essentially no decrease in optical density on cooling. Fe^{+2} (curve *D*) combines features of Mg^{+2} and Cu^{+2} in that it increases T_m but causes the retention of the maximum optical density when the solution is cooled.

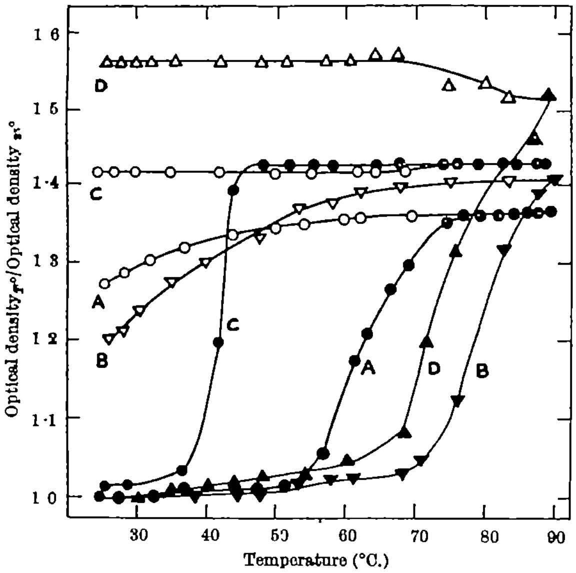

Fig. 1. Closed symbols represent heating curves and open symbols cooling curves for solutions of DNA which are 5×10^{-5} *M* in phosphate containing 5×10^{-3} *M* sodium nitrate and 10^{-4} *M* concentrations of the following: *A*, no divalent metal added; *B*, Mg^{+2}; *C*, Cu^{+2}; *D*, Fe^{+2}

In line with the previous interpretation of DNA melting curves, the effect of Mg^{+2} is considered as stabilizing the molecule. Such stabilization would be achieved by binding to the phosphate groups, and neutralizing the negative charges; the greater decrease in optical density on cooling may be explained by assuming that Mg^{+2} ions hold single chains of DNA in close proximity after the hydrogen bonds are split during heating, so that more of the hydrogen bonds can reconnect during the cooling process. Other metals that behave like Mg^{+2} are Ca^{+2}, Ba^{+2}, Mn^{+2}, Co^{+2}, Ni^{+2}, and Zn^{+2}.

The lowering in T_m by copper can be understood if Cu^{+2} helps to break the hydrogen bonds by displacing them through co-ordination to electron donor groups on the nucleotide bases. Such co-ordination would prevent hydrogen bond reformation on cooling, and thus also accounts for the complete irreversibility of the optical density increase. Cd^{+2} and Pb^{+2} also destabilize DNA, but not nearly to the same extent as Cu^{+2}.

The effect of Fe^{+2} can be explained by initial binding to phosphate, resulting in stabilization, and a subsequent change to binding of nucleotide bases, after the hydrogen bonds have been broken; reformation of hydrogen-bonds is thus prevented. No other metals have been found to behave in like manner.

It is concluded that the melting curves of DNA exhibit very different types of behaviour in the presence of 10^{-4} *M* concentrations of divalent metal ions, and that this behaviour can be related to the stabilization or destabilization of the DNA by these metal ions.

I thank Dr. Alexander Rich for his advice in this work, and Miss Barbara Randall for technical assistance.

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N-Terminal Amino-Acids formed during the Activation of Prothrombin

THERE is little information concerning the specific reactions involved in the biological conversion of prothrombin to thrombin. Recently it has been shown that the *N*-terminal amino-acid of thrombin differs from that of prothrombin[1]. The work reported here was undertaken to enumerate and identify the *N*-terminal amino-acids formed by the conversion of prothrombin to thrombin.

Prothrombin was prepared by a combination of the three classical techniques of adsorption on to barium salts[2], ammonium sulphate fractionation[3], and iso-electric precipitation[4]. This was followed by chromatography on 'Amberlite *IRC*-50' as described by Miller[5].

Human brain thromboplastin was prepared and the prothrombin activated by the method described by Miller[6]. Prior to activation a concentrated solution (1–2 per cent) of the prothrombin, prepared as described above, was dialysed overnight against a buffer 0·02 *M* in *tris*(hydroxymethyl)aminomethane and 0·15 *M* in sodium chloride adjusted to *p*H 7·4 with hydrochloric acid. The activation step was done at room temperature (22–23° C.) with constant shaking. Under these conditions quantitative conversion of the prothrombin was achieved in about 3 hr., after which the thromboplastic material was removed by centrifugation.

Determination of *N*-terminal amino-acids was made by forming the dinitrophenyl derivative as described by Fraenkel-Conrat *et al.*[7]. Tentative identification of the amino-acid derivatives was determined by paper chromatography using the 'toluene' system and afterwards 1·5 *M* phosphate at *p*H 6·1 for development.

Known dinitrophenyl amino-acids were run in conjunction with the experimental samples.

N-terminal amino-acid analysis of the prothrombin showed that 85–95 per cent of the *N*-terminal amino-acid present was alanine, 5–10 per cent was threonine, and 0–5 per cent was serine. No detectable *N*-terminal amino-acid was added to the prothrombin by the thromboplastin. After conversion of the prothrombin two new *N*-terminal amino-acids were found in quantities equimolar relative to the alanine. These were tentatively identified as threonine, and leucine (or) isoleucine. The finding of alanine as the *N*-terminal amino-acid of prothrombin is in agreement with the results of others[1,5]. Glutamic acid has been reported as the *N*-terminal amino-acid of thrombin[1]. In the foregoing experiments none was found.

Other investigators[8–10] have found evidence for the formation of an intermediate in the conversion of prothrombin to thrombin. The above results are consistent with this concept. If there is a reaction sequence:

$$\text{prothrombin} \xrightarrow{k_1} \text{intermediate} \xrightarrow{k_2} \text{thrombin}$$

where k_1 and k_2 are the rate constants, and if these are first-order reactions, the equation for the rate of formation of thrombin would be[11]:

$$T = P_0 \left[1 - \frac{k_2 e^{k_1 t} + k_1 e^{k_2 t}}{k_2 - k_1} \right]$$

Where T is the amount of thrombin present at time t, and P_0 is the initial prothrombin concentration. If $k_2 < k_1$ a plot of this equation (T versus t) gives a sigmoidal shape curve.

The curves obtained by plotting the experimentally determined thrombin-levels versus incubation time are also sigmoidal in shape[12], a fact which has heretofore been interpreted as indicative of the 'autocatalytic' nature of prothrombin conversion. In view of the direct evidence presented here for the occurrence of two reactions during the activation of prothrombin, the preferable interpretation appears to be that of sequential reactions rather than autocatalysis.

Further investigation has shown that per mole of *N*-terminal alanine more than 1 mole of *N*-terminal threonine may be formed; when this occurs, as much as a mole of *N*-terminal serine may arise.

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Participation of Adenine Nucleotides in the Proposed Direct Pathway of Biosynthesis of *N*-Phosphorylcreatine

EVIDENCE obtained in this laboratory[1,2] suggested that the phosphorylation of creatine by a phosphoryl donor, catalysed by a soluble enzyme system from rat muscle, might not require adenosine diphosphate (ADP). Morrison and Doherty[3] reach the opposite conclusion. Results obtained independently in this laboratory, by procedures already described[1,2], lead us to agree with their conclusion.

Either ADP or ATP is formed during the enzymatic incubation of adenosine diphosphate–ribose (ADP–R) with phospho(enol) pyruvate (PEP) (Table 1). It has previously been shown that they are not present initially in measurable quantities[2] and that they are not formed in the absence of PEP.

Table 1. EFFECT OF APYRASE ON THE PRODUCT OF THE INCUBATION OF PEP IN THE PRESENCE OF ADP–R OR ADP

Addition in first incubation	Concentration of addition ($M \times 10^3$)	*N*-Phosphorylcreatine synthesis, in last step µmoles/ml. in 20 min. Without apyrase treatment	With apyrase treatment
None	—	0·06	0·03
ADP–R	2·0	0·35	0·03
ADP	2·0	0·28	0·06

The reaction mixture contained in a total volume of 1 ml. 0·05 M *tris*-hydrochloric acid buffer *p*H 7·4; 0·04 M creatine; 0·01 M magnesium sulphate; 0·004 M PEP; 3 mgm. of rat muscle enzyme (ref. 1) and 2 μM ADP–R or ADP. After 20 min. at 37°, the contents of each tube was divided into two equal portions. To one portion was added 0·5 ml. of 0·3 N perchloric acid and to the other portion 0·8 mgm. of potato apyrase (ref. 4) and 1·25 µmoles of calcium chloride. It was incubated further for 20 min. at 37° C. and the reaction was stopped as above.

After neutralization with 2·8 N potassium hydroxide, the potassium perchlorate was removed by centrifugation. 0·2 ml. samples of all six neutral supernatants were added to a mixture containing *tris*-hydrochloric acid buffer *p*H 7·4 25 µmoles; creatine 20 µmoles; magnesium sulphate 5 µmoles; PEP 2 µmoles and 0·12 mgm. of pyruvate kinase plus 1·6 mgm. creatine phosphotransferase in a total volume of 0·5 ml.

After incubation for 20 min. at 37° reaction was stopped and *N*-phosphorylcreatine was determined (ref. 1).

Spectrophotometric analysis of a perchloric acid extract of the rat muscle enzyme[1] shows that it contains 0·2–0·6 µmoles of ADP or ATP per gm. of protein. This is in agreement with the finding of Morrison and Doherty (personal communication) that crystalline creatine phosphotransferase contains 0·1 µmole of ATP per gm.

We have not been able to find any radioactivity in an enzyme preparation obtained from rats injected with $NaH_2{}^{32}PO_4$.

Since ADP–R is enzymatically hydrolysed to AMP, the small amount of ATP present may start the autocatalytic formation of ADP in the presence of a phosphoryl donor. Thus a nucleotide which is bound in less than stoichiometric amounts to an enzyme may simulate a 'direct' phosphoryl transfer.

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Synthesis of Collagen

Gerber and Altman have suggested[1] that the mechanism involved in the synthesis of fibrous proteins is different from the mechanism involved in the synthesis of globular proteins. In particular, they suggested that collagen is synthesized by the addition of polypeptides containing 70–140 amino-acids to pre-existing collagen fibres. Although the communication raised a number of interesting questions, it should be noted that the conclusions which were reached are not entirely consistent with several recent reports. They stated that the mechanism underlying the biosynthesis of fibrous proteins must be unique, since experiments on the isotopic labelling of collagen and silk fibroin indicated that specific amino-acids are labelled to varying degrees in different parts of these molecules. It is now apparent, however, that a similar situation exists in the synthesis of a number of proteins and polypeptides, including ovalbumin, ribonuclease and insulin[2]. Recently, Dintzis[3] demonstrated non-uniform labelling of specific amino-acids in the synthesis of the globular protein hæmoglobin.

Also in support of their hypothesis, Gerber and Altman observed that most fibrous proteins are considerably larger than 140 Å., the diameter of the ribosomes on which protein synthesis is assumed to occur. Since several non-fibrous proteins are also larger than 140 Å., the problem raised by the relatively small size of ribosomes has been recognized for some time. Recent studies suggest that ribosomes may be considerably larger than was originally supposed[4]. In any event, it seems premature to conclude that the problem which exists in explaining the synthesis of fibrous proteins by ribosomes is entirely different from the problem of explaining the synthesis of large globular proteins. Our own observations in chick embryos[5] indicate that collagen is synthesized by the same ribosomal system which has been postulated for the synthesis of other proteins. Lowther *et al.*[12] have recently reported similar results in work on carrageenin granulomas.

Several other suggestions which were made in the paper also appear to be unlikely. Because the number of C- and N-terminal amino-acids increase after the degradation of collagen to gelatin[6,7], it was suggested that intramolecular cleavage of peptide bonds occurs when collagen is fractionated into soluble forms and that the resulting solutions of 'soluble' collagen consist of peptides from near the termini of the collagen molecule. It was further suggested that these soluble peptides represent 'building blocks' of collagen. Although some peptide bonds are ruptured by relatively small energies[7], the mild conditions generally used to solubilize collagen cannot be expected to rupture peptide bonds. "Neutral salt soluble collagen"[8], for example, is prepared by extracting tissues with dilute, neutral salt solutions at 4° C. It is very unlikely that peptide bonds are broken by this procedure. In addition, it may be pointed out that the molecular weight of neutral salt soluble collagen has been estimated as being 340,000–500,000 (ref. 9), a size which is considerably larger than the peptides postulated by the authors.

It was also suggested that the turn-over time of the peptide 'building blocks' of collagen can be determined from the turn-over time of free hydroxyproline in urine and the turn-over time of collagen itself can be determined from the turn-over time of the peptide-bound hydroxyproline in urine. In previously published experiments, Gerber *et al.*[10] injected proline labelled with carbon-14 into rats and found a turn-over time of about 0·5 days for free urinary hydroxyproline-^{14}C and 15 days for bound urinary hydroxyproline-^{14}C. Using different analytical techniques, we were recently unable to confirm their results and instead we found comparable rates of decay for both the free and bound hydroxyproline-^{14}C in urine[11]. My own results indicated that both the free and bound hydroxyproline in rat urine originate from pools of body collagen which are both synthesized and degraded rapidly.

Although the synthesis of fibrous proteins probably involves unique mechanisms not encountered in the synthesis of globular proteins, there is at present little evidence to indicate what these unique mechanisms may be. For the moment the similarities between collagen synthesis and the synthesis of other proteins are as apparent as the differences.

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Use of a Thin Layer of Calcium Sulphate for the Chromatographic Separation of Corticoids

In experiments where we sought a suitable adsorbent for the separation of corticoids by partition chromatography, we found that in calcium sulphate columns a good separation is obtained of less-polar corticoids when chloroform is used for elution. Based on this fact, a detailed analytical procedure was worked out for the fractionation of some corticoids in urinary extracts. The desirable properties of calcium sulphate were applied successfully in a method using thin layers, which substantially speeded up and simplified chromatographic analysis of steroid mixtures and separated the polar 17-OH-corticoids.

Calcium sulphate was prepared by precipitation of an aqueous solution of calcium chloride with a stoichiometric amount of sulphuric acid under constant stirring at a temperature of 70°–80° C. The precipitate of calcium sulphate was rinsed on a Büchner funnel with distilled water until the water gave a neutral reaction. The obtained precipitate was ground and spread in a 2–3 cm. thick layer and allowed to dry for 48 hr. at a temperature of 115°–120° C. Thus prepared, calcium sulphate was suspended in water (20 gm. calcium sulphate per 100 ml. water) with vigorous shaking. The suspension was rapidly poured on a thoroughly degreased glass plate 4 × 40 cm. and allowed to dry in a horizontal position for 2 hr. at 80° C. A compact layer of calcium sulphate was thus obtained which adhered firmly to the glass plate. After cooling, about 5 cm. from the lower end a mixture of standard steroids in alcoholic solution was applied. It was essential to try to keep the diameter of the spot as small as possible. The chromatogram was developed by immersing the lower edge of the plate into a chamber with a 2-cm. layer of the mobile phase at the bottom. After the chromatogram was inserted in a chamber, the latter was closed. As mobile phase we used an acetone/benzene mixture, 1 : 4. The development of the chromatogram was discontinued when the beginning of the mobile solution was 25 cm. from the start, which took about 60 min. After drying the chromatogram with a stream of warm air, we detected it using a spray of an alkaline blue tetrazolium solution[1].

When testing the separating capacity of the described method, standard preparations of pure corticoids were used:

Pregn-4-ene-17α : 21-diol-3 : 20-dione (*S*)
Pregn-4-ene-17α : 21-diol-3 : 11 : 20-trione (*E*)
Pregn-4-ene-11β : 17α : 21-triol-3 : 20-dione (*F*)
5β-Pregnane-3α : 17α : 21-triol-11 : 20-dione (*THE*)
5β-Pregnane-3α : 11β : 17α : 21-tetrol-20-one (*THF*)

By means of this technique a sharp separation was achieved, in particular of substances *S*, *E* and *F*, while the separation of *THF* and substance *THE* was only very slight. The mobility in relation to substance *F* of individual steroids is shown in Table 1. For a sharp separation of *THF* and substance *THE* the use of 3 per cent ethanol in chloroform proved very satisfactory (Table 2). In this system the substances were separated very sharply. This chromatogram was developed for 90 min. in an open chamber.

Table 1

Corticoid	R_F
THF	0·38
THE	0·41
F	1·00
E	1·43
S	3·43

Table 2

Corticoid	R_F
THF	0·54
THE	0·73
F	1·00

This technique proved suitable for the separation of corticoids in urinary extracts. The latter were prepared as follows: the urine was hydrolysed by means of β-glucuronidase and extracted with chloroform. After washing with 0·1 *N* sodium hydroxide the chloroform was dried with anhydrous sodium sulphate and evaporated to dryness. An aliquot portion corresponding to 0·3 mgm. of total 17-hydroxycorticoids estimated by Silber–Porter's method[2] was subjected to chromatography.

For these purposes the suspension of calcium sulphate (20 gm. calcium sulphate per 100 ml. water) on the two thoroughly degreased glass plates 4 × 40 cm. was heated in the horizontal position for 10 min. at 80° C. After 10 min. drying the calcium sulphate acquired a pasty consistency. In this state it could be divided into two equally wide chromatographic strips, which was done by a chisel 0·3 cm. wide. The strips are then further dried for 2 hr. at 80° C. After cooling, to one strip, about 5 cm. from the lower edge, urinary extract of corticoids was transferred dissolved in the smallest possible amount of a methanol/chloroform mixture, 1 : 1, and to the second strip a mixture of standard steroids in alcoholic solution was applied. One of the glass plates was developed in the system acetone/benzene, 1 : 4, the other in 3 per cent ethanol in chloroform. In urine samples of five normal men a *THF* spot, a strong *THE* spot, substance *F* and a trace of substance *E* were found. The preliminary experiments suggest that it will be possible to use the method successfully for quantitative analyses.

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Pyruvic Acid, a Unique Component of an Exocellular Bacterial Polysaccharide

PYRUVIC acid, a compound widely distributed in Nature as a metabolic intermediate, is a building unit for many compounds of biological origin. For example, in the biosynthesis of sialic acid—a carbohydrate associated with mucoproteins in animals—pyruvic acid is combined enzymatically with *N*-acetyl mannosamine by an aldol-type condensation[1,2]. Muramic acid—a carbohydrate constituent of bacterial cell walls—is thought to arise from a condensation of pyruvic acid, as phosphoenol pyruvate, with *N*-acetyl glucosamine[3]. Also, pyruvic acid has been isolated and identified as a component of a commercial red seaweed polysaccharide. Structural studies indicate the presence of one acid residue for each 51 hexose units[4,5].

Recently, we found a polysaccharide which contains 3·0–3·5 per cent pyruvic acid as a structural component. This high-viscosity, water-soluble gum is produced exocellularly by the bacterium *Xanthomonas campestris NRRL B*-1459 (ref. 6). In addition to pyruvic acid, the polysaccharide *B*-1459 contains D-glucose, D-mannose, D-glucuronic acid and acetic acid in the ratio 2·8 : 3·0 : 2·0 : 1·7 and has a repeat unit weight of approximately 1,580 (ref. 7).

The presence of pyruvic acid was discovered when hydroxamic acid derivatives were prepared from an ether extract of a polysaccharide acid hydrolysate. Paper chromatography of the hydroxamate preparation revealed hydroxamates of both acetic acid and pyruvic acid. The keto acid was identified definitely by preparing the 2,4-dinitrophenylhydrazone (DNP) derivative. This derivative had a melting point of 216° C., and its melting point was not depressed when it was mixed with an authentic sample of pyruvic acid DNP.

A reliable assay for pyruvic acid combined in the polysaccharide was established by modifying a method originally described for the determination of keto acids in fermentation liquors[8]. A 0·2–0·4 per cent solution of polysaccharide was hydrolysed in 1 *N* hydrochloric acid for 3 hr. A 2-ml. aliquot was removed and was mixed with 1 ml. of a 2,4-dinitrophenylhydrazine reagent (0·5 per cent in 2 *N* hydrochloric acid) for 5 min. The reaction mixture was extracted with 5 ml. of ethyl acetate, and the aqueous layer was discarded. After the ethyl acetate was extracted with three 5-ml. portions of 10 per cent sodium carbonate, the extract was diluted to 25 ml. with additional 10 per cent sodium carbonate. Concentration of pyruvate was determined by measuring optical density of the sodium carbonate solution at 375 mμ.

Highly purified samples of the polysaccharide *B*-1459 contained about 3 per cent of pyruvic acid and none was lost during periodate oxidation, sodium borohydride reduction, or treatment with alkali under conditions which readily effected deacetylation. In contrast, dextran *B*-512, gum karaya, and gum tragacanth contained no pyruvic acid.

In seaweed agar, pyruvic acid exists as a ketal linkage forming a 4,6-0-(1-carboxy-ethylidine) unit attached to galactose. The behaviour of the pyruvate bond in the polysaccharide *B*-1459 to acid and base hydrolysis suggests that it, too, is in a ketal form. Preliminary studies indicate that the pyruvate is attached to the glucose moiety. Since glucose is linked within the polysaccharide chain by glycosidic bonds at the 1 and 4 positions (unpublished data), pyruvate cannot be attached to glucose at the 4 and 6 positions. Therefore, the pyruvate–ketal linkage in the polysaccharide *B*-1459 is presumed to involve two glucose units to produce a polysaccharide with a novel cross-linked structure.

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Detection and Estimation of Squalene in Leaves

SQUALENE, $C_{30}H_{50}$, was discovered in the liver oils of squaloid sharks by Tsujimoto[1] and later found in olive oil by Thorbjarnarson and Drummond[2]. Values as high as 708 mgm. per 100 gm. have been reported by Fitelson[3] with much lower values, 5–28 mgm., in several other vegetable oils. More recently, Fuhrmann[4] reported a squalene content of 200 mgm. per 100 gm. in olive oil.

Squalene is usually identified by its crystalline hexahydrochloride. Three isomers, ranging in m.p. from 108° to 144° C., were separated by Heilbron *et al.*[5]. The method used for estimating total squalene is based on halogen uptake by the unsaponifiable fraction. This is inaccurate when phytoene, phytofluene or other unsaturated compounds are present. Identification is also unsatisfactory where very low concentrations of squalene are encountered.

By gas chromatography we have obtained a single sharply defined peak for squalene using a silicone-rubber column at temperatures between 240° and 290° C. Phytoene gave a broad peak 15–20 min. after the squalene had been separated. No peak was obtained for phytofluene. Results complement those of O'Neill and Gershbein[6]. The same peak, on which that for authentic squalene can be superimposed, has been detected in extracts of carrot roots, olive oil, the mould *Phycomyces blakesleeanus*, and the leaves of six species: *Acanthus*, alfalfa, carrot, elderberry, lettuce and olive.

For identification, we dehydrogenated the material with *N*-bromo-succinimide[7,8]. When fully dehydrogenated, squalene will have a lycopene-like spectrum[8]. In this test, one must exclude phytoene or phytofluene, which can be dehydrogenated to lycopene and which also yield small quantities of di- and tetra-dehydrolycopene, with 13 and 15 double bonds respectively. Exclusion can be assured by adsorption chromatography and a check on ultra-violet absorption at 286 mμ, the phytoene maximum.

The plant materials used were collected fresh or purchased locally. The following chemicals were used: squalene (Eastman technical grade, 90 per cent pure); aluminium oxide (Woelm); petroleum ether, passed over silica gel, free from ultra-violet-absorbing impurities to about 230 mμ; methanol (absolute).

The unsaponifiable fraction was prepared in the conventional manner. In two large-scale runs, alfalfa and olive leaves, the unsaponifiable fraction in about 20 ml. petroleum ether represented the extract from about 5 kgm. of leaf material.

The unsaponifiable fraction was chromatographed on alumina (activity, grade II) 25 × 4 cm., and washed with petroleum ether until 50–100 ml. eluate had been collected. Absorption at 286 mμ was negligible, showing the absence of phytoene.

In a previous test, 10 mgm. squalene were mixed with 4 mgm. phytoene and chromatographed on such a column, and each 25 ml. of eluate was examined for squalene and phytoene. Squalene was detected in the 2nd and 3rd fractions by dehydrogenation with *N*-bromosuccinimide. Phytoene did not appear until after the 5th fraction.

Authentic squalene, phytoene, and phytoene-freed unsaponifiable plant extracts were treated with *N*-bromosuccinimide. The maximum yield of material absorbing at 502, 470, 445 mμ in petroleum ether (lycopene-like spectrum) was obtained with a 1 : 2 ratio of squalene to *N*-bromosuccinimide, by weight.

A mole ratio of 1 : 1 has been recommended[7,8]; but whereas Zechmeister's laboratory was desirous of minimizing destruction of the conjugated system, in our case possible losses had to be counterbalanced by the necessity of obtaining a measurable quantity of fully dehydrogenated squalene.

The sample (authentic squalene, phytoene, plant extracts, etc.) containing 5–25 mgm. polyene was dissolved in 5 ml. chloroform and 2 ml. glacial acetic acid. *N*-bromosuccinimide in the ratio of 1 : 2 by weight was added and the mixture was gently refluxed. It slowly turned yellow and then brick red and finally brown. After 2 hr., the solution was taken up in petroleum ether, washed, dried and chromatographed on a small column of $MgO–SiO_2$, 5 × 0·8 cm., developed with petroleum ether containing acetone, then benzene and finally alcohol. The chromatogram was developed with petroleum ether containing increasing percentages of acetone, and fluorescent and yellow zones were eluted. On the addition of benzene, in the case of squalene, a pink zone separated from the upper brown. When eluted with alcohol, it showed a typical lycopene spectrum, and thus the presence of squalene was confirmed.

In the case of phytoene, two zones were detected above the lycopene. Zechmeister and Koe[9] had detected one, presumably with 13 double bonds in conjugation, maxima in benzene 540, 508, 475 mμ. The second had maxima at 555, 520, 492 mμ, and is presumably a tetra-dehydrolycopene, corresponding to the dehydrolycopene, $C_{40}H_{52}$, of Karrer and

Rutschmann[10]. The spectra showed *cis*-peaks, at 400 and 420 mμ, respectively.

Peaks for squalene were given on a silicone-rubber column ('Aerograph'), operated as follows: length of column, 5 ft.; temperature (*a*) 260°, (*b*) 244°; flow-rate (*a*) 80, (*b*) 96·8 c.c. per min.; retention time (*a*) 6·8, (*b*) 10·4 min.

Based on the ratios of areas under the curves, the following estimates (mgm. per 100 gm.) for squalene were made: olive oil, 156; olive leaves (air dried), 4·0; alfalfa dried meal, 2; *Acanthus* (fresh weight), carrot leaves and roots, elderberry, lettuce, 0·05–0·1.

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PHYSIOLOGY

Standard Bicarbonate Values of Whole Blood

THE convenience of the interpolation techniques[1,2] for the assessment of acid-base imbalance in patients has been widely recognized, and in clinical surgery the ability to differentiate rapidly between the metabolic and respiratory components of such a disturbance has special value. In particular, the standard bicarbonate value, defined as the bicarbonate content of oxygenated blood at 38° C. equilibrated at a $P\text{CO}_2$ of 40 mm. mercury provides a quantitative assessment of metabolic disorder. It was therefore disconcerting that when 63 arterial blood samples from a group of 22 patients anæsthetized with halothane were analysed, the standard bicarbonate values obtained (18·14 ± 1·94 m.equiv.) were significantly lower than those quoted by Astrup *et al.*[3] (22·9 ± 1·5 m.equiv.). Because there was no other evidence of metabolic acidosis in these patients and because the bicarbonate value of plasma (23·28 ± 2·82 m.equiv.) in a similar group under the same conditions fell within the range of Astrup's figures it was decided to establish our own standards.

Accordingly, blood samples obtained from conscious volunteers were separated into three aliquots and standard bicarbonate values determined for whole blood, for true plasma (that is, plasma equilibrated at a $P\text{CO}_2$ of 40 mm. mercury before separation from blood) and for separated plasma (that is, plasma equilibrated after separation from blood). The results obtained were as follows:

	Whole blood	True plasma	Separated plasma
Standard bicarbonate (m.equiv./l.)	18·89	19·44	23·14
Standard deviation	1·79	1·37	2·29

The accuracy of the interpolation method for determining carbon dioxide tension was assessed using 12 random samples of whole blood previously equilibrated in a tonometer with known tensions of carbon dioxide. The mean difference between the calculated and the known carbon dioxide tensions was + 0·183 ± 0·14 mm. mercury.

The accuracy of the method for the measurement of total carbon dioxide was assessed by deriving values from 16 arterial blood samples both by the interpolation method and by a colorimetric method in routine use in the biochemistry laboratories of this Hospital. The results obtained were 21·59 and 21·00 m.equiv./l. respectively. A similar comparison was made between the interpolation method and the Van Slyke technique[4] using 13 venous blood samples. The mean values obtained were 24·27 and 24·39 m.equiv./litre. No statistically significant difference was found in either comparison.

When the actual bicarbonate determinations derived from the colorimetric and Van Slyke techniques were substituted in the Henderson–Hasselbalch equation and using *p*H and $P\text{CO}_2$ values derived from the interpolation method the *p*K′ values obtained were 6·11 ± 0·03 and 6·10 ± 0·02 respectively.

A further analysis was carried out on a group of twelve conscious patients after surgery from whom 48 arterial blood samples were obtained. These gave a mean standard bicarbonate value of 18·95 ± 1·24 m.equiv./l., a figure which in the light of the evidence just presented probably reflects the normal standard bicarbonate in whole blood in man.

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Role of Genetic Factors in Susceptibility to Experimental Hypertension due to Chronic Excess Salt Ingestion

THERE is considerable evidence indicating a familial trend in human essential hypertension[1]. A familial disease could be due exclusively to common environmental factors, exclusively to common genetic factors, or to an interaction of the two.

We have been investigating the effects of chronic excess salt ingestion in both man and animals and have obtained evidence which supports the thesis that dietary salt plays an ætiological part in human essential hypertension[2]. However, it was observed that some individuals remained normotensive despite the fact that they were chronically consuming large amounts of salt. In salt-feeding experiments with rats, in a given group some never developed hypertension whereas a few developed fulminating hypertension after a short time on the diet. Such wide variations in response to excess salt consumption could be ascribed either to statistical limits of a homogeneous population or to variations in sensitivity to salt based on genetic differences. If the last were true, it should be possible by selective inbreeding to

Table 1

Strain	Original breeders		S_3		R_1	
Sex	♂	♀	♂	♀	♂	♀
No.	23	24	25	24	29	32
Systolic blood pressure (mm. mercury)						
Mean (± *S.D.*)	161·6 (±18·5)	157·6 (±24·2)	179·3 (±20·2)	182·8 (±18·3)	139·5 (±15·4)	136·1 (±14.5)
Median	164	161	182	178·5	143	139·5

Effect of L-triiodothyronine-sea salt regimen on systolic blood pressures of Sprague–Dawley rats: response of original breeding stock compared with third generation of animals inbred for sensitivity (S_3) and resistance (R_3) to hypertension from regimen.

By Student's *t* test, mean systolic blood pressure for S_3 > original breeders ($P < 0·001$) and R_3 < original breeders ($P < 0·001$).

separate strains that differ significantly in their liability to hypertension from excess salt consumption. This communication reports experiments on rats which confirmed this postulate. It seems probable that these observations are pertinent to man as well.

In rats, hypertension from chronic excess salt feeding usually develops slowly[3]. However, we have observed that the administration of L-triiodothyronine to intact salt-fed animals leads to marked hypertension in some animals within one month while animals treated similarly, except fed a low salt control diet, do not develop hypertension. This technique was used to isolate two strains of animals, one of which was resistant while the other was sensitive to the triiodothyronine–high salt regimen. After development of these two strains, salt-feeding without triiodothyronine led to the rapid development of hypertension in the strain known to be sensitive to the triiodothyronine–salt regimen whereas hypertension did not develop in the other resistant strain. The technique was as follows:

Twenty-three male and 24 female unselected 3-week-old Sprague–Dawley weanling rats constituted the initial breeding stock. Each received 50 µgm. triiodothyronine intraperitoneally on each of four successive days and then at about 2-day intervals ten 5 µgm. doses in the next 21 days. All were fed a chow containing 11·6 per cent sea salt containing about 7·3 per cent sodium chloride[3]; systolic blood pressures were measured in a standard fashion[3] 48 hr. after the last injection of triiodothyronine. Males and females with the highest blood pressures were selected for inbreeding as were those with the lowest pressures, and animals with intermediate pressures were discarded. The first generation of animals derived from these matings of parents with high or low blood pressures were called S_1 (sensitive) or R_1 (resistant) respectively; afterwards, no interbreeding between the *S* and *R* strains occurred.

The S_1 and R_1 animals were weaned at 3–4 weeks and immediately put on the triiodothyronine–sea salt regimen already described. Animals that responded in the S_1 strain with the highest, in the R_1 strain with the lowest, pressures were selected for further inbreeding and the remaining animals killed; usually brother–sister matings were possible. After two generations of such selective inbreeding there was evidence of a significant increase in the fraction of S_2 animals that responded with high blood pressure and a decrease in the fraction with normal pressures. Just the opposite was true for the R_2 strain. Therefore when the third generation was weaned, half the S_3 and R_3 animals of each sex were put on the triiodothyronine–sea salt regimen, while the other half were treated as described later.

Table 1 is a summary of the blood pressure response of the S_3 and R_3 animals to the standard triiodothyronine–sea salt regimen. It is compared with the response of the original breeding stock from which the two strains were derived. The difference in response between the S_3 and the R_3 animals was striking and highly significant, statistically. On the basis of this evidence it was concluded that a difference in susceptibility to the triiodothyronine–sea salt regimen had been developed after three generations of selective inbreeding although neither the S_3 nor the R_3 strains were as yet pure.

One hundred and thirty-one siblings of animals from the S_3 and R_3 strains shown in Table 1 received either the control low sodium diet or the same high sodium, sea salt chow used before but no triiodothyronine. Table 2 is a summary of the blood-pressure responses of these animals. The differences in blood-pressure response between the salt-fed S_3 and salt-fed R_3 animals were more striking than was indicated in Table 2: after 6 months of salt feeding none of the 39 R_3 animals had hypertension whereas after only 3 months on the same programme 49 of the 60 S_3 animals were hypertensive and many of them severely so. The 3-month lag was occasioned by differences in times at which litters were delivered as well as because S_3 animals tended to have smaller litters so that a longer time was required to get sufficient numbers of S_3 rats. Therefore, an increase in sensitivity to salt would tend to be minimized in the S_3 strain with differences in response of the two strains thereby being under- rather than over-estimated. When members of the same S_3 strain were maintained on the control low salt diet, hypertension did not develop. Thus, however strong the genetic tendency to hypertension may be in this strain, the addition of a high salt diet seems necessary to unmask it. Among the R_3 animals, the presence or absence of excess dietary salt did not seem to affect the blood-pressure response.

It seems reasonable to expect that similar genetic factors operate in man. If salt plays the important ætiological part that we said it does in human hyper-

Table 2

Strain	S_3				R_3			
Sex	♂		♀		♂		♀	
Diet	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow	11·6 per cent Sea salt chow	Control chow
No.	29	12	31	11	20	10	19	9
Systolic blood pressure (mm. mercury)								
Mean (± *S.D.*)	180·2 (±19·4)	125·8 (±6·8)	156·8 (±28·4)	112·2 (±7·3)	119·7 (±14·5)	120·1 (±8·5)	105·2 (±12·5)	106·4 (±10·7)
Median	180	126	146	114	121·5	119	108	104

Effect of high intake of salt without L-triiodothyronine on systolic blood pressures of Sprague–Dawley rats that were selectively inbred for three generations to develop strains sensitive (S_3) and resistant (R_3) to development of hypertension from salt. Blood pressures of S_3 strain after only 3 months whereas those of R_3 strain were after 6 months on same diet.

By Student's *t* test, the mean systolic blood pressures differed significantly ($P < 0·001$) as follows: S_3 salt-fed ♂ > S_3 control-fed ♂; S_3 salt-fed ♀ > S_3 control-fed ♀; S_3 salt-fed ♂ > R_3 salt-fed ♂; S_3 salt-fed ♀ > R_3 salt-fed ♀. By the same test, for comparably fed strains the mean systolic pressures for males exceeded that for females ($P < 0·01$).

tension, and given the usual genetically heterogeneous population characteristic of man, it would be illogical to expect all individuals on similar salt intakes to have similar blood pressures. On the contrary, one would expect to find individuals on high average salt intakes who did not develop hypertension as well as individuals on lower average salt intakes who did. It is unlikely that strains of humans can be found with the sensitivity to salt evinced by our rats, although studies of isolated populations might be helpful. In the absence of such genetic homogeneity, it is sufficient to re-emphasize[2b,c] that the available data relating intake of salt to hypertension in man indicate group rather than individual probability of developing hypertension.

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Iproniazid and Experimental Hypertension in Rabbits

MONOAMINO oxidase affects serotonin metabolism, degrading this substance to 5-hydroxy-indolyl acetic acid. Monoamino oxidase also affects epinephrine and norepinephrine metabolism[1]. Minz and Walaszeck[2] have shown that the substances of the hydrazid group such as iproniazid, inhibit monoamino oxidase, *in vivo* and *in vitro*; and produce a variation of the pressor effect of epinephrine applied on the brain cortex.

This pressor effect of cortically applied epinephrine is lowered by the previous action of iproniazid injected repeatedly into experimental animals; this was explained as being due to the raising of the serotonin-level in hypothalamus, resulting in inhibition of monoamino oxidase by iproniazid. This pressor effect was, on the contrary, exaggerated in the animals to which reserpine was given; in these animals serotonin was lowered as a result of reserpine injection.

We used 20 rabbits, in which arterial pressure was recorded with an intra-arterial catheter in the popliteal artery using a mercury manometer and a graphic recorder.

Increase of systemic blood pressure was obtained by intravenous injection of epinephrine (1 mgm./kgm. weight)[3]. In the control group, using 10 animals (average weight 1,700–3,000 gm.), the raising of tension was between 20 and 35 mm. mercury (Table 1).

In the other group 10 animals were used. An injection of iproniazid (450 mgm./kgm.) was given intravenously[4,5], and 60 min. after the injection the rise in systemic blood pressure varied from 20 to 45 mm. mercury; in 4 animals it was more than 35 mm. No variation was seen in initial blood pressure in the two groups—the control and those treated with iproniazid.

Table 1

Animals No.	Controls (mgm./mercury)	Animals No.	Iproniazid (mgm /mercury)
1	25	11	25
2	25	12	30
3	30	13	40
4	20	14	45
5	35	15	12
6	20	16	25
7	25	17	40
8	30	18	30
9	30	19	25
10	20	20	45

A single injection of iproniazid was given. The inhibition of monoamino oxidase was intense and acute; we suppose that this did not modify the hypothalamic serotonin-level, and that is why we did not obtain a lowered pressor response; on the contrary, it seems that those of the group pretreated with iproniazid have a higher pressor response than the controls.

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Changes in Mucoprotein in Serum and Aqueous Humour during the Sexual Cycle of the Rat

ŒSTROGENIC hormones appear to increase the acid mucopolysaccharide content in the connective tissue and the degree of polymerization. They also stimulate an increase in the mucopolysaccharides of the genital mucosa[1].

The mucoprotein content was examined in the serum and aqueous humour of 88 female rats at varied time-intervals during their sexual cycle and in 30 male rats of the same age. The quantity of mucoproteins was determined by the tryptophan method[2]. The total sugar content was determined in the aqueous humour by the same method. The qualitative degree of polymerization of the mucopolysaccharides was examined by the toluidine blue microtest[3] and the phenomenon of rhythmic crystallization in the aqueous humour[4].

A higher serum mucoprotein content was found in the female rats in œstrus than in those in diœstrus and moderate values were encountered in metœstrus and pro-œstrus. In the males, the mucoprotein content was the same as in females in diœstrus.

In the aqueous humour of female rats in œstrus, a lesser content of mucoproteins with a greater degree of polymerization and a 'fern-type' rhythmic crystallization were found. In female rats in diœstrus there is a greater mucoprotein content with a lesser degree of polymerization and a 'mixed coral-fern type' of rhythmic crystallization. In pro-œstrus and metœstrus, intermediate values were found. In the males, the mucoprotein values were similar to those found in pro-œstrus and metœstrus and the degree of polymerization and crystallization were similar to that found in diœstrus.

Table 1. MUCOPROTEINS IN SERUM AND AQUEOUS HUMOUR

	Serum		Aqueous humour			
	No. of rats	Mucoproteins Galactose–mannose (mgm./per cent)	No. of samples pooled	Mucoproteins Galactose–mannose (mgm./per cent)	No. of pools	Total sugar Galactose–mannose (mgm./per cent)
Diœstrus	40	190 ± 3	6	99·6 ± 8	4	178·4 ± 22
Pro-œstrus	15	200·5 ± 5	4	69·0 ± 8	2	140 ± 2
Œstrus	24	214 ± 6	9	51·8 ± 3	4	147·7 ± 7
Met-œstrus	18	200·3 ± 6	4	67·3 ± 9	3	160 ± 3
Males	30	188 ± 10	5	67·7 ± 5	3	174·1 ± 0·5

Quantitative results are presented in Table 1. From these results, we conclude that a cyclic variation in the content and quality of the mucoproteins of the serum and aqueous humour exists in the sexual cycle of the female rat. In the male rat, the values are similar to those found in the female rat in diœstrus. It may be supposed that the œstrogens are responsible for the variations seen.

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Effect of Altitude on the Body/Venous Hæmatocrit Ratio

THE concentration of red cells is not the same in all parts of the circulatory system[1], the cell plasma ratio being lower in the capillaries and small vessels than in the larger arteries and veins. The over-all cell percentage (body hæmatocrit) is determined from the direct measurement of both red cell volume and plasma volume, and from this the body/venous hæmatocrit ratio (*BH*/*VH* ratio) is calculated. In normal adults resident at sea-level the *BH*/*VH* ratio averages 0·91 (ref. 2).

Little is known of the constancy of the *BH*/*VH* ratio in varying physiological and pathological conditions. The ratio increases during pregnancy[3], and in patients with splenomegaly[4], and may decrease in congestive heart failure with fluid retention[5].

In subjects resident at an altitude of 5,740 ft. above sea-level there is contraction of the plasma volume[6], and an increase in red cell volume[7], compared with values at sea-level. It is not known, however, whether the *BH*/*VH* ratio remains at 0·91. To investigate this an investigation was undertaken, where red cell volume was measured with red cells labelled with chromium-51 and plasma volume with radioiodinated human serum albumin. Venous hæmatocrit was measured in a microhæmatocrit centrifuge, spinning at 12,000*g*. Observations were made on 36 young White adult males, 18 resident at sea-level (Durban) and 18 resident at an altitude of 5,740 ft. (Johannesburg). The results are shown in Table 1. The difference in the mean *BH*/*VH* ratio in the two groups is highly significant ($t = 5{\cdot}17$; $P = < 0{\cdot}01$).

Table 1. BLOOD VOLUME IN SUBJECTS RESIDENT AT SEA-LEVEL AND AT 5,740 FT.

No. of subjects	Altitude	Red cell volume (^{51}Cr) (ml./kgm.)	Plasma volume (RISA) (ml./kgm.)	Venous haematocrit (per cent)	Body haematocrit (per cent)	Body venous haematocrit ratio
18	Sea-level	30·53 ± 0·694	43·96 ± 0·799	45·83 ± 0·512	40·99 ± 0·358	0·902 ± 0·00965
18	5,740 ft.	35·72 ± 0·817	41·40 ± 0·584	48·11 ± 0·551	46·25 ± 0·549	0·962 ± 0·00830

The raised body hæmatocrit in subjects resident at a high altitude probably represents a compensatory mechanism enabling more red cells per unit volume to pass through the lungs in unit time. This compensation in capillaries is evidently not completely reflected in the larger vessels, and the *BH*/*VH* ratio is thus nearer unity in subjects resident at this altitude.

There is evidence from experiments on animals that the response to altitude is not uniform throughout the body vascular system. Thus in rats exposed to simulated altitudes of 20,000–22,000 ft. for periods of 1–4 weeks there is a greater increase in the capacity of the non-capillary vessels (arteries, veins, arterioles and venules) than in the capillary bed[8].

This observation has practical application in the routine clinical estimation of volume of blood. In practice, either the red cell volume or plasma volume are measured directly, and the other compartment then calculated from the body hæmatocrit, which is the product of the venous hæmatocrit and the *BH*/*VH* ratio. In subjects resident at altitudes above sea-level it is inaccurate to assume a *BH*/*VH* ratio of 0·91, and it is necessary to determine this value for subjects resident at any particular altitude.

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Plasma Clearance of *p*-Aminohippuric Acid by the Kidneys of Fœtal, Neonatal and Adult Sheep

AN investigation into the development of secretory activity in the renal tubules has been undertaken using *p*-aminohippuric acid.

Observations were made on fœtuses ranging from 89 days to 148 days (term), on lambs up to 2 weeks and on Welsh Mountain ewes. Pregnant ewes received a procaine spinal anæsthetic supplemented when necessary during the course of the experiment

Table 1. Glomerular Filtration-Rate and *p*-Aminohippuric Acid Clearance by the Sheep Metanephros at Different Ages

Age		No. of animals	Clearance of inulin or fructose (CIN) ml./min.	Clearance of inulin or fructose (CIN) ml./min./kgm.	Clearance of *p*-aminohippuric acid (CPAH) ml./min.	Clearance of *p*-aminohippuric acid (CPAH) ml./min./kgm.	Filtration fraction CIN/CPAH
Fœtuses	89–119 days	4	0·4 (0·2–0·8)	0·5 (0·4–0·8)	0·5 (0·2–0·9)	0·6 (0·5–0·8)	0·83
	121–140 days	5	1·7 (0·8–2·2)	0·6 (0·4–0·8)	2·3 (1·2–2·8)	0·9 (0·5–1·3)	0·67
	Full term	1	4·3	0·9	6·4	1·3	0·69
Lambs	1 day*	1	3·7	1·6	4·3	1·9	0·84
	1 day	1	9·0	4·5	12·5	6·3	0·71
	4 days	1	14·0	3·5	18·7	4·7	0·74
	13 days	1	11·4	3·5	22·1	6·7	0·52
Adults		3	91·4 (55·3–138)	2·8 (1·9–3·2)	417 (300–645)	12·7 (10–19·0)	0·22

Figures in parentheses indicate the range of values obtained.
* 24 hr. after delivery at full term by Caesarean section from comatose ewe.

by intravenous sodium thiopentone ('Pentothal'). Anæsthesia was induced and maintained in lambs by intravenous administration of sodium thiopentone. The ewes were placed in a cradle which held them lying at an angle of 45° above the horizontal. The dorsalis pedis artery and vein of one leg were then cannulated for subsequent arterial blood sampling and intravenous injections. The fœtus was delivered by Caesarean section and supported on a warmed platform placed beside the ewe's abdomen. The fœtal head was enclosed in a polythene bag of warm saline as soon as it was delivered in order to prevent the onset of respiration. The umbilical cord was kept slack and moist with warm saline. Cannulation of the fœtal bladder was performed following an abdominal incision under procaine local anæsthesia. An umbilical artery and vein were catheterized through tributaries for giving intravenous injections and the removal of blood samples.

Experiments on unanæsthetized ewes required a preliminary operation under thiopentone anæsthesia for cannulation of one dorsalis pedis artery and vein and for the passage of a Foley catheter (size 12 French gauge) into the bladder. Allowing 24 hr. for recovery it was then possible to obtain data over several weeks from each animal. For the duration of an experiment the animals were restrained by placing the limbs through holes in a canvas hammock slung sufficiently low to allow the animals to stand.

A priming dose of 20 per cent sodium *p*-aminohippurate and 10 per cent inulin (for determination of glomerular filtration-rate) was given intravenously followed by a maintaining dose made up in isotonic sodium sulphate, 0·6 or 0·9 per cent sodium chloride, administered by drip. The quantities to be injected were determined on a body-weight basis. In fœtuses no inulin was injected, instead the glomerular filtration-rate was calculated from the clearance of endogenous fructose[1]. The concentrations of *p*-aminohippurate were determined by the method of Smith *et al.*[2]. Inulin was hydrolysed and determined as fructose by the method of Chinard, *et al.*[3].

Steady plasma concentrations of *p*-aminohippuric acid were tried over a wide range. It was found that an increase in the plasma-level of *p*-aminohippuric acid to 10·0 mgm./100 ml. in any one fœtus gave similar values for plasma clearance. However, in lambs and adults most clearances were determined using plasma concentrations in the range of 1–4·5 mgm./100 ml.

Table 1 shows that the glomerular filtration-rate increases with age when expressed as ml./min. and does in fact attain adult values in the lambs when expressed as ml./min./kgm. The clearance of *p*-aminohippuric acid also increases with age expressed either as ml./min. or ml./min./kgm. but does not attain adult values. Calculation of the filtration fraction further reveals the lag in appearance of tubular secretory activity for *p*-aminohippuric acid.

In the adult kidney of several species *p*-aminohippuric acid is so efficiently secreted by the tubular tissue that its plasma clearance represents the effective renal plasma flow. The values found for adult sheep are similar to those obtained by McDonald and MacFarlane[4] and are assumed to represent the renal plasma flow. The low clearance values of this substance (as ml./min./kgm.) obtained in fœtuses and lambs compared with the adults suggest either an immature secretory mechanism or an inadequate distribution of plasma flow to the secretory cells.

It is hoped that work in progress on the direct measurement of renal blood flow and on the determination of the renal extraction ratio for *p*-aminohippuric acid will demonstrate the relative importance of these two factors.

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Effect of Moderate Low Temperature on the Accumulation of Liver Fat of Rats fed an Amino-Acid Unbalanced Diet

It has been reported by several workers that various kinds of unbalanced amounts of amino-acid in the diet lead to the accumulation of liver fat of rats. For example, the rats fed a diet containing 8–9 per cent of casein supplemented with sulphur-containing amino-acids and choline develop moderate fatty liver, but if the same diet low in casein is supplemented with both sulphur-containing amino-acids and threonine, the accumulation of fat is reduced[1,2].

In a previous report Yoshida, Ashida and Harper demonstrated a distinct decrease in liver fat accumulation due to threonine deficiency by moderate caloric restriction; however, this effect was not observed when the fatty liver was caused by choline deficiency[3]. From those results and the isotopic experiments[4] we suggested that rats fed this amino-acid unbalanced diet consume an excess of calories in relation to their intake of balanced protein and that the extra calories are used for the synthesis of fat. In subsequent work, it was demonstrated that only 20 per cent reduction of caloric intake prevented accumulation of fat in the liver due to threonine deficiency[5].

It is expected that when the caloric requirement of rats fed an unbalanced amino-acid diet is increased, an excess of calories in relation to their intake of balanced protein would be catabolized without accumulation of fat in the liver. Exposing the animals to cold is one of the means of increasing the caloric requirement of the animals.

The present communication describes observations made on the effect of the environmental temperature on the production of fatty liver. Moderate low temperature effectively prevented the development of fatty livers in rats fed the threonine-deficient diet, but the accumulation of liver fat due to choline deficiency was less affected at the same temperature.

Male weanling albino rats of the Wistar strain were used. The experiments consisted of two parts; in one a threonine-deficient diet was fed, in the other a choline-deficient diet. The threonine-deficient diet contained: casein, 8 per cent; DL-methionine, 0·3 per cent; sucrose, 82·5 per cent; sesame oil, 5 per cent; salt *B* (ref. 6), 4 per cent; and a complete vitamin mixture including 0·15 per cent choline chloride. The choline-deficient diet contained the same amounts of casein, salt *B*, sesame oil and sucrose, but choline chloride was omitted from the vitamin mixture and 0·5 per cent cholesterol was added. In both parts of the experiments, one group of rats was kept at 25° and the other group at 9°. The rats were fed *ad lib.* and the food consumption was measured daily. At the end of two weeks the rats were killed, their livers were removed and fat extracted with ether from the dried and finely powdered liver.

Table 1. EFFECT OF ENVIRONMENTAL TEMPERATURE ON LIVER FAT CONTENT OF RATS FED ON THREONINE- OR CHOLINE-DEFICIENT DIETS

	Threonine deficiency		Choline deficiency	
	25°	9°	25°	9°
Gain in 2 weeks (gm.)	23·4 ± 2·5*	22·7 ± 4·1	16·5 ± 2·1	13·1 ± 2·1
Food intake†	100	154	100	144
Liver fat (per cent)‡	24·7 ± 1·9	11·9 ± 0·7	28·1 ± 2·9	21·1 ± 2·7

* *S.E.*

† Food intake is expressed as percentage of the group at 25°.

‡ Liver fat is expressed as percentage of dried liver.

In the threonine-deficient experiment, the rats at 9° took about 150 per cent of the diet as compared with the group at 25°; however, the gains in body-weight for 2 weeks were essentially the same for both groups. This fact indicates the caloric requirement at 9° is significantly higher than at 25°. The liver fat of rats at 25° was 24·7 per cent on a dry weight basis; but the value decreased to 11·9 per cent when the rats were kept at 9°. Thus a moderate cold temperature prevented the fatty infiltration without appreciably reducing growth. These results quite resemble those obtained in the previous experiment[3], which showed a moderate caloric restriction in the threonine deficiency prevented the fatty liver.

The effect of moderate low temperature was not so effective in preventing the fatty liver caused by choline deficiency. The liver fat of rats was 28·1 per cent at 25°; at 9° it was 21·1 per cent, still higher than the normal range. Several workers reported the reduction of liver fat of choline-deficient animals when they were kept at 1° (refs. 7–10). Sellers stated that the choline-deficient diet must be restricted greatly to achieve the lowering of liver lipids at normal room temperature and the prevention of the liver fat accumulation at 1° was not due to the balance of energy requirement and caloric intake[7]. In threonine deficiency, deposition of fat in the liver was prevented by either moderate caloric restriction or moderate low temperature. These facts support our previous concept[3] that fatty liver caused by threonine deficiency is the result of a relatively high intake of calories in relation to the intake of balanced protein.

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PHARMACOLOGY

Biologically Active Citrulline Peptides

To the best of our knowledge no peptides have so far been synthesized with citrulline as a building component. This alone could be sufficient reason to study citrulline peptides, but our investigations were prompted by other considerations as well.

The interesting change in the pattern of biological activity which occurs when members of the adrenocorticotrophic hormone–melanocyte stimulating hormone (ACTH–MSH) group of the pituitary hormones are briefly heated in an alkaline solution[1] was tentatively explained[2] by the assumption that the arginine moiety of these molecules was converted into an ornithine residue. We have suspected that the possibility that hot dilute alkali could degrade the guanidino group of arginine to a ureido rather than to an amino group should also be considered, the more so since streptidine, for example, is rapidly converted in hot alkaline solution to streptureа but only very slowly to streptamine[3]. Thus, perhaps citrulline peptides might be responsible for the increased and prolonged activity of alkali-treated peptides related to MSH.

In the work recorded here citrulline-containing analogues of biologically active peptides were synthesized and examined. No melanophore-stimulating properties were exhibited by the pentapeptide L-histidyl-L-phenylalanyl-L-citrullyl-L-tryptophyl-glycine; instead, significant inhibition of the activity of α-MSH by this compound was observed. On the other hand, 1-L-citrulline bradykinin, that is, L-citrullyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine, shows bradykinin-like activity on the isolated rat uterus at a concentration of 0·1 γ/ml. In our experiments synthetic bradykinin prepared in our laboratory was active at 0·0001–0·001 γ/ml.; the corresponding nonapeptide with nitroarginine residues instead of the arginine moieties was active at 0·1 γ/ml. Neither the *N*-benzyloxycarbonyl derivative of this last-mentioned nonapeptide nor the octapeptide L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-L-seryl-L-phenylalanyl-L-arginine[4] was active in the same test, even at a concentration of 10 γ/ml. Each of the three nonapeptides was less than 0·01 as potent as bradykinin as a vasodepressor agent in the anæsthetized rat[5].

The hormone analogues, 8-L-citrulline vasopressin and 8-L-citrulline oxytocin, were active in several biological tests. Thus, 8-L-citrulline vasopressin was found to be about eight times less active than lysine vasopressin in the rat vasopressor test[5] and also in the dog anti-diuretic assay[6], but at least as active as lysine vasopressin on the isolated rat uterus. The 8-L-citrulline oxytocin showed about the same activity as oxytocin on the rat uterus, but half the activity in the avian depressor test.

The experience gained with citrulline-containing analogues of biologically active arginine peptides seems to indicate that the highly ionized character of the guanidino group contributes to, but, at least in certain instances, is not essential for biological activity. Some significance must undoubtedly be ascribed to the geometry of the arginine moiety.

In the synthesis of the aforementioned peptides the stepwise synthesis[8] by the nitrophenyl ester method[9] was applied with a few exceptions. The required *p*-nitrophenyl ester of *N* α-benzyloxycarbonyl-L-citrulline (m.p. 163–165°; $[\alpha]^{20}_{D} - 19°$ (*c*. 1, dimethylformamide)) was prepared via *N*α-benzyloxycarbonyl-L-citrulline (m.p. 115–117°). Since we did not succeed in preparing the *p*-nitrophenyl ester of *N*α-benzyloxycarbonyl-L-histidine, the azide of this protected amino-acid served in the last coupling step in the synthesis of the pentapeptide mentioned above. In the preparation of 1-L-citrulline bradykinin and also in that of bradykinin itself the *p*-nitrophenyl ester of *N*-benzyloxycarbonyl-*O*-benzyl-L-serine (m.p. 55–57°; $[\alpha]_{20}^{D} - 12°$ (*c*. 2, dimethylformamide) and the *p*-nitrophenyl ester of benzyloxycarbonyl-L-prolylglycine (m.p. 143·5–145°; $[\alpha]^{22}_{D} - 63°$ (*c* 1, dimethylformamide)) were used. The coupling of this dipeptide represents the only departure from the stepwise approach used in these studies.

The details of the synthesis of the citrulline peptides will be presented elsewhere.

We thank Prof. Aaron B. Lerner and Dr. M. Ruth Wright for the assays on the melanocyte-stimulating activity of the above-mentioned pentapeptide.

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Tryptamine and Serotonin Receptors

A RECENT communication by Vane *et al.* entitled "Tryptamine Receptors in the Central Nervous System"[1] concluded, on the basis of indirect evidence, that the receptors for tryptamine and for serotonin (5-hydroxytryptamine) were the same. Because the authors neglected to mention some earlier evidence of a more direct kind which showed clearly that certain receptors for these two substances were different, we wish to direct attention to this contrary evidence before the conclusion about their identity becomes widespread.

Two kinds of evidence suggest that the receptors are different.

(*a*) When a suitable antimetabolite of serotonin (such as 1-benzyl-2-methyl-5-methoxytryptamine or 1-benzyl-2-methyl-5-hydroxytryptamine) is given to a dog the rise in arterial blood pressure due to injected serotonin is completely prevented, but the rise due to injected tryptamine is not. This shows that the antimetabolite has blocked the serotonin receptors which control blood pressure, but not the tryptamine receptors. This experiment was published by us in 1957 (ref. 2).

(*b*) By storage of suitable tissues at 4° the ability to respond to tryptamine is retained, but the ability to respond to serotonin is lost. This experiment was carried out with sections of sheep carotid arteries and suggested[3] that the receptors for tryptamine were more stable than those for serotonin.

Although the experiments of Vane *et al.* were concerned with receptors in the central nervous system and our own dealt with peripheral ones, the conclusion of their communication implied (probably quite unintentionally) that the supposed identity of the two receptors might be generally true in many tissues. We therefore wish to direct attention to the direct evidence which indicates that at least in some parts of the body receptors for serotonin and for tryptamine are clearly not the same. Before their identity even in the central nervous system is accepted as established it would be desirable to have more direct evidence.

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WE were aware of the work to which Profs. Woolley and Shaw refer but it did not seem relevant to the central nervous system. We did not intend to give the impression that all responses to 5-hydroxytryptamine (5-HT) and tryptamine throughout the body are mediated by an action on receptor sites common to both drugs: indeed, there is substantial evidence that they are not. The blood-pressure response, on which Woolley and Shaw worked, provides an interesting example. The ratio of activities of 5-HT and tryptamine in several different biological preparations[1] suggests straight away that the blood-pressure response differs from other responses. The actions of 5-HT on blood pressure are a complex of: (*a*) stimulation of carotid chemoreceptors[2]; (*b*) stimulation of the heart; (*c*) liberation of catecholamine into the circulation from the adrenal medulla[3]; (*d*) direct action on vascular smooth muscle, unaffected by pretreatment with reserpine. The effects of tryptamine seem to be mediated by the local release of catecholamine in the vessel walls, for the pressor response is: (*a*) prevented by cocaine; (*b*) abolished by pretreatment of the animal with reserpine; (*c*) then restored by an infusion of noradrenaline[4]. Thus, for equipressor effects, there are receptors which are activated by 5-HT and not by tryptamine and vice versa. The antagonism by 1-benzyl-2-methyl-5-methoxytryptamine of the pressor response to 5-HT, but not to tryptamine, may be explained, as suggested by Woolley and Shaw, by supposing that the two amines act on different receptors.

In a much simpler system, the isolated rat stomach strip, 1-benzyl-2-methyl-5-methoxytryptamine, does not antagonize the direct actions of 5-HT or tryptamine[5]: but brom-LSD and LSD, both of which are good general antagonists of indoles, appear to antagonize 5-HT to a greater extent than tryptamine[6]. This difference disappears if the preparation is pretreated with an amine oxidase inhibitor[4]. In the same tissue, inhibition of amine oxidase selectively potentiates the response to tryptamine without altering that to 5-HT[7]. Here, then, tryptamine is acting on two receptors (smooth muscle and amine oxidase), whereas 5-HT, perhaps because of a diffusion barrier, is acting on only one of them (smooth muscle); the effects of amine oxidase inhibitors and brom-LSD can be explained on this basis.

To say that, in some sites, 5-HT and tryptamine may act on different receptors (including active spots on amine oxidase, chemoreceptors and different types of chromaffin cells) does not exclude the possibility that these substances act mainly at receptors common to both drugs peripherally and centrally. To explain the main actions we described, we thought it unnecessary to postulate different types of receptors for tryptamine and 5-HT in the central nervous system.

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ANATOMY

Centrifugal Fibres to the Retina in the Pigeon

DESPITE the description of centrifugal fibres in the retina of the bird by Cajal, Dogiel and others[1,2], and the finding of Wallenberg[3] of Marchi degeneration passing from the brain-stem to the retina, there is still considerable doubt as to the existence of a centrifugal fibre system in the visual system.

In a previous investigation of the avian visual pathway indirect evidence was obtained for such a centrifugal projection originating in the nucleus isthmo-opticus[4]. The relevant findings are that this nucleus undergoes complete cell loss following removal of the eye, and that the nature and time-course of the fibre degeneration in the isthmo-optic tract are strikingly different from that in all other components of the visual pathway. To prove that this nucleus is the site of origin of centrifugal fibres it must be demonstrated that localized lesions involving it result in fibre degeneration which can be traced through the associated pathway to terminate in the retina. Such degenerating fibres have now been traced, by the paraffin Nauta method, through the isthmo-optic tract into the dorsal part of the optic tract. From here the fibres can be clearly followed into the central region of the optic chiasma and thence into the contralateral optic nerve. In sections of the eye heavy degeneration is found in the optic nerve head, from which it spreads out in the optic nerve layer to all parts of the retina as recorded in the Marchi experiments of Wallenberg[3]. Occasional degenerating fibres can be traced through the ganglion cell layer and across the internal plexiform layer to terminate around cells on the inner aspect of the bipolar cell layer. The form of the axon terminals closely resembles those described by Cajal around the amacrine cells. The ipsilateral optic nerve and retina were completely free of degeneration in all experiments.

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PATHOLOGY

Experimental Production of Spermatic Granuloma in Rats

SPERMATOZOAL invasion of the epididymis is a recognized phenomenon in man, but when reviewing the literature on the subject it was found that no detailed systematic experimental study of the cellular reactions to spermatozoa in the interstitial tissues of the epididymis in animals has been made, nor has there been any such correlation with human lesions of this type.

Russell and Friedman[1] severed the vas deferens in rats, transplanted it to either the scrotal or abdominal walls, or to the retroperitoneum. 'Granulomatous' lesions developed at the resected ends, and no such lesions were found when the testes were removed. No histological descriptions were given other than the mention of pools of sperm surrounded by a wall of histiocytes (macrophages) nor did they state the time-interval between resection and killing of the animals. However, the legends of two of their photographs (very low power fields) carry the times 2½ and 3 weeks. The purpose of this preliminary communication is to describe the experimental production of spermatic granuloma by a variety of techniques with the histological changes found at different intervals post-operatively and to attempt to relate the findings to human material available to me.

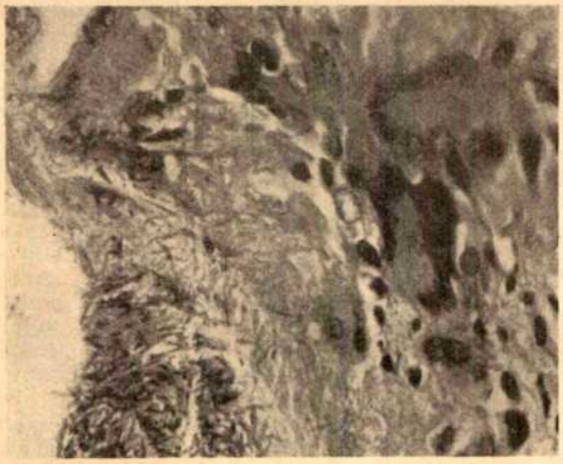

Fig. 1. Giant cells and epithelioid cells on right and aggregated spermatozoa on left in interstitial tissue of rat epididymis. Rat killed at 208 days

Adult male Wistar rats of average weight 250–300 gm. were anæsthetized with open ether. Five groups of experiments were performed. Group I, The seminal vesicles were divided and one half was removed to allow the seminal fluid to flow into the peritoneal cavity. Group II, The junction of the vas deferens with the epididymis was ligated. Group III, The rete testis was tied. Group IV, About 2 cm. of the beginning of the vas deferens was resected. The proximal end was diathermized and the tail portion of the epididymis was allowed to leak into the scrotal sac. Group V, Ligation of the body of the epididymis and the junction of tail of epididymis and vas was performed. The animals were killed at 2, 4, 7, 10, 14, 29, 39, 98 and 208 days.

In Group I no lesions developed; repair and organization of the resected seminal vesicles occurred almost immediately. To date, Group IV was the best type of experiment, of those used in this series, for examination of the cellular reactions to extravasated spermatozoa. After operation extensive extravasation of spermatozoa occurred, and at 2 days polymorphonuclear cells surrounded and intermingled with the spermatozoa.

At 4 days histiocytes of epithelioid character began to arrange themselves in a palisade at the periphery of the pool of sperms. The outline of the lesion gradually assumed a convoluted appearance after 14 days and the histiocytes began to group themselves into giant cells at 39 days approximately. 208 days post-operatively the spermatozoa appeared to be morphologically still intact in the main part of the lesion but were aggregated, suggesting agglutination. The cellular reaction at this stage was very striking and included numerous giant cells of Langhans' type (Fig. 1). The lesions now closely resembled tuberculosis and particularly in some foci where spermatozoa were not obviously present though the products of their disintegration may well have been.

The literature on human material from both autopsy and surgical sources shows considerable difference of opinion, and the varied manner of clinical and pathological presentation tends to suggest in some of the reports that ætiological agents other than the spermatozoa may be involved, for example, *Myco. tuberculosis* and *N. gonorrhoeae*.

This work shows that the spermatozoa *per se* are responsible for a definite type of cellular reaction similar to that excited by the tubercle bacillus, and may be caused by acid fast fractions known to be present in spermatozoa. These experimental lesions show a close similarity to primary spermatic granuloma in man at its various stages.

The final fate of the spermatozoa in these lesions and the mechanisms of healing are still being studied and a more detailed account will be published at a later date.

I thank the Medical Research Council of Ireland for a grant-in-aid for this work.

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Pulmonary Tumours in Mice induced by Oral Isoniazid and its Metabolites

Juhašz *et al.*[1] induced solitary or multiple pulmonary adenomas in 7 of 45 albino mice which received intraperitoneal injections of isoniazid in water for three months and survived for periods up to 7½ months. Mori *et al.*[2] incorporated the same compound in varying doses in the diet of *dd* mice for periods of 2, 3 or 4 months, obtaining 100 per cent of pulmonary 'tumours' in mice surviving the largest dose for 7½ months. Subcutaneous injection was also effective.

In the experiment reported here strain *Balb/c* virgin female mice, 8 weeks old, received equimolecular doses of isoniazid, hydrazine sulphate and the sodium salt of isonicotinic acid by mouth daily in watery solution over a period of 46 weeks, when the mice were killed (Table 1). All those which received isoniazid or hydrazine sulphate developed pulmonary tumours but only 19 per cent of those receiving the sodium salt of isonicotinic acid. Of 216 untreated virgins or breeders of the same strain 50 died before 46 weeks and 166 at periods varying from 47 to 121 weeks, but none had pulmonary tumours. Andervont and Dunn[3] observed 24 per cent of these tumours in breeding females of this strain 17–25 months of age.

Table 1. INCIDENCE OF PULMONARY TUMOURS FOLLOWING TREATMENT

Compound	Daily dose (mgm.)	No. of treatments	Total dose (mgm.)	No. of mice surviving for 46 weeks following start of treatment	Mice with tumours	
					No.	Per cent
Isoniazid	2·0	251	502	38	38	100
Hydrazine sulphate	1·13	251	283	22	22	100
Sodium salt of isonicotinic acid	1·30	251	326	21	4	19

The induced tumours were multiple in all the mice treated with the first two compounds, on an average 4 per mouse following isoniazid and 18 per mouse following hydrazine sulphate. Approximately 40 per cent of all the tumours were in the left lung, which is the smaller, and in the right lung, which has four lobes, the lobar distribution ranged from 13 to 22 per cent. The morphology of the tumours is shown in Table 2. A very large proportion had the structure of papillary or solid adenomas, but 15 tumours were judged on histological grounds to be undergoing malignant change. In addition 10 tumours were regarded as carcinomas on the grounds of atypical

Table 2. Incidence of Different Types of Pulmonary Tumours

Compound	No. of mice	Total No. of tumours	Morphology of the tumours: Adenoma		Adenoma becoming malignant		Carcinoma	
			No.	Per cent	No.	Per cent	No.	Per cent
Isoniazid	38	144	134	93	6	4·1	4	2·7
Hydrazine sulphate	22	393	378	96	9	2·2	6	1·7
Sodium salt of isonicotinic acid	21	5	5	100				

structure, or local invasion of lung alveoli or bronchioli. In one mouse treated with hydrazine sulphate metastasis to the liver and ovary had occurred.

Thus isoniazid has now been shown to induce pulmonary adenomas in mice from three sources, and occasional pulmonary carcinomas in *Balb/c* mice. Hydrazine sulphate has similar properties in *Balb/c* mice and one tumour metastasized to distant organs. The sodium salt of isonicotinic acid has very low tumour-inducing properties.

Krüger-Thiemer[4] showed that isoniazid is converted in the rat and ox to isonicotinic acid and hydrazine, which is rapidly degraded to ammonia. Our results suggest that it is possible that the tumours observed after the administration of isoniazid to the mouse are due mainly to the liberation of hydrazine. If this is so, any drug which liberates hydrazine on metabolism must be regarded as potentially carcinogenic and its use in medicine limited as far as is practicable.

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Virus-like Particles in the Cells of a Primary Carcinoma of the Liver

PRIMARY hepatocellular carcinoma of the liver (malignant hepatoma) is a tumour which is frequently encountered in the Bantu of Southern Africa. After an extensive review of the ætiology and pathology of liver cancer and cirrhosis in Africa, Steiner[1] came to the conclusion that "a likely cause for African liver cancer was viral infectious hepatitis with, possibly, prior malnutrition acting as an adjuvant". In view of the possible ætiological importance of virus infection we decided to conduct electron microscopical investigations of liver biopsies from Bantu patients with malignant hepatomas. The technique used in the preparation of biopsy material for cytological studies with the electron microscope has been described[2].

Surgical liver biopsies from three patients were studied. The diagnosis of malignant hepatoma was confirmed by light microscopical investigations of the same material. In one of these patients structural modifications of the cytoplasm of the tumour cells, suggestive of virus-like particles, were encountered. Two types of particles were observed: (*a*) The paranuclear cytoplasm showed the presence of dense zones containing a fine granular material (Fig. 1). The zones were more or less sharply defined, but no obvious limiting membrane could be discerned. These structures correspond to the 'viroplasm' one of the type of cytoplasmic inclusions described by Bernhard *et al.*[3,4] in the Shope fibroma. (*b*) In the paranuclear cytoplasm of other cells from the same tumour, a later stage in the development of the virus-like particles was observed (Fig. 2). In these cells membranes appeared within the matrix of the 'viroplasm' to form spherical corpuscles (Fig. 2, arrow 1). Some of the corpuscles contained granular material (Fig. 2, arrow 2), which appeared similar to the granular matrix of the surrounding 'viroplasm' (Fig. 2, arrow 3). Other corpuscles had no granular core (Fig. 2, arrow 4). These virus-like particles are again similar to cytoplasmic inclusions described by Bernhard *et al.*[3,4] in the cells of the Shope fibroma. According to Bernhard[5] the particles without any granular material should be regarded as "abortive

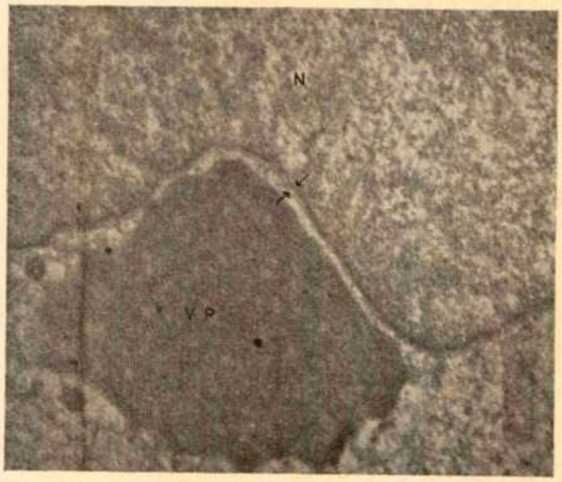

Fig. 1. 'Viroplasm' (*VP*) in the paranuclear cytoplasm of a malignant hepatoma cell. *N*, Nucleus. The arrows point to the double nuclear membrane. Note indentation of nuclear membrane by 'viroplasm'. (× 30,000)

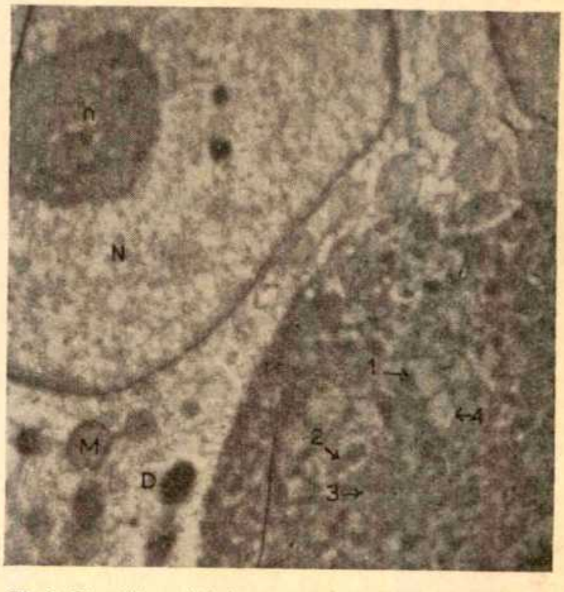

Fig. 2. Virus-like particles in paranuclear cytoplasm of malignant hepatoma cell. *N*, Nucleus; *n*, nucleolus; *M*, mitochondria; *D*, dense body. Arrow 1, Spherical corpuscle; arrow 2, granular material within corpuscle; arrow 3, 'viroplasm'; arrow 4, empty corpuscle. (× 10,000)

forms of the virus the membranes of which were closed before the granular mass could be included". Although there was a marked variation in the size of the spherical corpuscles, the majority had a diameter of approximately 240 mμ.

Virus-like particles have previously been visualized in the liver cells of patients with infectious hepatitis[6,7]. To our knowledge no such particles have been described in malignant hepatoma cells in human beings. It is obvious that the presence of these particles in hepatoma cells cannot be considered as final proof of the role of previous virus infection in the ætiology of this tumour. Our observations do, however, lend some support to the conclusion reached by Steiner[1] in 1960. A detailed report of the cytology and histochemistry of malignant hepatoma will be published elsewhere.

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IMMUNOLOGY

Oral Immunization of Guinea Pigs with Avirulent *Pasteurella pseudotuberculosis*

Immunity to pseudotuberculosis and plague is generally considered to be of a cellular nature[1-3]. Because of the common *R* antigen in *Pasteurella pestis* and *P. pseudotuberculosis*[4] and the stable immunity that this immunochemically common factor is able to induce against both infections[5], further experiments with avirulent strain 32/IV of *P. pseudotuberculosis* were thought to have some bearing on the elucidation of theoretical and practical problems of immunity in plague.

Virulent *P. pseudotuberculosis* bacteria, which in many respects behave like the enteric group, are often the cause of mesenterial lymphadenitis[6,7] in human beings. The aim of this investigation was to find out: (*a*) if avirulent strain 32/IV-bacteria could be transported to the intestinal tract, and if they were able to establish themselves in the mesenteric lymph nodes; (*b*) if the persistence in the mesenteric lymph nodes were sufficiently long to trigger off the formation of a demonstrable immunity.

The persistence of strain 32/IV after oral inoculation was investigated in 10 guinea pigs, of which two were killed after 1, 3, 7, 11 and 18 days, respectively. As can be seen from Table 1 the avirulent strain was isolated during this period five times from the mesenteric lymph nodes, three times from the small intestine and cæcum, respectively, and twice from the rectum. Although I used enrichment methods the bacteria were never found in the spleen, liver, lungs or heart blood.

Table 1. Persistence of *P. pseudotuberculosis* Avirulent Strain 32/IV after Oral Inoculation*

Isolation after	1 day		3 days		7 days		11 days		18 days	
Guinea pig No.	1	2	3	4	5	6	7	8	9	10
Site										
Heart blood	–	–	–	–	–	–	–	–	–	–
Lungs	–	–	–	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–	–	–	–
Liver	–	–	–	–	–	–	–	–	–	–
Mesenteric lymph nodes	+	–	+	–	–	+	–	+	–	+
Stomach contents	+	–	–	–	–	–	–	–	–	–
Small intestinal contents	+	+	–	–	–	–	–	+	–	–
Cæcal contents	+	+	–	–	–	+	–	–	–	–
Rectal contents	+	–	–	–	–	–	–	+	–	–

* 50 × 10^9 bacteria of strain 32/IV in 5-ml. saline were given *per os* through a urinary catheter of 1 mm. diameter.

Table 2 shows the results of oral compared with intraperitoneal immunization.

Obviously it is possible to protect guinea pigs against massive infection with *P. pseudotuberculosis* by the oral route. The avirulent bacteria are able to persist in the mesenteric lymph nodes for at least three weeks. No tests after that time were carried out. Since there was no evidence that strain 32/IV penetrated farther than the intestinal wall and the mesenteric lymph nodes, hæmatogeneous or further lymphatic dissemination apparently was not a factor in eliciting the immune response. The significance of humoral antibodies has not yet been investigated.

Table 2. Vaccination with *P. pseudotuberculosis* Avirulent Strain 32/IV* and Challenge with *P. pseudotuberculosis* Virulent Strain 14/1†

Mode of vaccination	No. of vaccinated animals	No. of survivors 30 days after challenge
Orally	8	7/8‡
Intraperitoneally	8	8/8
Controls		2/8§

* Strain 32/IV approximately, 50 × 10^9 bacteria orally. Strain 32/IV approximately, 10 × 10^7 bacteria intraperitoneally.

† Strain 14/I approximately, 10 × 10^7 bacteria intraperitoneally 3 weeks after vaccination.

‡ One guinea pig died intercurrently after a fortnight in a staphylococcal infection.

§ Six controls died within a week in *P. pseudotuberculosis* septicæmia, one of the survivors had a large abscess in the ventral abdominal wall from which the challenge strain was recovered as well as from the spleen and lungs.

Up to now, only a few strains belonging to *O*-group VI have been isolated[8]. It was considered necessary to investigate if this serological group in itself is endowed with certain antigenic properties which enable it to protect against strains belonging to other *O*-groups and another species. Strain 190/IV and strain Ikegaki[9] were tested. Both proved avirulent to guinea pigs[10], but gave after three weeks no protection against a standard test infection. It thus seems reasonable to conclude that *O* IV-antigen alone cannot be responsible for the immunity induced.

The effectiveness of oral immunization has traditionally been considered inferior to parenteral. In my experiments the avirulent strain 32/IV could be re-isolated from only 5 out of 10 guinea pigs. To leave the guinea pigs without feed 24 hr. before oral administration of the bacteria seems to result in better immunization.

In another series of experiments on guinea pigs intranasal instillation of a few drops of a 48 hr. broth culture of avirulent strain 32/IV conferred a solid immunity against massive infection with virulent *P. pseudotuberculosis* by the same route. The pathological changes and bacteriological findings in these animals will be described in a forthcoming communication[11].

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Immunological Properties of Electrophoretic Fractions of Human Growth Hormone

In a previous investigation we showed that human growth hormone, prepared by Raben's method, presented on agar gel electrophoresis two distinct strongly staining components and a less-distinctive faster moving component[1]. Component 1 moved between β- and α-globulin, component 2 corresponded to α-2-globulin and component 3 to α-1-globulin. Similar findings have been reported with starch gel electrophoresis[2,3]. Our previous work confirmed the suspicion of Boucher[4] and Hirschfeld *et al.*[5] that the human growth hormone preparations, obtained by both Raben's and Li's methods, are contaminated with human albumin.

In the present work the immunological properties of the individual components of human growth hormone were investigated. The hormone was prepared from human pituitaries according to Raben's[6] method, and the same batch, *A*–3, was used throughout. The immunological methods used have been described in detail[1].

Following agar gel electrophoresis[7] for $3\frac{1}{2}$ hr. and identification of the fractions by staining a longitudinal slice which had been cut off and transferred to another glass plate, the individual components (Fig. 1) were prepared for testing by cutting horizontal strips of gel. The pieces of gel containing the respective component were eluted with saline, centrifuged and the supernatant used. When tested by the Ouchterlony agar gel double diffusion test[8] using antiserum against whole human growth hormone, components 1, 2, 3 formed one precipitation line (Fig. 2). These lines were confluent between themselves and with the precipitation line formed by whole human growth hormone, indicating immunological identity. When tested by agar gel immunoelectrophoresis[7] using antiserum against whole human growth hormone, the level of the maximum convexity of the precipitation arc formed by the individual components differed corresponding to the electrophoretic mobility of the respective component. Fig. 3 illustrates the behaviour of components 1 and 2. None of the components gave a precipitation line with antiserum against human albumin by either agar gel double diffusion or immunoelectrophoresis. The eluate of the area of application (area 0 of Fig. 1) reacted with the antiserum to whole human growth hormone, both in agar gel double diffusion and agar

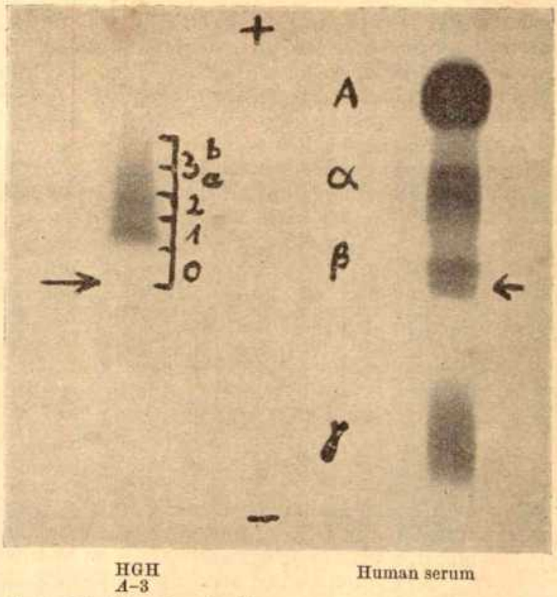

Fig. 1. Gel electrophoresis of human growth hormone (HGH, 0·35 mgm.) as compared with human serum. Arrows indicate origin. Stained with amido-black

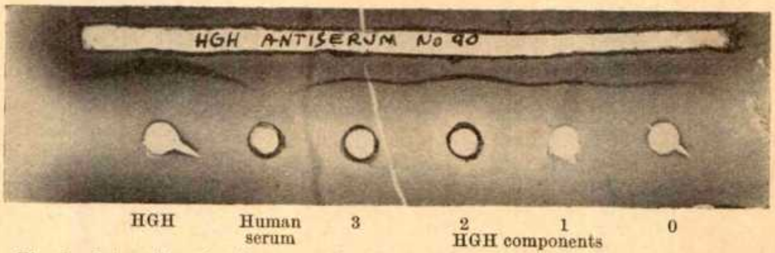

Fig. 2. Interaction of antiserum against whole human growth hormone (HGH) with individual components of human growth hormone and human serum as determined by the Ouchterlony agar gel double diffusion technique. Stained with amido-black

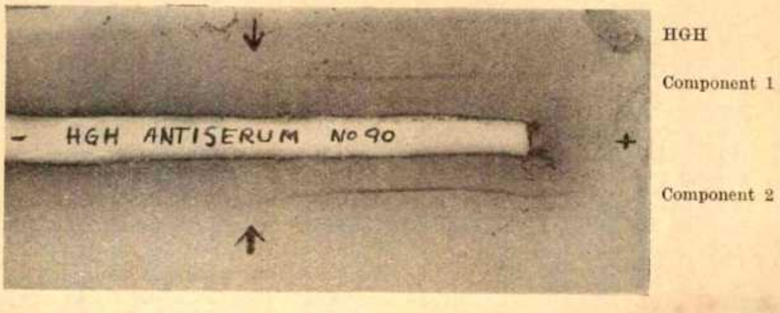

Fig. 3. Interaction of antiserum against whole human growth hormone (HGH) with individual components of human growth hormone as determined by agar gel immunoelectrophoresis. Arrow indicates origin. Stained by amido-black

gel immunoelectrophoresis, demonstrating that some hormone did not migrate from the application area, but its quantity was too small to be visible by the method of staining used. When testing the individual components with antiserum against human growth hormone and antiserum to human albumin by the hæmagglutination technique[9], it was found that all the components reacted with the antiserum to human growth hormone, but only component 3*b*, and sometimes 3*a*, reacted with the anti-albumin serum (Table 1).

Table 1. HÆMAGGLUTINATION REACTIONS OF ELECTROPHORETIC COMPONENTS (*C*) OF HUMAN GROWTH HORMONE (HGH) WITH ANTISERUM TO WHOLE HUMAN GROWTH HORMONE OR TO HUMAN ALBUMIN

Antibody Antigen	Anti-HGH	Anti-human albumin
Area of application 0	+	–
C 1	+	–
C 2	+	–
C 3*a*	+	+ or –
C 3*b*	+	+

As a further step in this investigation, antiserum to the individual components of human growth hormone was prepared by immunizing rabbits (component 3 was used non-divided). The gel strip containing the respective component was homogenized in water and the rabbits injected as follows: 0·1 ml. of a mixture of approximately 0·5 mgm. hormone component in 0·3 ml. water and an equal amount of complete Freund's adjuvant (Difco) was injected intradermally into each of two footpads: the remaining 0·4 ml. was injected subcutaneously. Two weeks later the procedure was repeated injecting the two other footpads. After a further two weeks about 1 mgm. of the respective human growth hormone component mixed with 0·6 ml. water and 0·6 ml. complete Freund's adjuvant was injected subcutaneously. Ten days after this subcutaneous booster the rabbits were bled; the antisera titres ranged between 1/10,000 and 1/12,000. When testing the interaction of the individual components with the different antisera by agar gel double diffusion, it was found that each component reacted with its own antiserum as well as with the antisera against the other components used. Similar findings were registered by the hæmagglutination technique (Table 2). The only difference encountered was the positive reaction between human albumin (1/1,000) with the antiserum to component 3. In the agar gel double diffusion test human albumin did not react with any of the antisera.

Table 2. HÆMAGGLUTINATION REACTIONS OF WHOLE HUMAN GROWTH HORMONE (HGH), ITS ELECTROPHORETIC COMPONENTS (*C*) AND HUMAN ALBUMIN WITH ANTISERUM TO THE INDIVIDUAL COMPONENTS OF HUMAN GROWTH HORMONE (ANTI-*C*)

Antibody Antigen	Anti-*C* 1	Anti-*C* 2	Anti-*C* 3
Whole HGH	+	+	+
C 1	+	+	+
C 2	+	+	+
C 3	+	+	+
Human albumin 1/1,000	–	–	+

The results of these experiments indicate that the components of human growth hormone, separated by agar gel electrophoresis, are immunologically identical and antigenically potent, and that the component contaminated with albumin can be singled out and separated. The formation of the precipitation arc at different levels by the individual immunologically identical components on immunoelectrophoresis explains the occasional two-peak precipitation line obtained when using whole human growth hormone[5]. The biological activity of the components of human growth hormone remains to be established.

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RADIOBIOLOGY

Protection of Primates against Lethal Doses of X-Radiation

THE protective effect of 2-amino ethylisothiouronium bromide (AET) against lethal doses of total body X-radiation administered to rats and mice is well documented[1,2]. In the dog, however, the protective effect of AET is obscured by the toxic response to the drug when levels sufficient to be protective are administered[3]. AET and cysteine administered simultaneously, however, were effective against a lethal exposure of X-radiation in dogs.

The protective effect of AET in primates has received only modest consideration. In the single investigation known to us[4], doses of X-rays were used which were lower than those utilized in the present report. Further studies concerning the effects of radio-protective agents in primates exposed to large single doses of X-radiation are of interest for several reasons. Positive protective effects of the two sulphydril-containing compounds would establish a continuity of the mechanism of action in the vertebrate kingdom. Furthermore, the phylogenetic relationship of monkey and man would suggest that knowledge of the active dose, toxicity of the drug, as well as the biological effects in the monkey could be more reasonably extrapolated to man. It is to these ends that the present report is directed.

Rhesus monkeys (*M. mulatta*) weighing between 4 and 5 kgm. were used. Radiation was administered with a General Electric Maxitron 250 X-ray tube operated at 250 kVp. and 30 m.amp., using filters of 0·5 mm. copper and 1 mm. aluminium. The half-value layer for the radiation was 1·5 mm. of copper. The animals were placed 150 cm. from the focal spot of the tube and the dose-rate in air at this distance was 11 r./min. The animals were completely immobilized in a wire cage and were rotated in the X-ray beam at the rate of 1 r.p.m. Measurements with a Victoreen meter at numerous places on the skin and in the abdominal cavity of a dead monkey under these conditions showed that the dose-rate at all places measured was within 10 per cent of the dose-rate in air at the centre of the field. Prior to each radiation session the dose-rate in air at the centre of the field was measured with a Victoreen meter which had been previously calibrated against a Bureau of Standards meter. AET and cysteine were rendered soluble, brought to pH 7 with 3 N sodium hydroxide, and administered intravenously immediately before exposure to X-radiation at a dosage-level of 100 and 500 mgm./kgm. body-weight respectively. Epinephrine was injected subcutaneously to avoid collapse of blood pressure.

A marked increase in the 30-day survival of rhesus monkeys receiving AET and cysteine prior to a total body X-radiation of 800 r. was observed. Five of the six drug-treated animals not only survived the 30-day period following exposure to 800 r., but are alive and in apparent good health at the time of writing, 90 days following irradiation. In contrast, 5 of the 7 unprotected animals died within 14–18 days following radiation.

A progressive decline in both leucocyte count and hæmatocrit was observed following X-radiation in non-treated animals. In the protected animals, however, the degree of depression was less dramatic (Figs. 1 and 2). Moreover, by day 21 following exposure a gradual recovery of both white cell counts and hæmatocrit was evident. A similar recovery pattern was noted in the two non-treated animals which ultimately survived the 30-day post-exposure period.

No obvious effect of the drug was observed in the injected animals. In contrast, the non-injected animals presented the classical symptoms of radiation sickness, namely, apathy, diarrhœa, vomiting and anorexia.

Six animals both treated and untreated had been ovariectomized six months prior to X-radiation. Since these animals were in good health and could not be utilized for breeding purposes they were included in this investigation. In view of the radio-protective effect of œstrogen in mice[5], it was conceivable that the operated animals might be more sensitive to X-radiation than intact monkeys. It was apparent, however, that the response of these animals, as indicated by both survival and hæmatological changes, was not affected by this operative procedure.

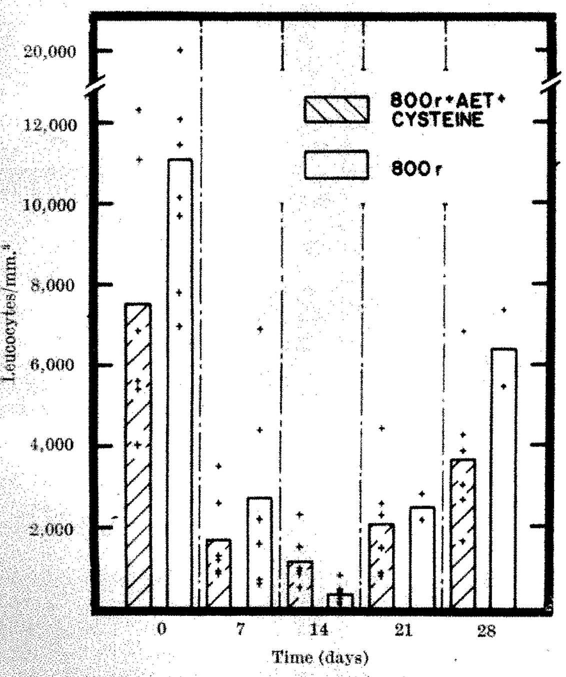

Fig. 1. Effect of AET and cysteine on leucocyte counts in irradiated monkeys. The figure demonstrates the changes in leucocyte counts/mm.³ of individual monkeys immediately prior to (0 days) and at weekly intervals after irradiation

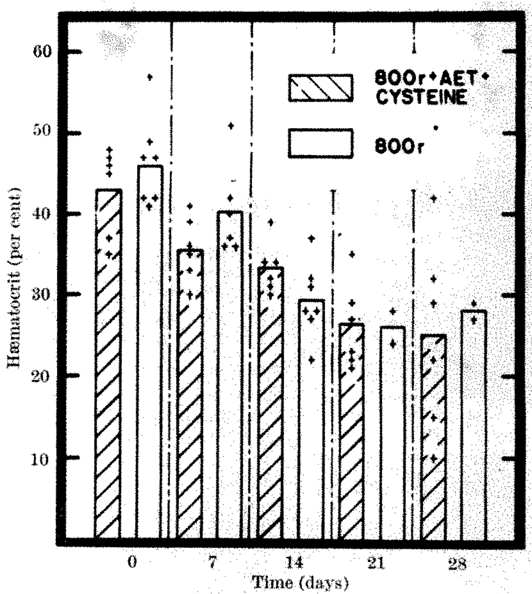

Fig. 2. Effect of AET and cysteine on the hæmatocrit of irradiated monkeys. The figure demonstrates the changes in the hæmatocrit of individual monkeys immediately prior to (0 days) and at weekly intervals after irradiation

These results clearly demonstrate the practicality of protecting primates against 800 r. administered during a period of 1 hr. It seems logical to conclude from these results that it is possible to protect monkeys against a dose of 800 r. administered at the rate of 11 r./min., provided that AET and cysteine are injected simultaneously before X-radiation.

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Curative Effect of Homologous Bone Marrow Transfusions in Rats receiving Lethal Doses of 'Myleran'

Weston *et al.* have reported on the curative effect of isologous bone marrow transfusions in aplastic anæmia in rats by 'Myleran', a radiomimetic chemical[1]. Talbot and Elson improved the survival of rats with isologous bone marrow after the administration of a lethal dose of dimethyl homologue of 'Myleran'[2]. The curative effect of isologous bone marrow also has been reported in mice by other investigators after administering lethal doses of thioguanine and nitrogen mustards[3–5]. Dunjic and Maisin confirmed the results obtained by Weston *et al.*, and investigated the relationship between the number of transfused bone marrow cells, and the survival of rats receiving a lethal dose of ‘Myleran’[6]. In the latter experiment the rats used were from a randomly bred strain. Further investigation established that individual antigenic differences between the host and the donor in randomly bred rats could be overcome either by administering higher lethal doses of ‘Myleran’ or by transfusing bone marrow on the day when immunological defence reactions of the host are at the lowest level[7].

In previously reported experiments the homologous bone marrow transfusions failed to restore the bone marrow aplasia induced by radiomimetic agents[2,4,5,7]. However, the trials were performed under experimental conditions (that is, the dose of agent, amount of injected bone marrow, time and route of injection) which allowed successful transplantation of isologous bone marrow in animals of inbred, as well as randomly bred, strains. I have already suggested that homologous bone marrow transfusions could produce more interesting results after administrations of higher doses of ‘Myleran’ and/or bone marrow cells[7]. In the present report the first successful results obtained with homologous bone marrow transfusions in rats receiving lethal doses of ‘Myleran’ are given.

The white male *L*-strain rats used were bred in the Institute by random mating. At the start of the experiment they were 3–4 months old, weighing 140–150 gm. Homologous bone marrow donors were female *R*-rats from an inbred strain purchased from R.I.T. Laboratories (Louvain, Belgium) when six weeks old. Methods of the preparation and mode of administration of ‘Myleran’ and suspensions of bone marrow cells are presented elsewhere[6,7]. The doses of ‘Myleran’ administered orally ranged from 20·7 to 41·4 mgm./kgm.; these were lethal between the eighth and fifteenth day for rats which were not treated. The $LD_{50/30}$ and *MLD* (maximum lethal dose) for the *L*-strain rats are, respectively, 14 and about 17 mgm./kgm. The bone marrow suspensions, containing 128 × 10^6 nucleated bone marrow cells were injected on the first or third day following the ‘Myleran’ administration.

Table 1. MORTALITY DATA OF *L*-STRAIN RATS TREATED WITH 128 × 10^6 HOMOLOGOUS BONE MARROW CELLS FROM *R*-STRAIN DONORS AFTER ADMINISTRATION OF SINGLE SUPRALETHAL DOSES OF ‘MYLERAN’

	No. of rats	mgm./kgm. of ‘Myleran’	Percentage mortality at 30 days	Distribution of deaths (in days)	Median survival time (in days)
Bone marrow injected on the first day	10	20·7	70·0	11–16	13
	11	27·6	63·6	9–19	13
	11	34·6	63·6	11–15	13
	10	41·4	80·0	8–14	10
Bone marrow injected on the third day	9	20·7	44·4	14–20	15
	12	27·6	58·3	10–16	11
	10	34·5	40·0	9–16	12
	10	41·4	60·0	8–18	11

The results are given in Table 1. The homologous bone marrow transfusions given on the first day following administration of ‘Myleran’ decreased the 30-day mortality-rate to values of between 80 and 63·6 per cent. When bone marrow transfusions were given on the third day, the decrease of mortality was more important and the mortality-rates varied from 60 to 44·4 per cent. The mortality-rates seem to be independent of ‘Myleran’ dosage for both the injection days and the dose-range of ‘Myleran’ used.

It is interesting to compare these results with those obtained previously with different donor–host combinations[7,8]. From my intra-strain experiments it is known that the mortality of highly inbred *R*-strain rats is completely suppressed if 32 × 10^6 nucleated bone marrow cells are transfused invariably on the first or third day after administration of ‘Myleran’ dose of 20·7–34·5 mgm./kgm.[8]. In order to decrease the mortality of randomly bred *L*-strain rats first, four times as many cells must be transfused, and secondly, this decrease of mortality is dependent on the dose of ‘Myleran’ and/or the time of bone marrow transfusion[7]. By transfusing 128 × 10^6 bone marrow cells on the first day, the mortality decreases with increasing doses of ‘Myleran’ and is almost completely suppressed for doses between 27·6 and 41·4 mgm./kgm. When the same amount of bone marrow cells is given on the third day, the mortality decreases independently of ‘Myleran’ dose to about 30 per cent. Furthermore, by increasing the interval between the administration of ‘Myleran’ and the injection of bone marrow to 5 days, the dose–response relationship again becomes dependent on the dose of ‘Myleran’ and its pattern resembles the one obtained by giving the bone marrow on the first day. On the other hand, in one of the inter-strain experiments, homologous bone marrow of randomly bred *H*-strain rats failed to decrease the mortality of *L*-strain rats after administering 20·7 and 27·6 mgm./kgm. of ‘Myleran’ 3 days before[7].

Thus the general pattern of dose–response relationship obtained in the present investigation partially resembles that encountered in randomly bred animals. As a comparison, Fig. 1 gives the relationship between the dose of ‘Myleran’ and the mortality at 30 days for randomly bred *L*-strain rats receiving bone marrow from donors of the same strain and from inbred *R*-strain donors on the first and third day after administration of increasing doses of ‘Myleran’. While the results obtained on the third day are quantitatively almost the same, the homologous bone marrow injected on the first day did not reduce the mortality to the same level. The present results confirm the fact that on the third day after administering ‘Myleran’, the immunological defence re-

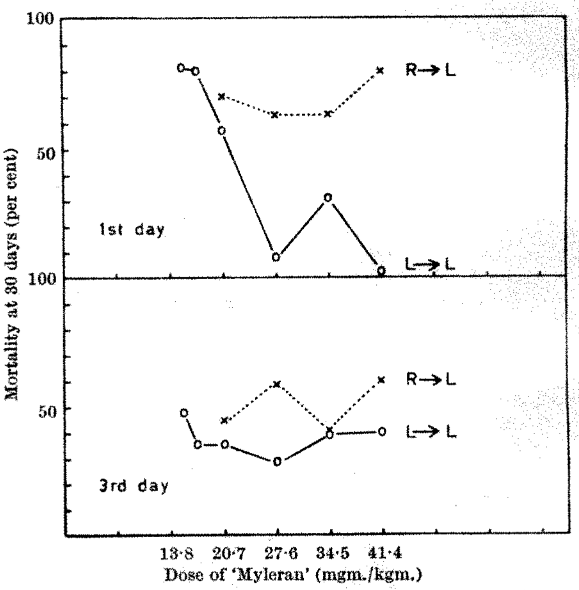

Fig. 1. Relationship between the dose of ‘Myleran’ and the mortality at 30 days of randomly bred *L*-strain rats receiving 128 × 10^6 nucleated bone marrow cells on the first or third day after ‘Myleran’ administration. *L*—*L*, Bone marrow from donors of the same strain (ref. 7); *R*—*L*, bone marrow from donors of the inbred *R*-strain

actions of the host are at the maximum of depression[7]. Also it can be assumed that quantitative differences on the first day indicate more or less a close antigenic relationship between the donor and host animals.

It can be concluded, in opposition to what was previously believed[2,4,5,7], that, under some experimental conditions, the homologous bone marrow treatment may be obtained following the administration of radiomimetic chemicals, and that the requirements for successful homologous bone marrow transplantation are similar to those met in bone marrow experiments with irradiated mammals[9].

This work was supported by grants from the Home Office (Ministère de l'Intérieur) of Belgium. The 'Myleran' (1 : 4-dimethanesulphonyloxybutane) used in this work was kindly supplied by Burroughs Wellcome and Co. Laboratories, London.

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Preparation of Iodine-131 Labelled Human Growth Hormone of High Specific Activity

RECENT procedures for the immunological assay of protein hormones in human plasma[1-3] require the routine preparation of hormones labelled with iodine-131 of high specific activity.

The present methods available[1,3,4] require the handling of 10–80 mc. of the isotope in volatile or organic solvents, and applied to human growth hormone[3] give low specific activity (5 μc./μgm.). Purification of the labelled hormones is required to remove damaged components.

The following method has been developed which allows the preparation in 1 hr. of 2–3 μgm. of labelled human growth hormone with specific activities of 250–590 μc./μgm. (up to 1·2 atoms of iodine per molecule). Human growth hormone (5 μgm.) is reacted with 2–4 mc. of carrier-free iodine-131 using chloramine *T* as the oxidizing agent; the efficiency of utilization of the iodine is 60–75 per cent. No purification of the product is required and it has been shown to be immunologically identical with unlabelled human growth hormone.

Reaction is carried out in the rubber-capped vial (10 ml.) in which the isotope is packaged ('*IBS*/3', Radiochemical Centre, Amersham). Care is taken that the vial is kept upright in transit. The iodine-131 (2–4 mc.) is used within 24 hr. of arrival. Storage of the material for 5 days results in the loss of iodinating activity.

The activity is counted using a scintillation counter with a well-type, sodium iodide crystal with the geometry modified such that 2 mc. in 0·05 ml. gives approximately 400 counts/sec. The rubber cap is removed and counted separately.

To the sample are added human growth hormone (5 μgm.) and fresh chloramine *T* (88 μgm.). These additions (0·025 ml.) are in 0·05 *M* sodium phosphate buffer (*p*H 7·5) and are made using an 'Agla' micrometer syringe by injection through the rubber cap. After gentle mixing, the vial is stood for approximately 1 min. and sodium metabisulphite is then added (0·1 ml. of a 2·4 mgm./ml. w/v solution in *p*H 7·5 phosphate buffer). This addition prevents any subsequent iodination of the dextran-gel bed by converting iodine to iodide. This residual iodide is diluted with carrier potassium iodide (0·2 ml. of a 10 mgm./ml. solution in the phosphate buffer). The reaction mixture is transferred to a dextran column followed by a single wash with further potassium iodide (0·4 ml. of a 10 mgm./ml. w/v solution in the phosphate buffer). The reaction vial and pipette are then counted.

Separation of labelled human growth hormone from the reaction mixture is carried out by gel filtration using a 1 gm., *G*50 dextran column. Equilibration of the gel and elution from it are carried out with *p*H 8·6 sodium barbitone buffer (0·07 *M*). Before the dextran column is used, crystalline bovine serum albumin (20 mgm. in 1 ml. *p*H 8·6 buffer) is passed through it, followed by 20 ml. wash with the same buffer. This 'presaturation' of the dextran with albumin allows the subsequent recovery of 3–4 μgm. of labelled hormone from the dextran in 80 per cent yield.

The elution pattern, determined by counting 1 ml. eluates, is reproducible and allows for a routine collection of a discard fraction (1·5 ml.), a protein peak (4·0 ml.) and a salt peak (3 × 5 ml. fractions) into calibrated counting vials. The protein peak is collected into a vial containing bovine serum albumin (0·5 ml. of a 5 mgm./ml. solution in *p*H 7·5 sodium phosphate buffer, 0·5 *M*, in normal saline). After counting each fraction the amount of labelled hormone eluted is calculated and this is then diluted to a final concentration of 50 mμgm./ml. with *p*H 7·5 sodium phosphate buffer (0·05 *M*) in normal saline containing 0·5 mgm./ml. of bovine serum albumin.

In control experiments without added hormone, radioactivity is recovered quantitatively from the vial and the column in the salt peak. The yield of the reaction in the presence of protein is therefore based upon the percentage of the total radioactivity collected in the salt peak. The iodinated protein is distributed between a fraction adsorbing to the glass surface of the reaction vial (26 per cent), the protein peak eluate (60 per cent) and a third fraction remaining adsorbed to the column (14 per cent). The recovery of the iodinated protein added to the column is 82 per cent.

Antisera to unlabelled human growth hormone when incubated with two preparations of labelled human growth hormone (237 and 590 μc./μgm.) were found to react with 95·5 per cent of the modified antigen. The extent of this reaction was judged by the transfer of the labelled hormone to the antibody region on electrophoresis of the incubation mixture. The preparation with a specific activity of 590 μc./μgm. contained 1·2 atoms of total iodine per molecule, assuming an isotope abundance of 90 per cent. This was assayed in terms of unlabelled human growth hormone by an immunoelectrophoretic method. Immunological activity was unaffected at this level of substitution as was human growth hormone substituted with iodine-127 up to 9·5 atoms per molecule. Substitution with up to 19·3 atoms iodine-127 per molecule resulted in a reduction of immunological reactivity to 1–2 per cent.

The undiminished immunological reactivity and the absence of degradation products in the labelled hormone appears to be due to the following factors: a low degree of iodination but with high specific activity; the use of carrier free isotope in a high efficiency reaction; and the rapid separation of the iodinated hormone from unreacted isotope.

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BIOLOGY

Inhibition of Cell Division by Carbon Dioxide

A DEMONSTRATION of utilization of carbon dioxide by propionic acid bacteria[1] drastically changed the long-entertained idea that carbon dioxide played only an indirect part in heterotrophic growth, by utilization of organic substances produced in autotrophic assimilation. After later evidence indicated that growth of many bacterial cultures stopped below a minimum concentration of carbon dioxide, the opinion gained support that heterotrophs, and indeed all living cells, can utilize, and possibly even require, carbon dioxide for growth and reproduction[2]. Reproduction on a cellular level means cell multiplication. The requirement for carbon dioxide in cell multiplication could not be proved or disproved until a technique was developed enabling separation of growth from cell division[3,4].

The effect of carbon dioxide on cell division was studied on synchronized cultures of the high-temperature alga, *Chlorella* 7-11-05 (ref. 5). Algal cells were synchronized by intermittent light–dark régime[6,7] and then grown for 8 hr. in a complete inorganic medium at 39° and 2,000 ft.-candles. During growth period in light, carbon dioxide was supplied by bubbling through algal suspensions a 5 per cent carbon dioxide in air mixture.

After 8 hr. of growth the majority of cells generally did not reveal any internal subdivision into daughter cells. However, potentially these cells were capable of cell division. If, after 8 hr. in light, cells were transferred in the same medium into darkness, they divided in the course of time to the extent that after 16 hr. in darkness 99 or more per cent of cells appeared as small daughter cells.

To observe the effect of carbon dioxide on cell division the 8-hr. cells were centrifuged, transferred into distilled water, and placed in darkness at 35°. The percentage of carbon dioxide in air, bubbled through algal cultures during dark period, varied from 0 to 100. The progress in cell division in a given algal suspension was recorded by placing it into one of the arbitrary categories characterized by the approximate percentage of cells which divided, or were in the process of cell division, at the time of observation, after 16 hr. in darkness. The categories were as follows: 0, 1, 10, 50, 90, and 99 per cent of divided cells referred to the corresponding initial number of mother cells. A degree of cell division between these categories was qualitatively indicated by placing a plus or a minus after corresponding percentages.

Table 1. A DEGREE OF COMPLETION OF CELL DIVISION IN SUSPENSIONS OF SYNCHRONIZED 8-HR. CELLS OF *Chlorella* 7–11–05 after 16 HR. IN DARKNESS AT 35° AND DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

Figures indicate numbers of experiments in which cell division proceeded to a degree shown in the left column.

Approximate percentage of divided cells in a particular experiment	Volume (%) of CO_2 in air					
	0	1	5	50	95	100
	Molar concentration of CO_2 in liquid in equilibrium with the CO_2 in air mixture					
	0	$2 \cdot 6 \times 10^{-4}$	$1 \cdot 3 \times 10^{-3}$	$1 \cdot 3 \times 10^{-2}$	$2 \cdot 5 \times 10^{-2}$	$2 \cdot 6 \times 10^{-2}$
0		16	9	3	2	1
0 +		3				
1 −		1				
1						
1 +						
10 −						
10	1					
10 +	1					
50 −						
50	2					
50 +						
90 −	1					
90	7					
90 +	8					
99 −	8					
99	23					
99 +	2					
Total No. of experiments	53	20	9	3	2	1
On No. of days	35	16	7	3	2	1

The results of 88 observations (experiments) are presented in Table 1. Of 53 experiments conducted in carbon dioxide-free air on 35 days, cell division was completed to 90 or more per cent in 48 (90 per cent) experiments. In 5 experiments the division after 16 hr. proceeded in less than 90 but not less than in 10 per cent of cells.

At 1 per cent carbon dioxide in 16 experiments there was no cell division, and in 4 experiments the degree of division was negligible. That some cells could divide at 1 per cent carbon dioxide was probably due to imperfect synchronization of cells. Some cells in 8-hr. cell suspension proceeded further in their development than others and were capable of division even in the atmosphere of 1 per cent of carbon dioxide in air, generally unsuitable for cell division. No cell division was recorded at 5–100 per cent of carbon dioxide.

Differences in the degree of completion of cell division in different experiments at 0 per cent carbon dioxide are understandable in view of the fact that even in air free from carbon dioxide cells were influenced by carbon dioxide produced by cells in the course of their dark metabolism. The amount of carbon dioxide evolved by cells into a volume of cell suspension per unit of time evidently depended on the density of cell population (size of inoculum), which differed in different experiments. The removal of carbon dioxide produced by cells depended on the degree of aeration (volume of gas bubbled through a volume of cell suspension in a unit of time) and on the degree of dispersion of the gas (size of gas bubbles) which, for technical reasons, varied in different experiments. Thus, a combination of a heavy inoculum with low aeration might create conditions under which the concentration of carbon dioxide produced by cells remained, during some experiments, high enough to retard cell division.

In darkness, and in the absence of other ions from the suspending medium, carbon dioxide acts on *Chlorella* cells as a powerful suppressor of cell division. Attention is directed to the fact that during 8 hr. of growth, cells in these experiments accumulated all necessary constituents and were capable of division after being moved into darkness. However, this capability was or was not realized, depending on the presence of carbon dioxide in the air bubbled through cell suspension during dark period.

Under natural conditions, carbon dioxide may act as a factor regulating cell division. In unicellular organisms metabolically produced carbon dioxide diffuses to the surface of a cell and escapes into the surrounding medium. If this carbon dioxide does not accumulate in the medium beyond a critical concentration, other conditions permitting, cell division proceeds in a succession of cells uninterruptedly for indefinite time. In multicellular organisms, an increase in bulk of embryonic tissues and resulting complications in transport systems may reach a point where accumulation of carbon dioxide will act as an inhibitor of cell division. The effect of carbon dioxide on cell division depends on several factors, particularly on the presence and concentration of other ions. Elucidation of these factors is of considerable interest in studies of normal and possibly neoplastic growth.

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Wart Structure of Gymnosperm Tracheids

THE wart structure of mature tracheids was first described by Kobayashi and Utsumi[1] and by Liese[2]. It was observed to consist of small excrescences (warts) (0·03–0·04μ diam.) on the inner surface of the cell wall. Subsequent surveys of the occurrence of these structures showed them to be of limited taxonomic significance[3,4].

In *Actinostrobus pyramidalis* the wart structure is large and readily seen in an optical microscope. Wardrop, Liese and Davies[5] concluded that the isolated structure consisted of a non-cellulosic membrane which formed a lining of the cell lumen and was extended in a series of diverticula protruding into the lumen. In some of these diverticula were strongly ultra-violet absorbing, nearly spherical bodies.

In a further investigation the wart structure in *Actinostrobus* has been shown by electron microscopic examination of sections after shadow casting to consist of a localized thickening of the cell wall in the form of a papilla (Fig. 1). In permanganate-fixed material the thickening could be seen to be covered by a strongly staining membrane (Fig. 2).

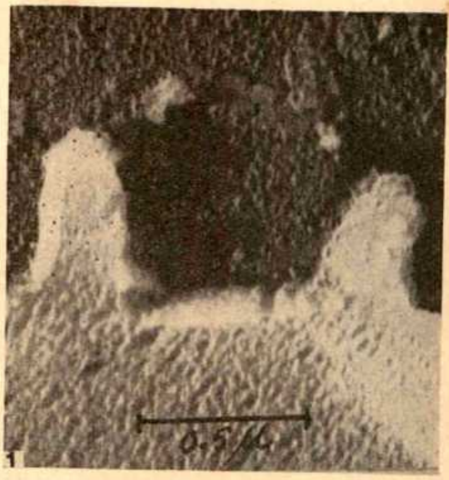

Fig. 1. Section through the wart structure of *Actinostrobus pyramidalis* showing the continuity of the wart structure with the cell wall

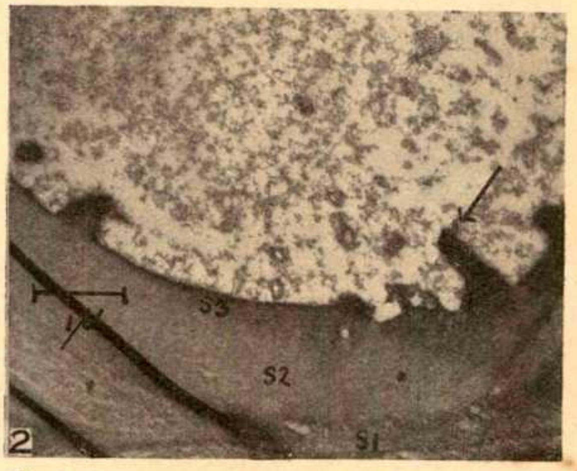

Fig. 2. Transverse section through a tracheid of *Actinostrobus pyramidalis* after fixation in potassium permanganate. Formation of the wart structure is almost complete and the heavily staining membrane covering it is apparent. The arrow indicates a spherical body at the tip of the wart structure

The first indication of the development of the wart structure was a localized withdrawal of the plasmalemma from the cell wall and the beginning of the formation of a localized thickening on the wall (Fig. 3), which continued until the wart structure reached its mature size (Fig. 2). Since the plasmalemma remained in contact with the wart thickening throughout the period of its formation, the associated spherical bodies previously described must arise within the cytoplasm. In cells which were conspicuously vacuolated the various cytoplasmic organelles were observed to be confined between the tonoplast and plasmalemma. At the death of the cell it might be anticipated that the tonoplast and membranous components of the cytoplasm would retract and dry on the surface of the lumen enclosing the remains of any cytoplasmic organelles between it and the plasmalemma. The spherical bodies associated with the wart structure of *Actinostrobus*[5] and some other species[6] are assumed to arise from these dena-

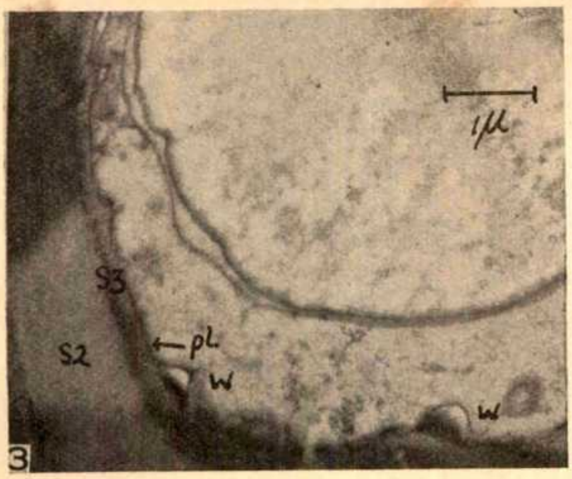

Fig. 3. As Fig. 2, showing the beginning of the formation of the wart structure (*W*) and the plasmalemma (*pl*) and the outer (S_1), middle (S_2) and inner (S_3) layers of the secondary cell wall

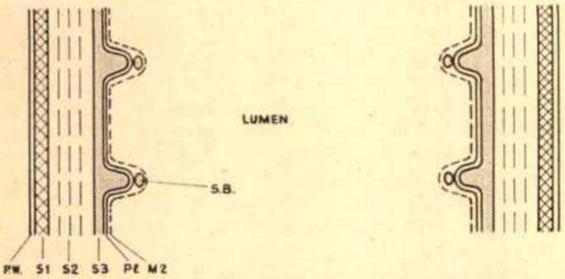

Fig. 4. Diagrammatic representation of the wart structure in *Actinostrobus pyramidalis* showing primary wall (*P.W.*). with three layers of the secondary wall (S_1, S_2 and S_3), plasmalemma (*Pl*) and spherical body (*SB*) and the second membrane system (*M*2) formed by the degenerating protoplast

tured organelles. As seen in mature xylem the wart structure may thus be represented as in Fig. 4.

From this account it is clear that the wart structure, as observed in mature cells, consists in part of a localized cell wall thickening and in part of residual components of the cytoplasm. It follows that the material previously isolated[5] consisted only of the cytoplasmic component, since the technique of isolation involved dissolution of the cell walls. Observed variations in the composition of the isolated material from different species is thus understandable[6].

From the manner of its formation it is reasonable to conclude that the cell wall component of the wart structure would be as significant a taxonomic feature as other forms of cell wall sculpturing, such as helical thickenings. However, differences in appearance between species resulting from the cytoplasmic component of the structure would not be expected to be significant in this respect.

Finally, it may be noted that Frey-Wyssling[7], in discussing the formation of secondary cell walls, has referred to the presence of a tertiary lamella between the cytoplasm and the cell wall which is present during the whole process of cell wall thickening, and of which the wart structure forms a part. From the present investigation, however, it would appear that the only limiting structure between the cell wall and the cytoplasm is the plasmalemma and the wart structure is a feature of the cell wall rather than one of the adjacent cytoplasmic membrane.

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Alteration of Sporulation in *Aspergillus niger* by Heat and Deuterium Oxide

ZAMENHOF[1] reported that unstable phenotypic variants sometimes result when bacterial cells or spores are heated. We wish to report the results of a similar investigation of the effects of heating spores of the mould *Aspergillus niger*. Our work has been extended to include the effect of heating the spores in a 99·5 per cent deuterium oxide medium, as well as heating in a water medium and in the dry state.

In this work we utilized *Aspergillus niger* A.T.C.C. No. 10581, and the minimal growth medium of Nicholas[2]; cultures were grown in a 30° C. incubator. Five-ml. aliquots of a washed spore suspension (about 10^8 spores/ml.), in 0·01 per cent sodium lauryl sulphate or 99·5 per cent deuterium oxide, was heated in a water or oil bath maintained at the indicated temperature. Dry spore preparations were prepared by lyophilization. After heating, a suitable aliquot was cooled quickly by dilution with ice-cold 0·01 per cent sodium lauryl sulphate; serial dilutions and pour plates were made by conventional methods.

Table 1 shows that spores suspended in deuterium oxide are considerably more resistant to heat inactivation than spores suspended in water. However, the most notable variation observed in heated spores involved the subsequent capacity for sporulation—inability of aerial hyphæ to differentiate into conidiophores, changes in the appearance and pigmentation of conidiophores with only minimum sporulation allowed, or delayed sporulation. The elicitation of non-sporulating strains by heating was confined almost exclusively to spores suspended in deuterium

Table 1. EFFECT OF HEAT ON *Aspergillus niger* SPORES

Heating conditions	Suspension medium	Survival (per cent)*	Sporulation variants among survivors (per cent)
3 hr., 70° C.	H_2O	0·001–0·0001	< 5
1 hr., 100° C.	H_2O	< 0·00001	—
3 hr., 100° C.	None (dry)	0·1–1	None detected
1–2 hr., 100° C.	D_2O	0·01–0·1	48

* Ability to form colonies on minimal media supplemented with 2 per cent 'Bacto' agar, 0·1 per cent Difco proteose peptone and 0·1 per cent Nutritional Biochemicals yeast extract.

Table 2. SPORULATION VARIANTS OF *Aspergillus niger* PRODUCED BY HEATING SPORES IN DEUTERIUM OXIDE FOR 1–2 HR. AT 100° C. TYPICAL RESULTS

	No. of strains isolated	Percentage
Green, irregular conidiophores	15	32
Blue, irregular conidiophores	1	2
Non-sporulating	6	13
Black, wild-type conidiophores	24	52

oxide. These sporulation anomalies have been observed to persist for several transfers, though reversion to wild-type usually occurs after several weeks.

The incorporation of 70–100 per cent deuterium oxide into culture media has been found to inhibit sporulation in *A. niger*[3,4], but recovery ensues after a short lag period in aqueous media. In such cases of organisms grown in deuterium oxide, it has proved difficult to differentiate between possible primary biological effects of deuterium and secondary isotope effects[5]. In the system discussed here, secondary isotope effects are relatively insignificant, in view of the short period of exposure and the known low metabolic rate of spores in general.

The replacement of exchangeable hydrogens by deuterium in proteins, and the resulting increased resistance to heat denaturation which has been reported in some cases[6] might explain the decreased rate of inactivation of spores heated in deuterium oxide, but such does not adequately explain the increased incidence of sporulation anomalies.

Alteration of the primary structure of deoxyribonucleic acid (DNA) under these conditions is likewise unlikely since no stable nutritional mutants have been detected under the stated conditions. Yet it is evident that an impression has been made on the information-bearing units which persists for a considerable period of time after exposure to deuterium oxide as previously reported by Hughes *et al.*[7].

A more probable explanation of the synergistic effect of deuterium oxide and heat in producing sporulation anomalies is an alteration of the hydrogen-bonded secondary structure of DNA. Temperatures of 70°–100° C. (depending on the solvent) have been shown to lead to an alteration of DNA *in vitro* from a predominantly helical configuration to a random coil structure[8].

DNA extracted from variant strains has been noted to exhibit a significantly different heat denaturation profile as is seen in Table 3. Rather than an abrupt increase in absorbance from 80° to 95° C. as is observed in the case of DNA extracted from control cultures, DNA extracted from aberrant strains exhibited a random absorbance increase over the entire temperature range. This type of behaviour has been previously noted in the case of DNA isolated from a hybrid strain of bacteria containing a random-coil type of DNA[10].

Table 3. CHARACTERISTICS OF DNA EXTRACTED FROM *A. niger* BY THE METHOD OF MARMUR (REF. 9)

Strain	T_m*	Percentage of hyperchromicity at 260 mμ
10581 control	90° C.	37
Non-sporulating 8–1–1	88° C.	12
Non-sporulating 9–28	57° C.	20
Slow sporulating 4–28	88° C.	20

* T_m defined as the temperature corresponding to the mid-point of the thermally induced absorbance increase. DNA solutions were made in 0·15 *M* sodium chloride, 0 015 *M* sodium citrate solution *p*H 7·0.

Thus the consistent isolation of 'denatured' DNA's from variant strains of *A. niger* which exhibit sporulation anomalies leads one to suspect that maintenance of the secondary structure in certain regions or in certain fractions of the DNA may be of major importance in so far as the capacity for development and differentiation is concerned, while it is somewhat less critical with respect to the capacity for growth.

The synergism between deuterium oxide and heat which has been noted suggests that alteration of the random coil vs. ordered helix equilibrium of DNA structure may represent one of the primary modes of action of deuterium oxide on biological systems.

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Embryonic Death in Aged Mice

THE breeding history of mice follows a fairly well established pattern[1,2]. Initially there is a period in which the size of litter is fairly constant. This is followed by a phase during which successive litters become progressively smaller until breeding ceases. This decline in size of litter could be due either to a gradual decrease in the number of oocytes produced or to the failure of an increasing proportion of the ovulated eggs to result in living offspring. Perry[3]. from a comparison of corpora lutea counts and embryo counts in slaughterhouse pig material, postulates that the decline in fertility with age in this species is due to failure of the ageing uterus to maintain all the fertilized eggs. Jones and Krohn[4], working with mice, showed a progressive decrease in the oocyte content of the ovaries of aged females. However, they concluded that the decline in size of litter is probably not due primarily to decreasing output of oocytes. Similarly, Biggers, Finn and McLaren[2] conclude that the gradual decline in size of litter in mice and the eventual cessation of breeding are probably due to factors concerned in the maintenance of the embryos in the uterus, and not to exhaustion of oocytes.

The experiments reported here were conducted to determine whether there is greater embryonic loss in aged mice than in young mice. Pairs of *TO* strain mice were allowed to breed continuously and records kept of all litters born, the young being removed soon after birth. When the mice had reached the declining phase of their reproductive life, laparotomies were performed and the uterus inspected. This was done at approximately the fifteenth day of pregnancy, when it is easy to distinguish normal embryos from regressing implantations.

Ten aged mice were examined, and the results are set out in Table 1. The breeding history of each mouse is given, followed by the number of normal embryos and old implantation sites found at laparotomy. From previous work it is known that the incidence of post-implantation embryonic loss in young mice of this strain is very low. However, to check this five young virgin females were mated and examined on the fifteenth day of pregnancy.

Table 1. Aged Mice

Sizes of all litters born	Appearance of uterus: Normal embryos	Appearance of uterus: Regressing implantations	Age: months	Age: days
M, 6, 7, 6, 10, 7, 11, 6, 9, 2, 5	6	5	10	2
6, 8, 10, 10, 9, *M*, 10, 8, 10, 7, 7, 6	3	4	9	21
10, 6, 10, 5, 6, 8, 2	3	12	9	17
12, 10, 10, 8, 6, 3, 5, 2	2	4	9	11
9, 4, 9, 10, 12, 7, 8, 8, 3, 4, 4	4	4	9	15
8, 7, 2, 10, 10, 7, 3, 11, 8, 9, 7	11	1	9	15
8, 10, 11, 10, 7, 3, 5, 3	4	5	9	15
11, 10, 13, 12, 4, 7, 7, 6, 8	7	4	10	18
9, 7, 7, 12, *M*, *M*, 9, 7, 7, 2, 6	5	3	10	11
8, 12, 8, 9, 10, 9, 7, 4, 9, 8, 3, 3	8	5	10	20
Young mice				
Virgin	7	0	Approx. 2 months	
,,	7	1	,,	
,,	9	0	,,	
,,	3	1	,,	
,,	8	0	,,	

M, litter mutilated by mother before it could be counted.

It will be seen that the embryonic loss in the aged mice is considerably higher than in the young mice, although the total number of implantations is not much less than the number of offspring per litter born during their early breeding life.

These results provide further direct evidence of the cause of the declining fertility in aged mice. However, they still leave open the question as to whether the failure of the implanted embryos to develop is due to structural changes in the uterus, changes in the endocrine environment or lethal factors in the eggs.

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A Phosphorus–Magnesium Interaction in Tomato Nutrition

Applications of phosphorus to an acid greenhouse soil initially low in this nutrient has resulted in increased tomato yield and reduced the incidence of manganese toxicity[1]. This observation has previously been noted[2]. Other effects accruing from phosphorus applications, however, are decreased leaf contents of nitrogen and potassium[3].

In further experimental work on the acid soil referred to[1] superphosphate and lime were applied at 0 and 2½, 6 and 0 and 10 and 20 oz. per sq. yd. respectively. Apart from the beneficial effect of both fertilizers on yield, plant growth and excess manganese symptoms it was further observed that some plants developed leaf chlorosis typical of magnesium deficiency. Soil and plant analysis confirmed this observation (Table 1).

The most notable feature of the results is that at the 'no calcium' level the available magnesium of the soil and the magnesium status of the plant decreased with increasing phosphorus application. The available soil magnesium content and plant uptake increased with rate of lime applied. While levels of available soil potassium were reduced by the phosphorus fertilizer particularly in the 'no calcium' treatment no clear-cut effect was discernible in the potassium status of the plants.

Table 1. Effect of Superphosphate and Lime on Available Soil Cations and Plant Uptake

Treatment	Soil Ca (lb. per acre)*	Soil P (lb. per acre)*	Soil K (lb. per acre)*	Soil Mg (lb. per acre)*	Leaves Ca (percentage oven-dried samples)	Leaves P (percentage oven-dried samples)	Leaves K (percentage oven-dried samples)	Leaves Mg (percentage oven-dried samples)
No P No Ca	2,367	5·5	1,000	185	3·6	0·08	5·5	0·70
Med. P No Ca	2,400	10·67	978	170	2·9	0·08	6·9	0·47
High P No Ca	3,833	19·67	754	120	3·7	0·16	5·3	0·36
No P Med. Ca	2,333	4·5	972	215	2·7	0·06	5·8	0·77
Med. P Med. Ca	2,633	11·83	976	235	2·9	0·08	5·8	0·86
High P Med. Ca	4,233	24·67	856	215	2·9	0·14	6·1	0·52
No P High Ca	2,733	5·33	925	255	3·1	0·06	5·3	0·86
Med. P High Ca	2·500	13·67	905	245	3·6	0·07	5·5	0·89
High P High Ca	4,800	18·17	942	255	3·5	0·14	5·2	0·72

* Extracted with sodium acetate buffer (Morgan's reagent).

While magnesium deficiency of tomatoes has been largely attributed to excess soil potassium[4,5] on this particular soil there has been an interaction between available soil magnesium, plant magnesium and applied phosphorus.

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An Unusual Parasymbiont of Marine Lichens

The ascocarps of lichens vary in form from one species to another. Their development in several species has been examined by Doppelbaur[1], but nothing is known of the mechanism controlling their morphology. Study of a recently identified lichen parasymbiont suggests that the thallus may have a determining role.

Deakin[2] described as *Sagedia marina* a plant which was collected on "rocks submerged by the sea at high-water, Meadfoot, etc., near Torquay, Devonshire". It has since been found elsewhere between high- and low-water marks on the coasts of the British Isles and of Western Europe[3,4]. Hitherto classed as a lichen, it has usually borne the name *Arthopyrenia marina* (Deakin) A. L. Sm., though von Keissler transferred it to the genus *Thelidium* Mass.[5] and (from the synonymous *Verrucaria leptotera* Nyl.) to *Pseudarthopyrenia* Keissl.[6]. But examination of the type specimen in the British Museum Herbarium shows that it is in fact a parasymbiont on *Verrucaria mucosa* Wahlenb.—that is, a parasite which does not harm the lichen but appears to share the thallus with the lichen's own ascocarps. Authentic material of *Arthopyrenia leptotera* (Nyl.) Arn. named by Nylander and also in the British Museum Herbarium belongs to the same species and the name is a later synonym. Since it is a parasymbiont the species should be transferred to the genus *Pharcidia* Körber. This will be dealt with elsewhere; but unfortunately the combination *Pharcidia marina* is precluded by its application (illegitimate) by Bommer[7] to the species now known as *Arthopyrenia consequens* (Nyl.) Arn. (syn. *A. sublitoralis* (Leight.) Arn.).

Material of the parasymbiont that I collected in July 1961 in Ireland, West Cork (vice-county *H*3), Tragumna Bay, on acidic shale shows an interesting state of affairs. It had for its hosts fertile plants of both *Verrucaria mucosa* Wahlenb. and *V. microspora* Nyl. in adjacent patches on rocks between high- and low-water marks. The involucrellum of the parasymbiont is found to take the form of the involucrellum of its host species (Figs. 1–4). With *Verrucaria mucosa* the ascocarp of the parasymbiont is only subcompound, as is the ascocarp of the lichen host, while with *V. microspora* it is fully compound (these terms have been previously defined[8]). There can be no doubt that the two groups of material represent the same species of parasymbiont, for apart from the involucrellum the ascocarps are identical in form and dimensions. The similarity of the spores is worth emphasizing because they are peculiarly distinctive with the central septum dividing off two equal, rather elongate cells, each of which shows two locules divided by an obscure septum.

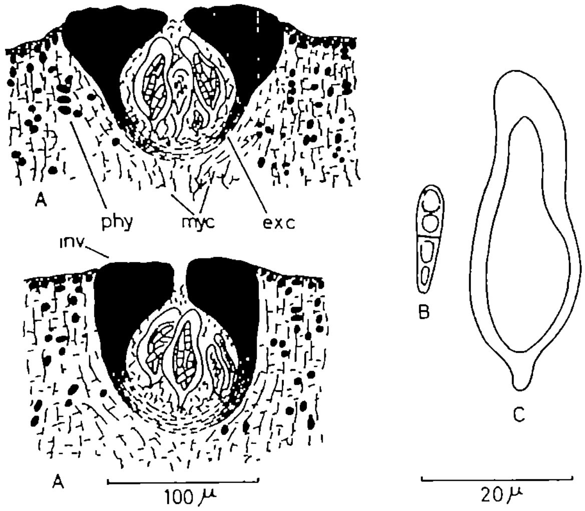

Fig. 1. '*Arthopyrenia marina*' on *Verrucaria mucosa*. Left, *A*, vertical sections of two ascocarps (*inv*, involucrellum; *exc*, excipulum; *phy*, phycobiont of host thallus; *myc*, mycobiont of host thallus); *B* and *C*, spore and ascus of '*Arthopyrenia marina*'

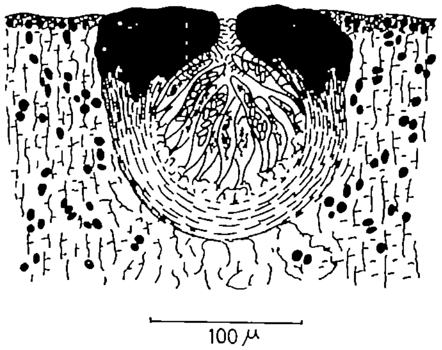

Fig. 2. *Verrucaria mucosa*, vertical section of ascocarp

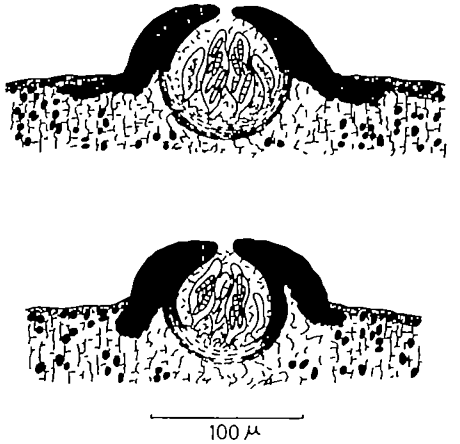

Fig. 3. '*Arthopyrenia marina*' on *Verrucaria microspora*, vertical sections of two ascocarps

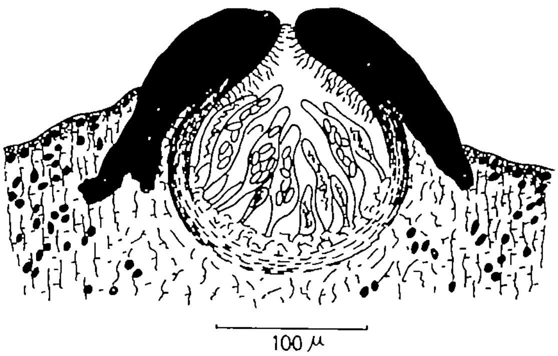

Fig. 4. *Verrucaria microspora*, vertical section of ascocarp

Doppelbaur[1] has shown that the involucrellum appears in the apex of the developing ascogone first as a ring of black plectenchyma. This then extends and thickens to assume the form of an inverted dish or bowl. The thallus could influence the course of this development, and it seems possible that the mechanism which controls the form of the involucrellum in *Verrucaria mucosa* and *V. microspora* is likewise shaping the ascocarps of the parasymbiont.

I thank the British Museum (Natural History) for facilities to examine specimens in its keeping.

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Effect of Polyvinyl Alcohol on *Chlamydomonas* Species

In previous communications it was shown that polyvinyl alcohol has a beneficial effect on the growth of peas and *Chlorella pyridinosa* and increase in chlorophyll in both those plants. When an appropriate concentration of polyvinyl alcohol was added, chlorophyll content was much greater than in the control aquarium to which polyvinyl alcohol had not been added[1,2].

We wished to discover how other species of Chlorophyta would behave if placed in a suitable concentration of polyvinyl alcohol. For this purpose we chose *Chlamydomonas* sp., which is to be found in abundance in the pool in the Academy grounds. Water taken from the pool was poured into jars.

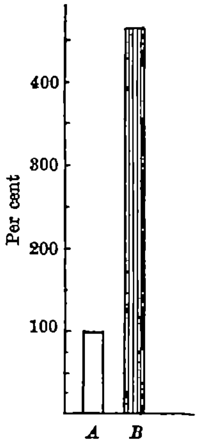

Fig. 1. Effect of polyvinyl alcohol on *Chlamydomonas* sp. *A*, Chlorophyll content in the controls; *B*, in the aquariums to which polyvinyl alcohol had been added

The appropriate concentration of polyvinyl alcohol being added to some, the others were left as controls. The greatest quantities of chlorophyll were present in the jars containing a concentration of 0·1 per cent polyvinyl alcohol. This concentration was used for the experiments on *Chlamydomonas* sp.

The experiment was begun on March 10, 1961. 10 days later (March 20) the chlorophyll content in the aquariums, that is, those without polyvinyl alcohol was determined. The results are given in Table 1 and Fig. 1. As these results show, 10 days after the addition of polyvinyl alcohol the number of *Chlamydomonas* sp. had increased considerably and the amount of chlorophyll was several times greater than that of the controls. The average chlorophyll content in the controls was only 0·072 mgm./l. whereas in the *Chlamydomonas* sp. culture to which 0·1 per cent polyvinyl alcohol had been added, it reached an average of 0·334 mgm./l. If the chlorophyll content of the controls is said to be 100 per cent (Fig. 1 *A*), then the chlorophyll content of the aquariums to which polyvinyl alcohol had been added increased by an average of 464 per cent (Fig. 1 *B*).

Table 1. Chlorophyll Content (mgm./l.) in the Cultures of *Chlamydomonas* sp.

Controls	With polyvinyl alcohol (0·1 per cent)
0·075	0·336
0·070	0·315
	0·350
0·072	0·334

From these experiments it is therefore obvious that polyvinyl alcohol has a beneficial effect on *Chlamydomonas* sp. as it had on *Chlorella pyridinosa* and *Pisum sativum* in our earlier experiments. It is worthy of note that Wetter[3] also observed the effect of polyvinyl alcohol on the growth of *Aspergillus niger*.

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ENTOMOLOGY

Effect of Cholesterol in the Larval Diet on Ovarian Development in the Adult House-Fly

The adult house-fly has been reported to require a dietary source of both protein and carbohydrate for ovarian maturation and to show little or no ovarian growth on an adult diet consisting solely of carbohydrates[1-5]. This requirement of the adult for protein could not be fulfilled by supplementing the larval diet with additional protein[4].

In the course of our investigations on nutrition and reproduction in the house-fly (*Musca domestica* L.) we observed that flies reared on larval medium supplemented with cholesterol developed mature ovaries when held on an adult diet of only sucrose and water. For these tests approximately 450 eggs from an *NAIDM* strain of flies were added to 75 gm. of cholesterol-supplemented 'CSMA' (Ralston Purina Co., St. Louis, Mo.) medium to which had been added 160 ml. of water. Flies reared on 'CSMA' medium not supplemented with cholesterol served as controls. After the pupæ were removed from the medium and cleaned the adults were allowed to emerge and then were kept on a diet of sucrose and water. At 6 days after emergence, and at subsequent intervals, the ovaries were removed from groups of 25 flies and the degree of ovarian development determined. The results in Table 1 indicate that under these experimental conditions, supplementing the larval diet with cholesterol results in ovarian maturation in about half the adult flies, even in the absence of a source of protein in the adult diet. Doubling the concentration of cholesterol appeared to give no increase in ovarian development. These results were confirmed with four other laboratory strains of house-flies.

Table 1. Ovarian Development in House-Flies reared on Cholesterol-Supplemented 'CSMA' Larval Medium and held on Adult Diet of Sucrose and Water for 6 Days

Cholesterol added to diet (Per cent dry weight)	Per cent flies with mature ovaries*
1	53±5
2	61±3
Control (non-supplemented)	2±1

*Means for 3 replicates ± *S.E.*, 2 groups of 25 flies per replicate.

This ovarian maturation occurred in the absence of any mortality, which indicated that it was not brought about by the female flies feeding on dead flies, as has previously been reported[4]. In addition, eggs from these flies were viable and produced larvæ which developed to adults.

To determine whether or not the activity observed was brought about by micro-organisms present in the larval medium, the tests were repeated with a synthetic larval diet and aseptic rearing techniques[6]. For comparison, the phytosterol β-sitosterol, which is one of the major sterols present in the 'CSMA' medium, was also tested. The cholesterol used in these tests was purified through the dibromide (m.p. 149·5° C.), and the β-sitosterol (m.p. 139–140·5° C.) was found to be greater than 97 per cent pure by gas-liquid chromatographic analysis. 25–68 per cent of the flies reared aseptically on the synthetic larval diet containing the various concentrations of cholesterol and held on an adult diet of sucrose and water were found to contain mature ovaries, whereas those flies reared on the diet containing β-sitosterol showed only slight ovarian development (Table 2). This lack

Table 2. OVARIAN DEVELOPMENT IN HOUSE-FLIES REARED ASEPTICALLY ON A SYNTHETIC MEDIUM CONTAINING VARIOUS CONCENTRATIONS OF STEROLS AND THEN HELD ON AN ADULT DIET OF SUCROSE AND WATER

Sterol in diet (Per cent dry weight)	Per cent flies with mature ovaries* Days 6	9	12
Cholesterol			
0 125	25±2	46±3	46±3
0 250	35±6	47±9	68±9
0 500	41±5	60±9	57±12
β-Sitosterol			
0 125	3±1	5±2	4±1
0 250	3±2	3±2	2±2
0 500	2±1	1±1	3±2

* Means for 3 replicates ± *S.E.*, with 2–3 groups of 25 flies per replicate.

of equivalence in the biological activity of cholesterol and β-sitosterol is in agreement with the results of our prior biochemical studies with the house-fly, using tritiated β-sitosterol, in which no conversion of β-sitosterol to cholesterol could be detected[7].

These results point to an interesting physiological role for cholesterol in the house-fly, an insect which has previously been shown to require a dietary source of sterol for growth and development[8,9] and for sustained viable egg production[10]. It is not known whether cholesterol as such is responsible for this effect, or whether it serves as precursor for an essential steroid metabolite which regulates gonadal development.

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Population Density and Atypical Behaviour in Male Odonata

A 7-YEAR study of the behaviour and population ecology of imaginal dragonflies in Dorset, England, has revealed two features common to all fifteen species studied.

(1) The upper limit of population density (the highest steady density) of males by water is determined by the behaviour of the insects themselves. The number of insects per unit area of water edge differs from species to species, but each has a characteristic highest steady density.

(2) Male dragonflies often exhibit atypical and apparently dysgenic behaviour.

The values of highest steady density for several species were checked by field experiments. In one type of experiment a population at highest steady density was collected out. When there were many surplus individuals near the pond it was quickly recolonized: the new population was found to agree closely with the original. In another type of experiment individual insects were caught elsewhere and released at a pond the population of which had been counted previously. The new population density was found frequently to equal, but never to exceed, the highest steady density recorded for the species under natural conditions. The highest steady densities, both natural and experimental, which were obtained in the study-area during 1954–60 are given in Table 1. It will be seen that the highest steady densities have an approximate inverse relationship to the size of insect. By using the figures in Table 1 it is possible to predict the approximate upper limits of male dragonfly populations of any pond the circumference of which is known. Many such predictions were made on ponds outside the study-area and were found to agree closely with the observed values.

The control of population density and hence dispersal was observed to result both from aggressive and homosexual attack. The latter was the commonest type of abnormal behaviour observed. Attempts to mate with males and females of other species were also recorded but much less frequently. Much inter-specific fighting occurred between certain species which superficially closely resembled each other (for example, *Pyrrhosoma nymphula* and *Ceriagrion tenellum*). Abnormal behaviour also occurred in habitat selection; males were often observed defending territories by waters (pools in salt marsh, etc.) which were shown to be unsuitable breeding places. Poor discrimination in selecting habitat appeared to occur when dragonflies were particularly abundant. By contrast female dragonflies were very rarely observed to oviposit in unsuitable habitats.

The work of Mayer[1] and my own more recent work[2] suggest that male dragonflies can distinguish between males and females, and between their own species and others, and have accurate habitat selection mechanisms. Therefore, it would appear that the frequently observed reactions to sub-optimal stimuli described here are atypical and are due to a lowering

Table 1. HIGHEST STEADY DENSITY OF MALE DRAGONFLIES BY WATER

Family	Species	Length (mm.) (ref. 1) (min.)	(max.)	Highest natural steady density recorded 1954–60. No of males per 100 m. of pond edge	Highest experimental steady density recorded 1954–60. No. of males per 100 m of pond edge
Aeshnidae	*Aeshna cyanea*	71	76	1	
Aeshnidae	*Aeshna juncea*	71	76	1	
Aeshnidae	*Anax imperator*	76	81	2	
Cordulidae	*Cordulia aenea*	48	51	2	
Libellulidae	*Libellula depressa*	48	48	8	
Libellulidae	*Orthetrum coerulescens*	40	43	15	
Libellulidae	*Sympetrum striolatum*	36	40	16	
Libellulidae	*Sympetrum danae*	30	33	17	
Libellulidae	*Libellula quadrimaculata*	46	48	28	(17)
Coenagriidae	*Ischnura elegans*	30	33	40	40
Coenagriidae	*Pyrrhosoma nymphula*	33	38	44	44
Coenagriidae	*Enallagma cyathigerum*	30	38	60	(60)
Lestidae	*Lestes sponsa*	33	40	65	(57)
Coenagriidae	*Coenagrion puella*	33	36	115	(61)
Coenagriidac	*Ceriagrion tenellum*	28	33	147	129

Note Figures in parentheses were obtained in a series of less than 20 experiments in each case of the threshold of response. The threshold of response appears to be low unusually often in all the species of Odonata investigated.

It must be concluded that either much dragonfly behaviour is dysgenic or that the atypical behaviour observed has some biological advantage. When females or suitable breeding areas are scarce, discrimination in choice of mate and habitat appears to decrease. Therefore the occurrence of normal or abnormal behaviour appears to be dependent on density. What seems to be a wasteful abundance of dysgenic behaviour in dragonflies may be part of behaviour mechanisms dependent on density based on threshold effects.

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MICROBIOLOGY

A Copper Requirement for the Sporulation of *Bacillus megaterium*

AN important relationship exists between metal ions and bacterial sporulation[1,2]. In previous mineral studies with spore-formers c.p. or reagent grade chemicals were used, and although important metal associations with sporulation have been revealed the presence and effect of unsuspected trace amounts of metallic ions were not considered.

This communication describes experiments in which purification procedures and additions of extremely pure chemicals give a highly pure medium which has been used to follow trace element requirements during bacterial sporulation.

The unpurified medium utilized consisted of the following c.p. or reagent grade chemicals: 1 gm. sucrose, 1 gm. NaCl, 0·2 gm. $MgSO_4 \cdot 7H_2O$, 2·4 gm. KH_2PO_4, 11 gm. $(NH_4)_2HPO_4$, 10 mgm. $FeSO_4 \cdot 7H_2O$, 10 mgm. $ZnSO_4 \cdot 7H_2O$, 7 mgm. $MnSO_4$ and 5 mgm. $CaCl_2$ in 1 litre of distilled de-ionized water, final *p*H 7·1–7·3. The phosphates were sterilized separately prior to the addition to the medium. Purification of this medium to eliminate trace contamination was accomplished by the replacement of most medium components with Matthey spectrographically pure standardized chemicals (spec.-pure) or by purification of some medium ingredients. NaCl, $MgSO_4 \cdot 7H_2O$, $MnSO_4$, $CaCl_2$, KH_2PO_4, $ZnSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$ were available in this form; however, sucrose and $(NH_4)_2HPO_4$ were not. Sucrose was purified by passage through a cation exchange column. Elimination of trace contamination by this procedure was indicated by a decrease in iron content as measured by the orthophenanthroline method[3]. Further examination for specific ions was not necessary since subsequent experiments showed that a trace metal contaminant resided in the $FeSO_4$, $ZnSO_4$, $MnSO_4$ or $CaCl_2$ portion of the medium. $(NH_4)_2HPO_4$ was purified by the 8-hydroxyquinoline method[4].

All other materials used were cleaned according to accepted procedures for the removal of trace contamination[4,5]. Polypropylene ware was substituted wherever possible[6]. All water used was first distilled and then de-ionized by passage through a cation exchange column. A spore inoculum of *Bacillus megaterium* collected from a 60-hr. culture of the unpurified synthetic medium by centrifugation in a Servall *SS*–1 angle centrifuge at 1,200*g* for 15 min. at 6° C. was used. The spores were stored at − 20° C. until use, when they were defrosted at 6° C., washed twice and standardized with distilled deionized water. A 1-ml. inoculum of 500 Klett units, as measured in a Klett–Summerson photoelectric colorimeter, consisting of about 20×10^7 spores per ml. (dry wt., 0·5 mgm.) was used per 100 ml. of medium in 350 ml. Erlenmeyer glass flasks or 500 ml. Erlenmeyer polypropylene containers. Flasks or containers were incubated at 30° C. on a reciprocating shaking machine operating at 78 four-in. strokes per min. Five-ml. aliquots were removed at various time intervals for turbidimetric determinations on a Klett–Summerson photoelectric colorimeter and direct counts in a Petroff–Hauser Chamber. Cell types were distinguished by dark contrast phase contrast microscopy. Non-refractile rod-shaped cells were scored as vegetative cells; rod-shaped cells containing a distinct refractile body were considered sporangia; and oval or spherical highly refractile bodies were counted as free spores.

Growth and sporulation patterns of *B. megaterium* in the unpurified medium in glassware and polypropylene were similar. The growth peak, indicated by turbidimetric measurements and total counts, was reached at approximately 28 hr. Sporangia started to appear at 24 hr. while spores first appeared at 28 hr. Complete sporulation, greater than 90 per cent, occurred by 38 hr. These results mean that polypropylene substitution for glassware had no effects on growth or sporulation. However, purified medium in polypropylene supported good vegetative growth only, with a growth peak at 24 hr. Sporangia or spore formation was never observed.

This suggested that the unpurified medium was deleted of some important component(s), vital for sporulation, during the purification and substitution procedure. This missing component(s) was shown to be part of the four salts: $FeSO_4$, $ZnSO_4$, $MnSO_4$ or $CaCl_2$. When these four spec.-pure salts were replaced with c.p. or reagent grade salts, the medium became conducive to sporulation. A spectrographic qualitative analysis of a mixture of these four salts showed traces of aluminium, copper, strontium, magnesium, silicon, cobalt, and molybdenum. In the quest for this component(s) necessary for sporulation, the following compounds were added to the purified synthetic medium: spec.-pure $CoSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and c.p. or reagent grade $NiCl_2 \cdot 6H_2O$, $NaSiO_3 \cdot 5H_2O$, $AlCl_3$, $LiCl_2$, CrO_3, H_3BO_3 and $BaCl_2 \cdot 2H_2O$. Only the addition of the copper salt (0·05 μgm./ml.) induced sporulation. The concentration of the $CuSO_4 \cdot 5H_2O$ must be within the 0·025–0·10 μgm./ml. range to stimulate maximum sporulation. Below the lower limit sporulation is decreased until 0·01 μgm. per ml. is reached when no sporulation occurs. Greater than 0·1 μgm. per ml. results in inhibition of germination of the spore inoculum, and although eventually the cells will germinate and cells reproduce after a long lag phase incomplete sporulation occurs by 62 hr.

These results indicate that copper must be added as a supplement to the purified medium to facilitate sporulation and suggest that this element may be the trace contaminant component of the regular salts which is absent from the medium upon replacement with spec.-pure salts.

Occasionally, the addition of copper alone did not stimulate sporulation; however, in all cases, the addition of molybdenum as spec.-pure $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (50 μgm. per ml.), although not having an effect on sporulation when added alone, resulted in sporulation when added with the copper.

Although copper has never been implicated as a requirement for sporulation, previous studies[6] indicate its presence both in vegetative cells and spores.

The specific function of minerals in sporulation is not known. In all probability, they act catalytically in the activation of many enzyme systems. They may accelerate the complex enzymatic systems involved in intracellular protein degradation and resynthesis postulated[7] as the basic mechanism in sporogenesis.

It is now possible to study in more completeness, without concern about trace contamination, the role of other metals in sporulation. A more detailed publication reporting experiments indicating when the copper requirement occurs during the sporulation cycle and the effects of deletion of various metals from the Cu–Mo supplemented purified medium is in preparation.

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Lysogeny in the Genus *Lactobacillus*

We have reported[1] the isolation of 14 phages active on *Lactobacilli* by the use of an enrichment technique in which 10 batches of sewage were incubated with groups of *Lactobacilli*. Each group comprised 12 different strains. These experiments have now been repeated with one modification: the sewage in the mixtures has been replaced by an equal volume of tap water. No phage was isolated. It would appear that none of the 120 strains of *Lactobacillus* used was lysogenic and that the phages originally isolated were present in the sewage.

Using the same media and techniques, lysogeny in many strains of the species previously used was investigated as follows. Fifteen strains of *Lactobacilli* were grown in all combinations of two according to Scholtens's method[2] by sub-culturing two drops of an overnight culture into 40 ml. of broth. Cultures were incubated for 14 days, centrifuged to clarity and the supernatants spotted on lawns of 102 strains of *Lactobacilli*. No phage activity was demonstrated. Another batch of 15 strains was similarly investigated using the same 102 indicator strains, again with negative results. Afterwards, 200 strains were grown singly in broth for 10 days. Cultures were then spun to clarity and the supernatant spotted on lawns of these 200 strains. Although a number of diffusible antibiotics[3] was obtained, no phage activity was demonstrated. Finally, the bacilli in 10 ml. of overnight broth cultures of 50 strains were deposited by centrifugation and resuspended in equal volumes of sterile distilled water in Petri dishes. The suspensions were irradiated with a 30-W. Hanovia Sterilamp from 25 cm. The lamp delivers more than 80 per cent of its output at a wave-length of 2357 Å.

At intervals of 5, 15 and 30 sec., 3 ml. were pipetted into 10-ml. pre-warmed broth and incubated in the dark overnight. The cultures were then centrifuged to clarity and the supernatants spotted on lawns of 109 strains of *Lactobacilli*. Two of the 50 strains proved lysogenic. They are both strains of *L. fermenti* (Nos. 535 and 544). The phage liberated by strain 535 forms small clear plaques in *L. fermenti* strains 222*a* and 196. The other phage forms larger turbid plaques on *L. fermenti* strains *F*1, 184 and 525. Strains 535 and 544 were actually used in all three other experiments listed above. These experiments were afterwards repeated with the two strains. Results were again negative. So far the only method of demonstrating the lysogenic property of the strains is by ultra-violet irradiation.

The 16 phages we now possess have been tested for lytic activity on large numbers of strains of other genera of the family Lactobacteriaceae. No activity has been demonstrated. Tests on numerous strains of Enterobacteriaceae were also negative.

In summary, 92 *L. acidophilus* strains, 42 *L. salivarius*, 30 *L. casei*, 72 *L. casei* var. *rhamnosus*, 19 *L. plantarum*, 76 *L. fermenti*, 12 *L. brevis* and one each of *L. bulgaricus* and *L. helveticus* were examined for lysogenicity by one or more methods. Two *L. fermenti* strains were found to be lysogenic by ultra-violet induction. This, we think, is the first report of lysogenic *Lactobacilli*. The host-range of *Lactobacillus* phages appears to be genus-specific.

This work is submitted with permission of Prof. C. L. de Jager, honorary director of the Nutrition and Dental Health Research Group of the South African Council for Scientific and Industrial Research.

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GENETICS

Twin Data in Support of the Lyon Hypothesis

The Lyon hypothesis[1,2] suggests that in the *XX* mouse and human female one *X* chromosome is genetically inactive after a certain stage in embryogenesis; that this 'inactive' *X* chromosome forms the Barr body, which can be identified at about the sixteenth day in the human embryo; that it is a random proposition which of the two *X* chromosomes in a given cell becomes the genetically inactive one; that once it is decided which *X* chromosome is to be the genetically inactive one in a given cell all descendants of this cell abide by the decision and have the same *X* chromosome inactive; and that the germ cell line does not participate in this process of *X* chromosome differentiation. The hypothesis suggests a mechanism: (1) of dosage compensation for the 'excess' genetic material in females; (2) of the observed phenotypic variability in females heterozygous for X-borne recessive mutations. The hypothesis requires the existence, in heterozygous females, of two populations of cells with regard to a particular trait for which the female is heterozygous. Beutler[3] seems to have indirect evidence for such, in females heterozygous for glucose-6-phosphate dehydrogenase deficiency. All the genetic and cytological evidence supporting this attractive hypothesis[1-4] will not be reviewed here. Some further evidence in its support will, however, be presented.

The Lyon hypothesis would predict that for many metrical characters intrapair differences are greater for monozygotic female twins than for monozygotic male twins, on the assumption that at least one of the genetic factors determining the character is on the X-chromosome. Because the random decision as to which X-chromosome is to be the genetically inactive one is made at a time when relatively few anlage cells are present, the possibility for chance deviations between two female MZ co-twins exists. If it is a matter of chance as to which X chromosome is genetically inactive and if the outcomes in the several cells are at least in part independent. then the number of cells in which a particular X chromosome becomes inactive may vary from none to all. In male twins, however, there is only one X chromosome, and thus there can be, from this source, no genetic variation between male MZ twins.

With these considerations in mind, the results from the Hereditary Abilities Study of the Institute of Human Biology of the University of Michigan were checked. In connexion with other reports[5-8] most of the necessary calculations had already been made on the IBM 650 of the University of Michigan Computer Laboratory.

The within pair variance of the twins is given by:

$$S_w^2 = \frac{1}{N}\,\Sigma(x_A - x_B)^2$$

where x_A and x_B are the values for twins A and B, and N is the number of pairs.

Table 1. No. of Variables with Specified Values for $F = S_{w♀}^2/S_{w♂}^2$

Nature of measures	< 0·10	0 10– 0 19	0 20– 0·49	0·50– 0 99	1·00– 1·99	2·00– 4·99	5 00– 9·99	10·00 or more
P	1	3	3	8	11	16	12	1
C				9	9	5	1	
A					13	28	7	
H			3	1	6	11		
B		1	8	27	28	28	6	
ψ	2	4	16	86	155	67	8	
Total	3	8	30	131	222	150	34	1
	Significant increase in S_w^2 for males			No significant F ratio		Significant increase in S_w^2 for females		
	41 variables			353 variables		185 variables		

Table 1 summarizes the comparison by F test of the within pair variances of 21 female and 23 male pairs of monozygous twins. The ages of the twins varied from 12 to 18 years, with identical mean ages in the two groups (16 years 4 months). Monozygosity was established by means of 8 characteristics (7 blood groups and secretor). Although there were occasional missing values, we will here, for ease of presentation, assume equal degrees of freedom for all variables. The variables are broken down into the following six areas: P for polygraph measures, C for cardiovascular data, A for anthropometric data, H for hæmatological data, B for biochemical data and ψ for psychological data. Examples of the variables analysed are change in heart-rate after loud noise (P). resting systolic blood pressure (C), stature (A), erythrocyte count (H), urinary beta-amino-isobutyric acid (B), and verbal score on Chicago Primary Mental Abilities test (ψ).

From Table 1 it is evident that for the metrical characters studied monozygotic female twins show significantly greater intrapair differences than do monozygotic male twins much more often than the converse. The only evident explanation for the finding is that afforded by the Lyon hypothesis. If the female co-twins were at different phases of their menstrual cycles at the time of study, this might possibly account for increased female intrapair difference in regard to some characters, but could scarcely account for the findings with regard to anthropometric traits.

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GERONTOLOGY

Senescence and Senility

NEEDHAM[1] has recently claimed that Minot's paradox[2]: "Senescence is at its maximum in the very young stages and the rate of senescence diminishes with age", is false. Instead of the relationship $\dot{S} = \dot{G}$ (where $\dot{S}$ = rate of senescence, $\dot{G}$ = specific growth-rate) indicated by Minot he advocates the use of the relationship $\dot{S} = k/\dot{G}$ in which, however, $\dot{G}$ refers to growth "in the broadest sense .... perhaps in fact anabolism in toto". It will be argued here that Minot's paradox is derived correctly from the simplest model of a living system; and that Needham's relationship gives only an approximation to the increase in senility in a more complex model. Secondly, it will be suggested that the changes under discussion bear no direct relationship to the rates of anabolism and catabolism at any time, and that changes in senescent systems are not related to changes in specific growth-rates.

Let S be the senility of any living system, presumably a 'function of state' for that system. Then dS/dt will be the senescence-rate, $\dot{S}$, for that system. Similarly, if G is the size, or more accurately the state of development of the system, then dG/dt, or $\dot{G}$ will be the rate of growth or development. The system can pass through a series of theoretically defined states in a continuous manner (that is, at any time the system comprises contributions from several states):

$$E_1 \xrightarrow{k_1} E_2 \xrightarrow{k_2} E_3 \xrightarrow{k_3} E_4 \;.\;.\;.\; E_j \xrightarrow{k_j} \;.\;.\;.\; E_n$$

The actual system then equals ΣE_j at all times, and in material terms $\Sigma E_j(t = t_1) = E_1(t = t_0) = E_0$. As this is constant, at all times $\Sigma\Delta E_j = 0$, that is, the sum of all changes decreasing given E states must be equal to the sum of all changes increasing other E states. If, therefore, the initial rate of change, $(\mathrm{d}E_1/\mathrm{d}t)_{t_0}$ ($= k_1E_0$ if first-order kinetics are assumed), is the maximal one, then it will also be the maximal rate of transition of the whole system (ΣE_j at all times) to its final state (E_n). These two rates, however, only correspond necessarily to the rates of growth and senescence in the simplest model, where $n = 2$. In this case, the rate of growth (disappearance of E_1) equals the rate of senescence (appearance of E_2), and for the first order case (exponential decay of E_1) the rate of senescence is greatest when $E_1 = E_0$.

If $n > 2$, intermediate states exist, and it then becomes necessary to define the state (or set of states) constituting maturity (E_m), and the state (or set of states) constituting senility. It is reasonable to assume that the former precede the latter, and that the most senile states are the terminal ones. The growth, or development rate, can then be considered the sum of all processes leading to E_m, and the senescence rate the sum of all processes leading away from E_m. Under simple conditions, given here, the absolute rate of growth will have a maximum value $\dot{G}_{\max}$ at a time t_g and the absolute rate of senescence a maximum value $\dot{S}_{\max}$ at a time t_s, where t_s is later than t_m (the time when E_m is maximal) and t_m is later than t_g. The differential rates of growth and senescence are given by: $\dot{G}_{\text{net.}} = \dot{G}-\dot{S}$ and $\dot{S}_{\text{net.}} = \dot{S}-\dot{G}$. Before E_m is maximal (t_m), $\dot{G}_{\text{net.}}$ is positive and $\dot{S}_{\text{net.}}$ is negative; after t_m, $\dot{G}_{\text{net.}}$ is negative and $\dot{S}_{\text{net.}}$ is positive. But always: $\dot{G}_{\text{net.}} = -\dot{S}_{\text{net.}}$. This relationship is quite different from that which gives $\dot{G} = \dot{S}$ for the system where $n = 2$ (in that case, $\dot{G}_{\text{net.}} = \dot{S}_{\text{net.}} = 0$).

The empirical relationships given by Needham[1] can be summarized as follows. If the growth-rate equals x, then according to Needham, Minot's postulate predicts that 'senescence' $= k-x$, while it is more reasonable to think that 'senescence' $= k/x$. Here 'k' corresponds to k_1E_0 above and 'x' to $\dot{G}$ above. For the system where $n = 2$, '$k-x$' is, however, proportional to the amount of senile state E_2 and not to the rate of its attainment (senescence). For systems where $n > 2$, 'k/x' can likewise sometimes be an approximation to the amount of a later state than E_2 that is present (cf. the relationship $(E_3)_t \propto t^2$ for the analogous chemical system where $n = 3$ and t is small[3]). In a system involving a sequence of first order changes, the rate of change in the system after E_2 may be proportional to the amount of E_3 or of a subsequent state. But there is no reason to believe that the value of $\dot{S}$ or $\dot{S}_{\text{net.}}$ is ever of this particular reciprocal form, while the actual relation expressed by Minot of the form $\dot{S} = \dot{G}$ is at least accurate for $\dot{S}$ in the simplest system.

If it is assumed that, at each stage, each contributing state maintains itself partly stable against induced change, we have at each point the condition:

$$\begin{tikzcd} {} \arrow[r] & E_j{}^1 \arrow[r, "k^1{}_j"] \arrow[d, shift left, "K_j"] & {} \\ & E_j{}^s \arrow[u, shift left] & \end{tikzcd}$$

($k^1{}_j$ = rate of change of $E_j{}^1$
K_j = equilibrium between $E_j{}^1$ and $E_j{}^s$)

where $E_j{}^1$ is the labile form and E_j^s the form stabilized by some anabolic processes, then the rates of change k_jE_j can be written $\left(\dfrac{k^1{}_j}{K_j + 1}\right)E_j$. That is, the rate of transition will be determined at each stage not only by the growth-determining and senescence-determining rate constants ($k^1{}_j$) but also by the active equilibrium K_j, determined by metabolic processes and environmental influence. As indicated by Needham, even the states composing senile systems are capable of anabolism maintaining large positive K_j values. This anabolism cannot itself be a measure of growth- or senescence-rates, which are determined by the transitions between the several E states. If the values of $k^1{}_j$ and K_j are independent of the amounts of various E states at any time (that is, the k_j values are time invariant) then the relation given here 't_s is later than t_m which is later than t_g' is probably true. The kinetics of such a system have been described for the corresponding chemical reactions[4]. If the k_j values are highly dependent on the E states, then the values of $\dot{G}$ and $\dot{S}$ will vary in a complex manner to which no simple generalizations can apply.

In particular, the fact that the rate of shrinkage in man decreases in extreme old age cannot lead to any conclusions about $\dot{G}$ at that time. The rate of shrinkage is not necessarily equivalent to the differential ageing rate, $\dot{S}_{\text{net.}}$, as defined here. It probably deals with changes in states lying between E_m (the mature state) and E_n (the final senile state) and hence must diminish when the times giving maximal contribution from these intermediate states of senility have passed. Even if it were equivalent to $\dot{S}_{\text{net.}}$, as $\dot{S}_{\text{net.}} = \dot{S}-\dot{G}$, a decrease can be due to either an increase in $\dot{G}$ or a decrease in $\dot{S}$, and cannot definitively be attributed to either. But in this case the latter would seem the more likely effect; there is no mathematical or grammatical need to equate the most senile with the most senescent.

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The application of formal mathematics to the present problem of senescence is very desirable, and Dr. Nicholls's treatment therefore is a welcome contribution. The main object of my previous communication was to show that the foundation of Minot's quantitative estimate of senescence was very shaky. The alternative proposed by me not only resolves his incongruous paradox, but also is in harmony with a good deal of empirical knowledge concerning senescence, growth and anabolism in general. Its theoretical foundations do need to be tested more fully. however, for it is probably not the only one which would be consistent with the empirical observations.

Nicholls claims to show that Minot's relation is after all more plausible than mine, and while I do not feel that he has established either this or his other main claims, I think that the approach is very valuable. For this reason and also in the hope of clarifying a number of aspects of the problem, it is worth examining his thesis in more detail.

One of its weaknesses is the large number of premises required to develop it, and not all these are well founded in the general theory of growth. A second shortcoming is that it is rarely clear how the really significant conclusions actually emerge from the mathematics. The theory of consecutive reactions has been little applied in this field so that details and documentation which would not be necessary to the physical chemist are indispensable in this context.

It is not evident that he does more in support of his main contention than show Minot's relation to be relevant to the theoretical and biologically trivial case of a development which consists of only a single step, $E_1 \rightarrow E_2$. On the other hand he admits that mine gives a reasonable approximation to more complex situations, and these are the type likely to be found in actual ontogenesis. Moreover, it is difficult to reconcile the main contention with his final conclusion that changes in senescent systems are not related to changes in specific growth-rate at all—since it was the essence of Minot's theory that these changes give a quantitative measure of the senescent process. Another surprising conclusion is that the changes under discussion (presumably senescence and growth) bear no relation to the rates of anabolism and catabolism—for growth is by definition anabolic.

The explanation lies mainly in the fact that although he ostensibly used the growth component of development, symbolized by $\dot{G}$, in practice he used sometimes the differentiation component and sometimes development as a whole. Confusion of growth with development as a whole is shown by such expressions as "growth or developmental rate" and "size, or more accurately the state of development". Development consists of the two components, growth: purely quantitative and differentiation: qualitative. Since Nicholls's sequence $E_1 \rightarrow E_2 \rightarrow$ etc. is characterized by ΣE_j = a constant (or $\Sigma \Delta E_j = 0$) it excludes any auxesis, and defines purely qualitative changes. Auxetic changes are the only ones relevant to pure growth, on the other hand.

That his $\dot{G}$ does represent differentiation-rate is shown also by the need he eventually feels to define state(s) of maturity, rather than of maximal size, although he still visualizes $\dot{G}_{\max.}$ as the maximal rate of growth rather than of differentiation. It is no doubt useful to obtain a relation between senescence and differentiation, but this is not what Minot did; he related it to growth—if only because measurements of growth were the only quantitative data available. This led to his conclusion that the rate of senescence was maximal at the outset; but Nicholls is led to postulate a maximal rate later than the stage of maturity, a decision much more in accord with the evidence in my previous communication.

He implies that in this I related the specific growth-rate to the rate of senescence rather than to its cumulative effect, the state of senility, but this is not so, although no doubt the phrase "state of senescence" is more open to misunderstanding than "state of senility". I followed Minot in relating S to $\dot{G}$ and not $\dot{S}$ to $\dot{G}$, and it is interesting that this form is also the one indicated by Nicholls's treatment. Misunderstanding appears to have originated in Nicholls's own initial formulation as $\dot{S} = \dot{G}$ rather than $S = \dot{G}$ (or more precisely $S = k - \dot{G}$). He also defined $\dot{G}$ initially as the specific growth-rate (rate per unit of mass) but later G is defined as size so that $\dot{G}$ (dG/dt) is the absolute or gross growth-rate. There are other minor points which increase the difficulty in following his treatment; one is that while E_1, E_2, etc., are amounts of material in the successive states of development, E_0 is not the first member of this sequence but the initial concentration of material in state E_1.

He expresses the differential rate of $\dot{G}$ and $\dot{S}$ as the difference $\dot{G} - \dot{S}$, rather than as the ratio $\dot{G}/\dot{S}$ which is more usual in growth studies. The rationale for this lies in the essentially multiplicative nature of growth. This was one of my main reasons for advocating the relation $S = k/x$ rather than the linear form $S + x = k$ (S = degree of senility and x = specific growth-rate). If we may follow Nicholls in supposing a very close affinity between senescence and differentiation, then there is perhaps some further evidence in favour of the first relation from such cases as the behaviour of iris cells *in vitro*. Here the degree of differentiation is measured by the concentration of melanin granules, and in a general way this is inversely related (that is, by dilution) to the growth- and proliferation-rates of the cells. Nevertheless the relation undoubtedly needs a more rigid mathematical derivation.

Irrespective of the contribution of Nicholls's approach to the solution of the present problem it is a potentially valuable approach. Even if it only provided a quantitative relation between differentiation and time, it would fulfil a long-standing hope of morphogeneticists. There seems no formal reason, however, why it should not be extended to include also the growth component. The theory of consecutive reactions naturally was first applied to the more simple case of closed systems, with an expendable amount of initial raw material, E_1, but in order to grow, and indeed even to maintain its bulk, an organism is necessarily an open system, with a continuous inflow of raw material, from yolk-depots or other food. It will be evident that under these conditions the first step, $E_1 \rightarrow E_2$, of the sequence no longer has the unique attribute of an exponential progress curve. It is also worth considering the further possibility that raw materials feed in at various points of the sequence. This could be done by substituting open tributaries for the stabilizing backwaters, $E_j S$.

The theory should be applied with due caution. Benson[1] emphasizes that at present even inanimate systems when investigated in detail appear to "display an anarchistic tendency to become unique laws unto themselves". There may be a further danger in attempting to compare a sequence of ontogenetic processes directly with a sequence of chemical reactions; as indicated above the early exponential fall in specific growth-rate does not have its basis in an initial, first order, chemical reaction. The danger is not only of confusing processes of two very different degrees of complexity but also of confusing two very different time scales, the slow ontogenetic and the rapid metabolic[2]. The body fabric which, in quantity and quality, changes slowly along the axis of ontogenesis is at each point the overall steady state maintained by all contemporary systems of (rapid) metabolic reactions. The slow shift of the steady state with time has a more obscure driving force, perhaps hormonal as opposed to enzymatic, and might also require a different mathematical theory from that of reaction sequences.

In conclusion, it seems fair to say that Nicholls's approach does not decide the present issue in favour of Minot's view but that it is a tool of great potential value for the mathematical definition not only of growth and senescence but also of differentiation.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, May 7

University of London (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Prof. Frank Fenner: "Reactivation of Animal Viruses".* (First of four Almroth Wright Lectures.)

University of London (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. I. M. Glynn: "Sodium and Potassium Movements in Living Cells".* (Further lecture on May 14.)

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. A. W. Haslett: "Limits of Explanation".

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. S. Welch: "The Signalling Problems associated with Large-Register-Controlled Automatic Telephone Exchanges".

University of London (in the Physics Lecture Theatre, Queen Mary College, London, E.1), at 5.30 p.m.—Prof. R. Righini (Florence). "The Abundance of Isotopes in the Solar Atmosphere".*

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. A. Winegarten: "Agriculture". (First of three Cantor Lectures on "The Development of Natural Resources".)

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Prof. L. Lacqué (Paris). "The French Chemical Industry".

Women's Engineering Society, London Branch (at "Hope House", 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Mr. P. F. Mariner. "Microminiaturization".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. John H. Hemming and Mr. Christopher S. Lambert. "The Iriri River Expedition".

Monday, May 7—Tuesday, May 8

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at Church House, Great Smith Street, London, S.W.1)—Spring Meeting.

Tuesday, May 8

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Sir Keith Murray. "Major Issues in the Expansion of University Education".

Royal Society of Medicine, Experimental Medicine and Therapeutics Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Annual General Meeting. Dr. P. L. Mollison. "The Reticulo-endothelial System and Red Cell Destruction" (Presidential Address).

University of London (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. Sir Willis Jackson. "The Partnership between Science and Electrical Engineering" (Inaugural Lecture).

Institution of Polish Engineers in Great Britain, Group for Economic, Social and Management Studies (at 55 Princes Gate, Exhibition Road, London, S.W.7), at 7 p.m.—Dr. S. A. Young: "Technical Snobbery and its Damaging Effects".

Tuesday, May 8—Saturday, May 12

Institute of Metal Finishing (at the Hotel Metropole, Brighton)—Metal Finishing Conference.

Tuesday, May 8—Wednesday, May 23

Building Research Station (D.S.I.R.) (at the Building Centre, Store Street, London, W.C.1)—Exhibition—"Research in Building".*

Wednesday, May 9

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. K. R. Dumbell: "Temperature and Infection".*

Imperial College of Science and Technology (in association with the Surface Activity Group of the Society of Chemical Industry, in the Mining Lecture Theatre, Royal School of Mines, Prince Consort Road, London, S.W.7), at 5 p.m.—Prof. F. Sebba: "Ion Flotation" (Lecture and Demonstration).

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. G. de Zoeten: "Aim and Facilities of KEMA Laboratories".

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. A. J. Harrison and Mr. D. Chamberlain: "The Integrated Radar Systems for S.S. *Canberra*".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. T. Lyddon Gardner: "The Cosmetics Industry".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. S. S. Duncan: "The Operation of a Combined Climatic and Random Vibration Test Facility".

Society of Instrument Technology, Control Section (at Manson House, 26 Portland Place, London, W.1), at 6.15 p.m.—Annual General Meeting. 7 p.m.—Mr. A. J. Young and Mr. I. Gray: "Computer Control of Processes".

Thursday, May 10

British Steel Founders' Association (at the Criterion, Piccadilly, London, W.1), at 9.45 a.m.—Technical Conference.

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. T. A. J. Prankerd: "Glucose-6-phosphate Dehydrogenase Deficiency".*

University of London (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. W. B. Bonnor: "The Future of Applied Mathematics" (Inaugural Lecture).*

Chemical Society (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7.30 p.m.—Prof. H. B. Henbest: "Stereoselectivity in the Reactions of Cyclic Compounds" (Tilden Lecture).

Friday, May 11

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Problems associated with (i) Blood Pressure Measurements and (ii) Storage Devices".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. G. M. Carstairs: "Psychotic Illness and Artistic Production".

Monday, May 14

University of London (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Dr. J. L. Gowans: "The Role of Lymphocytes in Immunological Reactions".* (Second of four Almrith Wright Lectures.)

British Society for the History of Science (at the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. A. W. Badcock. "Physical Optics at the Royal Society to 1800".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Adventure in Nepal", Colour films with commentary by Mr. J. B. Tyson.

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. J. Macdonald: "Forestry". (Second of three Cantor Lectures on "The Development of Natural Resources".)

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W.C.2), at 6.45 p.m.—Annual General Meeting, followed by Dr. H. G. Taylor: "The Work of the Electrical Research Association"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer (preferably with an active research interest in one aspect of experimental botany including microbiology) in Botany—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 11).

Lecturer in the Department of Zoology—The Secretary, The University, Edinburgh (May 12).

Lecturer in the Department of Mathematical Physics—The Secretary, The University, Edinburgh (May 14).

Lecturer or Assistant Lecturer in Statistics in the Department of Mathematics—The Registrar, The University, Leeds 2 (May 14).

Lecturer (with qualifications in either pure or applied mathematics) in Mathematics—The Registrar (Room 22, O.R.B.), The University, Reading (May 14).

Research Assistant (with special interests in animal learning) in Psychology—The Registrar and Secretary, The University, 38 North Bailey, Durham (May 14).

Assistants (2) in Mathematics—The Secretary of University Court, The University, Glasgow (May 15).

Assistants (3) in Organic, Inorganic and Physical Chemistry; and a Lecturer in Physical Chemistry—The Secretary, The University, Aberdeen (May 19).

Lecturer and an Assistant Lecturer in Psychology—The Secretary, The Queen's University, Belfast, N. Ireland (May 19).

Baxter Chair of Mathematics in Queen's College, Dundee—The Secretary, The University, College Gate, St. Andrews, Fife (May 21).

Lecturers (2) in the Department of Natural Philosophy—The Secretary, The University, Edinburgh (May 21).

Junior Research Fellow (with a good first degree in zoology) in Locust Physiology in the Department of Zoology, University College of Rhodesia and Nyasaland, for work on water relations of locusts—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 22).

Senior Lecturer in Bacteriology; a Lecturer in Pathology (Morbid Anatomy); and a Lecturer in Hæmatology and Blood Transfusion at University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W.C.1 (May 28).

RESEARCH ASSISTANT (with a science degree) IN THE DEPARTMENT OF BOTANY, for work on glycollate formation during photosynthesis—The Registrar, University College of Wales, Aberystwyth (May 31).

DEMONSTRATOR IN THE DEPARTMENT OF BOTANY—The Registrar, The University, Liverpool (June 2).

UNIVERSITY LECTURER IN THEORETICAL PHYSICS (with emphasis on low or high energy nuclear physics preferred)—The Secretary of the Faculties, University Registry, Oxford (June 5).

LECTURER/SENIOR LECTURER IN APPLIED MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House Pall Mall, London, S.W.1 (Australia and London, June 8).

RESEARCH FELLOW (preferably specialized in the fields of agricultural geography or geomorphology) IN GEOGRAPHY at the Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 8).

EDWIN CUTHBERT HALL CHAIR OF MIDDLE EASTERN ARCHÆOLOGY in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 15).

ASSISTANT DIRECTOR (with previous experience in a computing laboratory and the qualifications and experience to teach mathematics at Diploma in Technology level) OF THE COMPUTING LABORATORY—The Registrar, Bradford Institute of Technology, Bradford 7

FIELD ASSISTANT or ASSISTANT WARDEN (man or woman, with an honours degree in botany, together with a general interest in other field subjects) at Malham Tarn Field Centre, near Settle, Yorks—The Secretary, Field Studies Council, 9 Devereux Court, Strand, London, W.C.2.

MATHEMATICIANS (with at least an honours degree in mathematics, and preferably previous experience with computers) at the Computation Centre, National Research Council of Canada, for work involving the analysis and programming of problems which arise in the various scientific and engineering divisions of the Council—The Employment Officer, National Research Council, 100 Sussex Drive, Ottawa, Ontario, quoting file E.R.-210.

MEDICAL LABORATORY TECHNICIAN (qualified A.I.M.L.T., science degree or equivalent)—The Administrative Officer, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex.

PLANT VIROLOGIST (under 45, with an honours degree in botany or agricultural botany, specialized in plant pathology, and preferably a knowledge of population genetics) with the East African Agriculture and Forestry Research Organization, to study either (*a*) virus diseases affecting agricultural crops, or (*b*) the genetics of resistance of plants to disease—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting Ref RC.264/348/02/T.

READER IN THE DEPARTMENT OF CHEMISTRY—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH FELLOW IN LOW TEMPERATURE PHYSICS, to study nuclear and electronic interactions in ferromagnetic and antiferromagnetic metals at very low temperatures—The Secretary and Registrar, The University, Southampton.

SENIOR BIOCHEMIST (with previous experience in research, and preferably a higher degree) in the Institute of Dental Research—The Acting Director, United Dental Hospital of Sydney, 2 Chalmers Street, Sydney, N S.W., Australia.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Federation of British Industries. Industrial Research in Manufacturing Industry, 1959–60 Results of a Survey including Commentaries and a Statistical Report. Pp. 129 (London Federation of British Industries, 1961.) [62

Department of Agriculture and Fisheries for Scotland Spaced–Plant Trials at East Craigs. 9 Varieties of Timothy, *Phleum pratense* L 1956–1958 Pp 7) Edinburgh Department of Agriculture and Fisheries for Scotland, 1962.) [62

Central Advisory Water Committee. Sub-Committee on the Growing Demand for Water—Final Report. Pp. iii+43. (London H.M. Stationery Office, 1962.) 3s. 6*d*. net. [62

Ministry of Housing and Local Government Circular No 3/62 Clean Air Act, 1956. Smoke Control Areas. Pp 11 1*s* net Circular No. 4/62 Clean Air Act, 1956. Smoke Control in the Black Areas Pp. 3. 5*d*. net. (London H.M. Stationery Office, 1962) [62

Agricultural Research Council Radiobiological Laboratory. Radioactivity in Milk—Interim Report, December 1961. (ARCRL 6) Pp. vi+14. (London H M. Stationery Office, 1961.) 2*s* [62

The International Journal of Production Research, Vol. 1, No 1 (November, 1961) Pp v+65. Subscriptions (payable in advance). Members of the Institution of Production Engineers 84*s* p.a. including postage. Single issues 25*s*. each Non-members 105*s*. p.a , including postage. Single issues 30*s*. each. Published quarterly. (London Institution of Production Engineers, 1961.) [62

Negretti and Zambra, Ltd Publication G/45/2 (1961) · Indicating and Recording Gauges—Vacuum, Pressure, Differential. Pp 57. (London · Negretti and Zambra, Ltd., 1961) [62

National Coal Board. The Relation between Pneumoconiosis and Environmental Conditions an Analysis of the Results of the First Series of X-ray Surveys in the National Coal Board's Pneumoconiosis Field Research. By D. Hicks, J. W. J. Fay, J. R. Ashford and S. Rae Pp. viii+40+31 tables + Appendix 1–14+23 figures. The Study of the Composition of Respirable Dust in the Pneumoconiosis Field Research. By J. W. J. Fay and D. Hicks. Pp. ii+25+6 tables + Appendix 1–3. (London National Coal Board, 1962) [62

English Electric Valve Co., Ltd. Abridged Valve Data. Pp. 42. (Chelmsford English Electric Valve Co , Ltd., 1962.) [72

Department of Scientific and Industrial Research Building Research Station. Factory Building Studies No. 11 : Thermal Insulation of Factory Buildings. By G. D. Nash. Pp iv+23 (London H M. Stationery Office, 1962) 3*s* net. [72

Building Research Station Digest, (Second Series). No. 19 : The Lighting of Drawing Offices Pp. 4. (London . H.M. Stationery Office, 1962.) 4*d*. [142

North Staffordshire Journal of Field Studies, Vol. 1, 1961. (Incorporating *The Transactions of the North Staffordshire Field Club*.) Pp. iii+140. (Keele . University of North Staffordshire, 1961.) 25*s* [142

Field Studies Council. Annual Report, 1960–61. Pp. 29. (London : Field Studies Council, 9 Devereux Court, W.C.2), 1962.) [142

Hopkin and Williams, Ltd "M F C." Materials for Chromatography including Gas Chromatography. Pp 9. Chemicals for Science and Industry. Activated Alumina . Some Notes on Available Types, their Properties and Applications Pp. 4. (Chadwell Heath, Essex : Hopkins and Williams, Ltd., 1962.) [142

Other Countries

Smithsonian Miscellaneous Collections. Vol 144, No 1 A Further Study of the Lower Eocene Mammalian Faunas of Southwestern Wyoming By C. Lewis Gazin Pp. v+98+14 plates (Publication 4474) (Washington, D.C : Smithsonian Institution, 1962.) [62

University of Florida. Florida State Museum Gainsville. Director's Report, 1960–1961. Pp. 24. Bulletin of the State Museum—Biological Sciences. Vol. 6, No 2 : Osteology of Living and Fossil New World Quails (Aves, Galliformes). By J. Alan Holman. Pp. 131—233. 1.55 dollars. (Gainsville, Flas. · Florida State Museum, 1961.) [62

United States Department of Agriculture. Leaflet No. 500 Milky Disease for Control of Japanese Beetle Grubs. By Walter E Fleming. Pp 6. (Washington, D C. Government Printing Office, 1961) 5 cents [62

Research in Public Health Administration. Selected Recent Abstracts. Pp. 52 (Baltimore, Md Research in Public Health Administration Project, School of Hygiene and Public Health, The Johns Hopkins University, 1962) [62

National Academy of Sciences—National Research Council. Nuclear Science Series—Radiochemical Techniques. NAS–NS 3104 . Rapid Radiochemical Separations By Yusuru Kusaka and W. Wayne Meinke Pp. vi+125 (Washington, D C. Office of Technical Services, Department of Commerce, 1961) 1 25 dollars. [62

The Administration of South West Africa : Marine Research Laboratory. Investigational Report No 3 The Pilchard of South West Africa (*Sardinops ocellata*) and Marsbanker (*Trachurus trachurus*), Bird Predators, 1957–1958. By J. P. Matthews. Pp. 35. (Windhoek · Secretary for South West Africa, 1961.) [62

Yale Peabody Museum of Natural History. Postilla. No. 54, (October 30, 1961) : The Dentition of *Ourayia*—Its Bearing on Relationships of Omomyid Prosimians. By Elwyn L. Simons Pp 20. No. 57, (November 30, 1961) · The Phyletic Position of *Ramapithecus* By E L. Simons. Pp 9 (New Haven, Conn. Yale Peabody Museum of Natural History, 1961.) [62

Smithsonian Institution · Bureau of American Ethnology Bulletin No 183 : Seneca Thanksgiving Rituals By Wallace L. Chafe. Pp. iii+302. (Washington, D.C Government Printing Office, 1961.) 2.25 dollars [62

Smithsonian Institution, Washington. United States National Museum. Contributions from the Museum of History and Technology. Paper 26 : Holcomb, Fitz, and Peate Three 19th–Century American Telescope Makers. By Robert P. Multhauf, Julia Fitz Howell, F. W Preston and William J McGrath, Jr. Pp. 155–184 30 cents. Paper 28 : The Development of Electrical Technology in the 19th Century. 1 The Electrochemical Cell and the Electromagnet By W. James King. Pp. 231–271. 35 cents. (Washington, D C. : Government Printing Office, 1962.) [62

Smithsonian Miscellaneous Collections Vol 143, No 3 Supplement to the Annotated Subject-Heading Bibliography of Termites, 1955 to 1960 By Thomas E Snyder Pp iii+137. (Publication 4463). (Washington, D.C. Smithsonian Institution, 1961) [62

Phoenix, Vol. 1, No 3, (December, 1961) Bilharziasis/Schistosomiasis Pp. 16 (Ann Arbor, Michigan Michigan Memorial–Phoenix Project, The University, 1961) [62

Angewandte Chemie, Vol. 1, No 1, (January, 1962). (International Edition in English) Pp. 1–62 Subscription rate, Volume 1, 1962 · 107*s*. 6*d*. ; 15 dollars (plus postage) Single copies 2 dollars. (Weinheim/Bergstr : Verlag Chemie, G.m b H. ; New York and London · Pergamon Press, 1962) [62

Presidential Address delivered at the Cento Scientific Symposium, held at Lahore, Pakistan, January 8, 1962. By Dr. I H. Usmani. Pp. 14. (Karachi Pakistan Atomic Energy Commission, 1962.) [62

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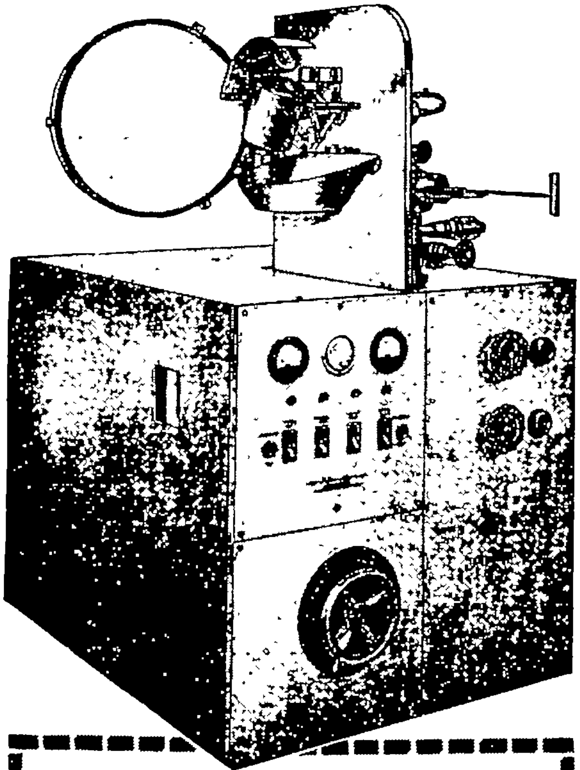

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APPOINTMENTS VACANT

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

The Council of the Marine Biological Association invite applications for a post at the Plymouth laboratory to do research on the adsorption of chemical substances on to fine particles in the sea. Candidates should have had experience of chemical and physico-chemical methods, preferably those involving the use of radioactive materials, and should be capable of relating the work to biological problems. Candidates should have had at least two or three years research experience and the appointment will be in the equivalent grades of Scientific Officer or Senior Scientific Officer of the Civil Service according to age and experience The appointment will be for two years, renewable annually, in the first instance

Forms of application may be obtained from the Secretary of the Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, and should be returned to him in quadruplicate not later than May 31, 1962.

SCHOOL OF TROPICAL MEDICINE

(UNIVERSITY OF LIVERPOOL)

Research Assistant with a degree in biochemistry or physiology required to work on various aspects of tissue metabolism in relation to parasitic diseases. Suitable study for higher degree. Salary according to age and qualifications.

Please apply to the Dean, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, 3.

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The salary for this position will be in accordance with N.J.C., Grade 2, £700 to £775, depending on age and qualifications.

Applications, giving details of age, education, qualifications or experience, should be sent to the Assistant Regional Personnel Officer, Central Electricity Generating Board, North Thames Division, West Farm Place, Chalk Lane, Cockfosters, Barnet, Herts, to arrive not later than May 12, 1962

F. W. SKELCHER,
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AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF APPLIED PHYSICS

PHYSICISTS OR ENGINEERS

Applications are invited from physicists or engineers to the professional staff of the Organization's Division of Applied Physics, located in the grounds of the University of Sydney, New South Wales

The Division of Applied Physics is one of the two Divisions comprising the National Standards Laboratory and is responsible for the establishment and maintenance of standards of mechanical and electrical measurements in Australia.

The research activities of the Division generally are concerned with the application and refinement of modern developments in physics in order to achieve better standards of physical measurement The current programme of applied and fundamental research includes optical interferometry and the development of wavelength standards, the development of the cross-capacitor as a basic electrical standard, the absolute determination of g at Sydney, microwave physics and the dielectric properties of solids. It is planned to undertake the determination of other physical constants and to start work on masers and lasers.

APPOINTMENT NO.: 750/247

Several research workers are needed to take part in the above programme and there will be ample opportunity for them to develop their own research interests Those interested may obtain further details of the individual projects and the facilities available by writing directly to the Division.

The minimum qualifications required are at least a University Honours degree in Science or Engineering with Physics as a major subject together with several years' postgraduate research experience

By mutual arrangement, the appointments for the posts may be either for an indefinite period or for a fixed term of three years

Salary: Dependent on qualifications and experience within the ranges:

£A 1,541 to £A 2,296 per annum or £A 2,426 to £A 2,751 per annum.

APPOINTMENT NO.: 750/248

Applications and enquiries are also invited from graduates or diplomates in Science or Engineering or those holding equivalent qualifications who would be interested in assisting in the research programmes outlined above.

Applicants should possess a University degree in Science or Engineering with Physics as major subject or hold equivalent qualifications

Salary: Dependent on qualifications and experience within the ranges·

£A.1,101 to £A.1,651 per annum or £A.1,651 to £A 1,981 per annum but would not be less than £A.1,321 per annum.

Salary for women will be £A 188 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid for the appointee and his dependent family and for fixed term appointments return fares will also be paid. Further particulars may be obtained on applications to:

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London W.C.2,

to whom applications (quoting relevant appointment number) should be addressed by June 23, 1962

COUNTY BOROUGH OF STOCKPORT

STOCKPORT COLLEGE FOR FURTHER EDUCATION

Lecturer in Chemistry required in Science Department. Candidate must hold an honours degree of a British University Industrial and/or teaching experience is desirable. Duties to commence on September 1, 1962. Burnham Technical Salary Scale for Lecturers. Agreed Conditions of Tenure.

Further particulars and application forms from the Principal, Stockport College for Further Education, Wellington Road South, Stockport, to be returned by May 12, 1962

ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF NATURAL PHILOSOPHY

Applications are invited for the post of Senior Lecturer on the salary scale £1,975 by £75 to £2,275. Applicants should be physicists with a Ph D degree who have extensive experimental experience in electronics and are interested in plasma physics

Application forms, to be lodged by May 31, 1962, may be obtained from the Secretary, George Street, Glasgow, C 1

INSTITUTE OF DENTAL RESEARCH

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SENIOR BIOCHEMIST

Applications will be received until June 15, 1962, for the position of Senior Biochemist in the Institute of Dental Research. Previous experience in research is essential and a higher degree is desirable. Salary at a weekly equivalent of £A.2,457 to £A 2,782 per annum

Further information and details of travel expenses may be obtained from the Acting Director.

C. C. BOWIE,
Secretary

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VACANCY FOR MINERALOGIST-PETROgrapher with sound training in principles of geochemistry Opportunities for research available Applicants should have a Ph D. or equivalent Probationary lectureship for two years leading to permanent appointment. Appointment to be effective September 1, 1962. Deadline for applications, July 1, 1962.—Apply to J. W Ambrose, Department of Geological Science, Queen's University, Kingston, Ontario, Canada.

EDUCATION AND TRAINING FOR MANAGEMENT

In a valuable memorandum* submitted to the Robbins Committee on Higher Education, the Association of Teachers of Management has clarified much of the confused thinking which abounds in circles where management training is discussed.

The Association makes it clear that, before the role of higher educational institutions in management education can be considered, education and training for management must be defined. This analysis involves, first, the question of the type of institution which is appropriate and the resources which it should command. Secondly, there is the type of course, the level and nature of any awards, and the student groups at which such courses are directed. The part to be played by such activities must also be set against the total provision of education and training which can be expected in this field.

In a clear analysis of the nature of education and training for management, four main divisions are distinguished: (1) education about business organization and management; (2) education for management; (3) training in management skills; (4) training in procedures and procedural skills. There is a considerable overlap between these four divisions, and a fully comprehensive scheme for education and training must embrace all four.

Education about business organization and management is defined as the processes by which men transform scientific and technical knowledge and raw materials into wealth through organization and management. This is only partially achieved by present teaching in the relevant disciplines of economics, psychology and sociology and through descriptive treatments of commerce and engineering. "Some knowledge of these complex processes is appropriate for any educated person who may enter commerce, industry or administration and no man can be said to be properly educated without some such knowledge".

The Association defines education for management as the process of developing in a man the capacity to think out problems of management in a disciplined way. This presupposes both knowledge about management and procedures and procedural skills. At present the volume of knowledge available does not constitute an academic discipline in its own right, and for many academic people this is the main obstacle to the creation of suitable courses in management. Management is an area of interaction of a number of disciplines, economics, politics, psychology and sociology, of much that is derived from engineering practice, and of a growing body of knowledge not attributable to any of these. Even if and when a body of knowledge tantamount to a new discipline emerges, the distinction between 'education about' and 'education for' management will remain.

Considerable support would be given to the Association's views on education about and education for management. They will not escape criticism, however, for the way in which they have ignored the manager's overriding responsibility for controlling people, for getting results through them, and the need to educate himself to do this. This, in turn, should have direct bearing on the subjects studied at higher educational institutions and, even more important, the way in which they are taught. The influence of a discerning tutor may be of more importance in the education of a manager than the subjects studied.

By training in management skills the Association includes the ability to analyse situations, to use and co-ordinate procedures, and to make decisions. These skills are distinct from purely procedural skills such as work study, production planning or distribution. Higher educational institutions should be concerned with education for and about management and with training in management skills, procedures and procedural skills only in so far as such training contributes to such education.

The choice of institutions to engage in any higher education for or about management should, in the Association's view, be governed by three basic conditions. It must engage in research; the development of this field of study calls for substantially more research than is being undertaken at present. In addition, the practising manager ought to be confronted with those carrying out research at frontiers of their disciplines but who are doing so on problems of organization, administration and management. So far as possible, both students and practising managers should be involved in the research process.

The second basic requirement must be close contact with commerce, industry and public administration on the part of the staff and of the institution as a whole. Such contact is to be encouraged provided that full academic freedom is safeguarded.

Many people in industry and commerce would agree with this worthy sentiment and would have to admit that, so far, their doors have been almost completely closed to full-time university students who wish to examine their policies and practices too closely. As industry gets more experience of dealing with research students, more and more facilities should be made available.

How the process could be considerably accelerated is bound up with the Association's third basic condition about the necessity for teaching staffs to be recruited from the relevant disciplines and from relevant processional training. Such staff should be attached to departments of management studies. In the future such teaching staff ought to be recruited from three main sources: (1) the universities, for example, psychologists and economists; (2) graduates from universities and other higher institutions after research in industry and commerce; (3) graduates and others who have taken an advanced course in management, at postgraduate-level, whether at a university or other institution.

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* *Education and Training for Management.* (Secretary, A. E. Thomas, Scottish College of Commerce, Glasgow.)

Here, surely, is the rub which indicates why existing departments of management studies carry little real weight in industry*. Unless these departments can recruit members of staff who have had actual experience of managerial conditions in industry for some time, they will continue to be regarded as academic institutions run by men of little managerial experience with untested theoretical contributions to make to industry and commerce. Salary differences, of course, make recruitment of practising managers difficult. With the increasing tendency of higher educational institutions to encourage consultancy, however, many existing managers, and especially management consultants, would accept teaching posts and could use their previous knowledge and experience to open industrial and commercial doors to research students. Equally important, they could continue to carry out part-time assignments in industry and commerce and so ensure that their teaching is constantly revitalized with examples taken from managment in practice. The Association's lament that they have little opportunity and less encouragement to undertake research and the somewhat churlish attitude to part-time consultancy may stem from the distressing fact that, at present, even in colleges of advanced technology, many teachers of management subjects have been largely drawn from comparatively minor departments of commerce and industrial administration in technical colleges where the contribution to management theory or management practice has not shown particularly distinctive features. The development of education and training for management is unlikely to make rapid progress until some kind of sandwich device which has been so successful in training doctors is used to bring about greater union between higher educational institutions and industry and commerce. The appointment of a vigorous and successful managing director to become the first Research Fellow in management studies at Balliol College, Oxford, should have considerable influence on the direction of management education in Britain.

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* *New Ways in Management Training.* By C. Sofer and G. Hutton. (London: Tavistock Publications, 1958.) 18*s*.

THE RESPONSIBILITY OF SCIENCE TO SOCIETY

Science in the Cause of Man

By Gerard Piel. Pp. ix + 298. (New York: Alfred A. Knopf, 1961.) 5 dollars.

DR. PIEL is a comparatively young American thinker and writer who is publisher and editor of the *Scientific American*. In this book he has gathered together a number of papers and addresses delivered in the past ten years. This shows that Dr. Piel is thinking about the same problem as C. P. Snow, of the gap between scientists and the humanities, and on the need of communication to the general public of informed scientific reflexion. This is an extremely difficult task, and Dr. Piel's book is to be welcomed as a contribution towards solving the problem of which is he well aware. The essays discuss questions such as the history of science, the role of technology, education, disarmament, censorship, population problems, and the necessity of moral responsibility in scientists. Throughout the book there runs a note of urgency, well summed up in a comment by Lord Boyd-Orr: "Dr. Piel gives a lucid account of the forces which have put human society on a knife-edge balance between total destruction in a forty-eight-hour war and a wonderful new era of almost inconceivable wealth".

For Dr. Piel the great aim of science is to understand not only the external world but also man himself. He writes: "It is the great destiny of science not to ease man's labours or prolong his life, noble as these aims may be, nor to serve the ends of power, but to enable man to walk upright without fear in a world which he at length will understand and which is his home" (p. 111). There is a flavour of the old eighteenth-century hope in words of Dr. Piel where he admits that men have lost the old absolutes in which they once found purpose in their lives. "Science has brought forward no adequate substitute for the system of values that it has destroyed. But the demands of the human spirit cannot be denied . . . each man must have conviction in his own worth as an individual and purpose that fulfils the personal miracle of his existence." But this can only be grounded in man himself. He writes elsewhere: "There is general recognition of the idea that the cosmos is a vast impersonal system ordered by laws of mechanics" (p. 68). Dr. Piel does not realize how difficult it is to reconcile the two demands. The ruthless break between the impersonal system and the human person so easily leads to the treatment of persons impersonally. If laws of mechanics govern the cosmos they all too easily govern men, and all depends then on who possesses the power to govern. Dr. Piel has a high doctrine of man, but this impersonal system has thrown up this strange creature man and maybe is not entirely unresponsible for him. If man has no grounding for the best that is in him in any reality greater than himself, he will find all too unhappily that he has little worth in himself, and the purpose he sets before himself may well lead to a conflict with purposes other men set before themselves and destruction come on him. The appeal to rationality is not strong enough to overcome the despair of man in a system which is fundamentally impersonal and mechanistic.

But there is so much in Dr. Piel's book with which all men of good will must agree. On education he writes: "Without a grounding in science, man is cheated of full participation in the life of his century". He wants scientists and technologists to become more responsible. "They have an inescapable and direct moral responsibility for the practical uses to which their work will be put." But how to work this out he scarcely tells us. He realizes the problem of security; the danger that science will be put to uses for defence, and the use of weapons against which the conscience revolts; the danger of Government pressures, the weakening of academic freedom. But what is the poor scientist to do? He cannot dig: to beg he is ashamed; and Dr. Piel throws out the challenge but seems too little aware of what this involves, short of a general strike of scientists and technicians against all work which might be useful in war.

American writing is often turgid and heavy. Dr. Piel, however, has a lucid style and is not without wit. He is on the side of the angels, even though he rejects their reality. His book throws much light on the American scene, political and educational. His sympathy is broad, and his picture of science as both beautiful and illuminating has its attraction. His book represents the best in American scientific humanism, and opens up problems of the modern age which must engage the attention of all responsible men.

MARCUS KNIGHT

THE NATURE OF TIME

The Natural Philosophy of Time

By Dr. G. J. Whitrow. Pp. xi+324. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1961.) 42*s*. net.

TIME is probably the most fundamental observable in science, and it is curious that the idea of time is so much taken for granted. We learn how to measure it with remarkable accuracy without concerning ourselves with what it is we are measuring, and very few undergraduate courses in, for example, applied mathematics or physics, deal honestly with questions involving the nature of time. Relativity theory touches them, but even there it is assumed that every observer has a built-in clock about which he does not need to ask questions—one result of which is the now famous clock paradox.

The late E. A. Milne was probably the most eminent theoretical physicist who refused to take time for granted but asked—and to some extent answered—fundamental questions about the role of time in modern science, and since Dr. Whitrow was a student and collaborator of Milne's it is not surprising that it is from him we receive this long overdue account of the subject. Nor is it surprising that the author has devoted more than half the book to the various mathematical problems that arise in the study of primitive clocks and time-scales and the position of time in relativity theory, because it is to this part of the subject that he has made his own major contributions. Nevertheless, two very substantial chapters, of a non-mathematical nature, are given to the ideas and observations contributed by philosophy, psychology and biology to the subject, and in view of the large amount of material collected here it is to the author's credit that he has been able to present it in such an interesting way. A critical account, with adequate references, is given of the various philosophical theories and comments on the nature of time, and the more recent contributions of experimental psychology and biology are described clearly and fitted into an unfolding pattern. Even if the mathematical parts are ignored, the book is well worth reading for these chapters alone.

The remaining four chapters are concerned with the logical and mathematical aspects of the subject, starting in Chapter 3 with a study of the postulates of temporal instants, order and measurement, and going on in Chapters 4 and 5 to the study of time in the mathematical framework of relativity and cosmology. In Chapter 4 there is described a system of eleven axioms following formal definitions of the time and distance assigned by an observer to a distant event. The consequences of these are worked out without undue mathematical complication—needing in fact little more than the idea of a continuous function—and the resulting structure is compared with that of Einstein's special theory of relativity and also with that given axiomatically by A. A. Robb. In Chapter 5 there are an account and some discussion of the position of the time parameter in various standard space-times of general relativity and cosmology, and in Chapter 6 there is a brief discussion of the nature of time in modern physics, dealing particularly with asymmetry, the causal and statistical theories, the Minkowski diagram and, finally, precognition. In this second part of the book there is much to interest mathematicians and physicists as well as philosophers, and the whole book should undoubtedly be read by every scientist and natural philosopher. The printing and production are excellent.

A. G. WALKER

HIGH-ENERGY NUCLEAR PHYSICS

Kinematics of Nuclear Reactions

By A. M. Baldin, V. I. Goldanskii, and I. L. Rozental'. Translated by Ronald Peierls. Pp. xii+223+57 tables. (London: Oxford University Press, 1961.) 38*s*. net.

AT the heart of every experiment in high-energy nuclear physics, involving the collision of particles, lie two basic topics, one being the kinematics of the reaction and the other the theoretical interpretation of the reaction. This book, intended by the authors to be, primarily, for experimental physicists, deals with these two subjects. The first half is concerned with the classical theory of relativistic kinematics and contains the derivation of the mathematical equations required to solve two-body, and, under certain limiting conditions, multi-body kinematics.

This includes such topics as the Lorentz transformation, four-vectors, the Jacobian and other kinematical transformations necessary in going from the laboratory system of co-ordinates into the centre-of-mass system of the two colliding bodies. Decay kinematics of particles which decay into two others are also treated in detail. Appendix 1 contains graphical illustrations of the results of computations based on the formulæ derived in the first part, and a fair selection of the two-body reactions has been made. One might have included some graphs of decay kinematics, but, obviously, it is not practicable to cover all possibilities and still retain a book of reasonable size.

The second part is concerned with the quantum mechanical aspects of the interactions between particles and requires an introductory course in quantum mechanics for full appreciation and understanding. A discussion on the properties of the scattering (S)-matric and its applications is lucid, and topics like diffraction scattering, the optical theorem and the principle of detailed balance are dealt with in a straightforward manner. The chapter dealing with collisions of particles with spin becomes more mathematical in treatment and introduces vector coupling (Clebsch–Gordon) coefficients and the W, X and Z (Racah) coefficients. Other subjects discussed include the phenomenon of polarization and photo-reactions, such as photo-meson production and nucleon Compton scattering. In the second appendix to this part there are some 57 tables of varying complexity giving values of W, X, Z, and Clebsch–Gordon coefficients, and the work involved in the preparation of these must have been considerable.

The authors state that the first half of the book was written by two of them and the second half by the third. The result of the *troika* has been, in effect, to produce two separate books within the same cover, and an opportunity has been missed, one feels, to bridge the gap which tends to arise between theory and experiment as subjects become more and more specialized. As an experimentalist, I felt the need in the second part for more links between theory and experiment to see the full significance of the theoretical development, without having to go to other sources for this information.

One can commend the book as a reference of importance to students of the physics of high-energy particles. They may well wish to derive the equations in Part I for themselves, and it is good to have the answers set down methodically. As regards calculations of kinematics, however, electronic computers now eliminate much of the tedium of calculations, and publications like the *Oxford Kinematical Tables* (H.M.S.O.) go a long way to solving some of the two-body problems. The latter half of the book will be read more easily by physicists who have a good grounding in those topics going collectively under the name "theoretical option". The translator has been successful in turning the Russian text into freely flowing English, and the quality of reproduction is excellent. W. GALBRAITH

THE POWER OF PHYSICS

Physics and Politics

By Prof. Max Born. Pp. vii+86. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 12*s*. 6*d*.

THE four articles that comprise this volume are, as Dr. Kathleen Lonsdale admits in her foreword, not easy reading. They demand of the reader a concentrated attention and an understanding of physical science far exceeding that required by Bagehot's earlier classic under the same title. The first of these articles, "Modern Physics: a Veteran's View", was written for the volume. The other three are lectures given during 1958–60, the last giving its title to the book, while the other two discuss the concept of reality in physics and the limits to our image of the universe. Nevertheless, though even a Nobel Prize-winner can scarcely give to so slender a book the status of Bagehot's classic, and its appeal is probably more restricted, the two books have more in common than at first appears.

Essentially, Born's book is an exposition in the context of to-day of Bagehot's main thesis of the function of discussion in the evolution—or dissolution—of society. Even in 1876, Bagehot could write: "The issues of life are plain no longer. To act rightly in modern society requires a great deal of previous study, a great deal of assimilated information, a great deal of sharpened imagination", and Born's book demonstrates the truth of this comment to-day, independently of whether his main argument is accepted or not. In brief, all four articles elaborate the view that the advent of nuclear weapons has made warfare inconsistent with survival: the choice lies between the resolution of differences by discussion and the collapse or obliteration of civilization.

On the broad impact of physical science on politics, or the practical relations between the scientist and Government he has little or nothing to say, and although he provides one encouraging example of discussion and *rapprochement* of views between scientists of differing political views, once he leaves the strict field of science itself and its image of the universe, he seldom approaches Bagehot in penetration. Less satisfying than, for example, the late Prof. A. H. Compton's *Atomic Quest*, the book is moving in its sincerity and the deep, almost passionate, concern of the scientist for the consequences of advances in nuclear physics and the intense desire through discussion and interpretation to bring home to the world of politics, to society as a whole, the implications of the situation and the necessity for reconciliation and disarmament. R. BRIGHTMAN

A PRACTICAL ACCOUNT OF REACTOR THEORY

Thermal Reactor Theory

By A. D. Galanin. Second revised and authorized edition. Translated from the Russian by J. B. Sykes. (International Series of Monographs on Nuclear Energy. Division X: Reactor Design Physics, Vol. 3.) Pp. xiv+412. (London and New York: Pergamon Press, 1960.) 100*s*. net.

THIS book compares favourably with the standard Western texts on reactor theory being, in general, more comprehensive and more frankly technological. This is appropriate, since reactor science has ceased to be a purely logical exercise and is now a technological discipline.

The book begins with a description of the slowing down and diffusion of neutrons. The customary disquisition on nuclear theory is very properly omitted, though there is a useful compilation of nuclear data in the appendixes. This section and the five which follow it (on one- and two-group theories of homogeneous reactors, on heterogeneous effects, on critical sizes, and on perturbation theory) are fairly conventional, though the treatment of resonance absorption is different from the standard Western approach. This is followed by an extensive discussion of burn-up (the time variation of isotopic composition), problems of control and stability, transport effects, thermal spectrum and the treatment of heterogeneous effects which is associated with the names of Feinberg and Galanin: much of this material is not to be found in other books. All in all, this book is perhaps more useful to those who are studying thermal reactor theory for the first time than anything else which is now available.

However, this is not the book for which practical reactor physicists are waiting. Some of its theoretical treatment is rather out of date, and it makes no mention of the vital role of the digital computer in modern reactor calculations, though there is a short section on the numerical calculation of critical size. (In fairness to Galanin, it should be said that, although the book is dated 1960, the manuscript was finished in 1957: he would no doubt write a very different book to-day). On the theoretical side, neither collision probability techniques nor finite difference methods of solving the transport equation (such as that of Carlson) are mentioned. The discussion of thermal spectrum is limited to a perturbation solution of the Wilkins and Wigner–Wilkins equations, and to a rather unrealistic calculation of mean neutron energy. The only application of this work is to the thermalization correction to the migration area.

It seems unlikely that a single book meeting all these needs will now be written, and a series of monographs is perhaps a better solution. An excellent start has been made by Dresner[1] with a text on resonance escape. There is an urgent need for further works of the same quality on subjects such as thermal spectrum, numerical techniques and methods of solving the transport equation.

The translation of Galanin's book by J. B. Sykes is impeccable and the production is adequate. The price seems very high.

D. C. Leslie

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[1] Dresner, L., *Resonance Absorption in Nuclear Reactors* (Pergamon Press, London, 1960).

MARKOV PROCESSES AND THEIR APPLICATIONS

Elements of the Theory of Markov Processes and Their Applications

By Prof. A. T. Bharucha-Reid. (McGraw-Hill Series in Probability and Statistics.) Pp. xi + 468. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1960.) 89*s*.

It can be said straight away that this is a most interesting work, of considerable value to those concerned with either the theory or the applications of stochastic processes.

The first third of the book covers the general theory of Markov processes. Branching and diffusion processes are treated in particular detail. Although the treatment is straightforward, and does not bring in measure–theoretic concepts, the description 'elements' errs on the side of modesty. The presentation is exact, and a number of advanced and novel topics are included. For example, there is the material on limit theorems for branching processes, which has hitherto lain rather inaccessibly in the Russian journal literature. Another welcome section is the elementary treatment of the Kolmogorov differential equations on p. 64 *et seq.*

The remaining part consists of a truly encyclopædic study of applications: biology (populations, epidemics, genetics), physics (cascades, counters, fission detectors, reactors), astronomy (brightness fluctuations, galaxy distribution, radiative transfer), chemistry (reaction kinetics), queues, telephone traffic, and machine servicing. There are more than 600 references in all.

The author has rendered a real service by combing the literature, particularly the journals, in so many disciplines and languages, and by presenting the results clearly, capably and concisely. One is also impressed by the international balance achieved: the work gives a faithful account of activity in America and in both Western and Eastern Europe.

The theoretical section of the book is fairly definitive and well rounded. This is understandably much less true of the applied section, which is virtually a report on what has been done in the various fields, rather than a digested and unified account. So, results are presented in detail, but rather baldly and indiscriminately. I, for one, would not criticize the author for this: had he done otherwise the book would never have been written. Indeed, I would be more prone to criticize him when he occasionally weakens to the encyclopædist's temptation and writes as though a subject were in a tolerably complete state when this is scarcely the case. For example, the accounts of ecological competition and of reaction kinetics tend to give a more satisfactory picture than is justified. By its nature the book is concerned with frontier subjects, the development of which is often ragged, and just herein lies a principal value of the work.

Mr. Bharucha-Reid has succeeded in an ambitious task. I warmly recommend his book to anyone, student or research worker, at all interested in random processes.

P. Whittle

NEW EMPHASIS IN THE STUDY OF FORAMINIFERA

Ecology and Distribution of Recent Foraminifera

By Prof. Fred B. Phleger. Pp. viii + 297. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1960.) 60*s*. net.

Because of their abundance and wide distribution in marine sediments and in the plankton of past and present oceans, the shells of Foraminifera have proved valuable in stratigraphic studies and much taxonomic information has resulted. Following success in the dating of rocks, and to a lesser extent in foraminiferan taxonomy, many students of the group are now engaged in the field of palæoecology, or more specifically, in the reconstruction of fossil environments. Knowledge of the distribution and ecology of contemporary Foraminifera is of importance in such studies, and it is opportune that a book has now been written bringing together, for the first time, much of the available information on the subject.

Unlike so many papers reporting foraminiferan studies, this book includes a full account of the methods used by the author; he rightly points out that unsatisfactory methods of collection and subsequent treatment of sediment samples has rendered many published accounts less valuable.

Another obstacle to synthesizing the vast amount of previous work on foraminiferan distribution is the taxonomic confusion which exists in the group. This, of necessity, is avoided by the author, who relies on detailed studies, such as that carried out in the Gulf of Mexico, to illustrate his discussion. Nevertheless, it is unfortunate that space was not found for an appraisal of those aspects of foraminiferal distribution which would have clarified certain statements such as " . . . probably there are few benthonic species which are world-wide in distribution except those in the deep sea" (p. 45). This calls for comment; especially in a book of this title.

Interesting accounts are given of the existence of depth zones for Foraminifera, of the various faunas characteristic of different environments, of the importance of establishing and assessing the living components of a foraminiferan sample and of the contamination of faunas by displacement and currents. The least-documented part of the book appears to be that dealing with the ecological factors affecting the depth distribution of Foraminifera. It is here that the estrangement of many students of the group from their colleagues working with other marine animals is very evident. The section on food has no reference to H. Sandon's *The Food of Protozoa* in which four pages are devoted to the Foraminifera. Furthermore (p. 123), there is reference to work on the effect of pressure on marine bacteria which, the reader is told, is the only specific evidence known to the writer concerning the effect of pressure on marine organisms. There are, however, other works on this subject to which the reader's attention ought to be directed.

Prof. Phleger's book is a useful contribution in a very difficult field of study. No great criticism is implied when I suggest that now the first book on the subject has been written a second and more comprehensive one must surely follow. R. H. HEDLEY

ENDEMIC DISEASES IN THE TROPICS

The Control of Disease in the Tropics

A handbook for medical practitioners. By T. H. Davey and W. P. H. Lightbody. Pp. xii+22. (London: H. K. Lewis and Co., Ltd.) 47*s.* 6*d.* net.

SINCE the appearance of the first edition of this book in 1956 it has remained without a serious rival. Books devoted to the treatment of tropical diseases are much more numerous. Does the lack of books on the prevention of disease indicate a lack of interest in this subject in developing countries? If this is so, it is a great pity as the techniques of disease control have developed so rapidly that many of the major endemic diseases are now much more susceptible to attack.

These advances are reflected in the second edition of this book. More emphasis is placed on the treatment of tuberculosis with the new drugs now available. Wide-scale treatment by this method gives hope of eventual control without the need of expensive buildings and equipment. Similarly, the emphasis on the control of leprosy has shifted in line with the success of the sulphones; segregation is giving way to mass treatment on a wide scale.

The chapters on malaria have, perhaps, not followed the present trends quite so noticeably. A few paragraphs are devoted to the principles and practice of malaria eradication, but this seems scarcely enough for a programme that is operating in malarious countries with an aggregate population of more than a thousand million people. It may be argued that malaria eradication is operated by specialists. It is likely that its very considerable success would be even greater if the medical officer with general public health responsibilities had a better understanding of its methods and problems.

It is unwise to paint too optimistic a picture of the prospects of control of all the great endemic diseases of the tropics. No spectacular advance has been made against schistosomiasis, hookworm and filariasis; methods of control are known but have not been shown to be applicable in practice in many areas in the tropics. It would be useful for a book of this nature to indicate which methods of control are already successful and which require development for effective use on a large scale. An appreciation of this difference is essential for the rational planning of health services.

Two new chapters are included, one on poliomyelitis, which is increasingly being recognized as a problem in the tropics, and the second on health education. This subject, which forms a meeting ground between the physician and the social scientist, and which often suffers from a lack of definition, has been dealt with in a most straightforward manner; the importance of the part to be played by all grades of health workers is emphasized.

Almost every page has been revised in order to bring it up to date. There is no risk in forecasting that this edition will be as successful as the first.

M. J. COLBOURNE

HIGHLAND BRITAIN

The Peak District

By Prof. K. C. Edwards, assisted by Dr. H. H. Swinnerton and R. H. Hall. (The New Naturalist: a Survey of British Natural History.) Pp. xvi+240+28 plates. (London: William Collins, Sons and Co., Ltd., 1962.) 30*s.* net.

IN the darkest days of the War, almost as a gesture of defiance as well as of faith, began the publication of Collins's *New Naturalist Series*. The first three titles appeared in 1945 and the present volume is No. 44—apart from 18 monographs. The series was an instantaneous success, and the editorial board has never swerved from its original aim of "maintaining a high standard of accuracy combined with clarity of exposition in presenting the results of modern scientific research" to the British public. Many experienced publishers shook their heads knowingly at this deliberate refusal to 'popularize' science, but how wrong they have been proved. From the beginning it was agreed that there should be a number of regional volumes, interpreting the natural history of the area very widely, and not neglecting man and his activities. The London region was followed by Snowdonia, the Weald and Dartmoor and, in a more general view, the sea coast.

The present volume covers broadly the area of the Peak District National Park—the first to be designated as such under the Act of 1949. The days when one individual could, in the Gilbert White tradition, claim to cover adequately the whole field of natural history—including man—are past, yet a volume written by a team is apt to be irregular and to lack cohesion. The solution here is that Kenneth Edwards, professor of geography in the University of Nottingham, has acted as principal author and as editor. Two leading members of his team, nearly all closely associated with the University or with local institutions, are named on the title-page, but eight others are thanked in the authors' preface for their contributions or assistance.

For more than a quarter of a century, Prof. Edwards, from his base at Nottingham, has explored the Peak District, for the most part on foot. As a well-known field-man, he has guided successive generations of youth hostellers and members of the Ramblers' Federation and many field societies as well as his own students to appreciate the influence of a varied physical environment both on wild life and on man.

As an island of 'Highland Britain' surrounded by industrial England, the outcrops of the Millstone Grit, the Coal Measure sandstones and shales, the massive Carboniferous limestones and the old volcanic 'toad stones' have afforded sharply contrasted land forms and habitats—from intimate wooded dales to windswept moorland heights and boggy hollows. Man's interest has varied with the passing of time, and to-day competing interests are strong—from mining and quarrying, through farming, grazing and forestry to the needs of water supply, sport, recreation and nature conservation.

In a well-balanced text, supported by numerous maps and diagrams and a fine selection of pictures, the authors are to be cordially congratulated in giving due consideration to each of these varied and often conflicting interests.

L. DUDLEY STAMP

THE NEW CAMBRIDGE RADIO TELESCOPE

By Prof. M. RYLE, F.R.S.
Mullard Radio Astronomy Observatory, Cavendish Laboratory, Cambridge

THE Department of Scientific and Industrial Research has recently announced the award of a grant for the construction of a new radio telescope which has been designed at Cambridge. Like the two existing large telescopes[1,2], the new instrument will make use of the principle of aperture synthesis[3] and will enable a further increase in resolving power and sensitivity to be achieved.

The instrument has been designed primarily: (*a*) to obtain detailed information on the structure of the more intense radio galaxies; (*b*) to extend the statistical information concerning the number-flux density relationship to considerably weaker sources; (*c*) to investigate any non-uniformities in their angular distribution; these observations are aimed particularly at the further investigation of the cosmological problem. It will also be used in high-resolution studies of the emission from the Galaxy, and in exploring the detailed structure within galactic sources.

In the design of an instrument having a resolution of the order of 1 min. of arc, the limits set by the ionosphere make it necessary to operate at higher frequencies than that used in the present large interferometer (178 Mc./s.). Until recently, receiving amplifiers for these higher frequencies have had comparatively poor noise performance; but the development of new types of amplifier now allows the operation of 'unfilled apertures' of the Mills Cross[4,5] or synthesis type with adequate sensitivity.

Although the cylindrical parabolic reflectors which have been used as the individual aerial units in the present Cambridge instrument have been shown to provide a very inexpensive form of construction, there are difficulties in extending the same technique to the higher frequencies now envisaged. These difficulties arise partly because of the attenuation inherent in providing a line feed, and partly in the mechanical design of closely spaced wire reflector surfaces. The first limitation can be overcome by using a large number of independent pre-amplifiers in the manner proposed for the Benelux Cross[5], but the maintenance of sufficiently accurate phase equality involves considerable technical problems.

An alternative two-dimensional form of synthesis has therefore been adopted in which the relative positions of two small circular aerials are altered to occupy, in turn, all the positions present in the synthesized aperture[3]. One method of achieving such a two-dimensional synthesis is by the use of transit observations with the two elements mounted on mutually perpendicular railway tracks; this system is, however, inconvenient to use since the observations, of necessity, provide information concerning a complete declination strip of the sky, and a very large number of observations must be completed before a map of any part of it is available.

In the new instrument a single railway track is used to provide a variable spacing in an east–west direction. Since the rotation of the Earth carries this axis through one complete revolution in 24 hr., extended observations of a given area of sky at each separation of the elementary aerials allow the sampling of those components of a large aperture contained within a ring (Fig. 1). By combining the observations made with different separations of the elementary aerials, it becomes possible to synthesize a complete elliptical aperture, which may be regarded as the projection of a circular aperture the plane of which is normal to the Earth's rotation axis. The symmetry of the system is such that observations are needed for only 12 hr. at each spacing of the elementary aerials.

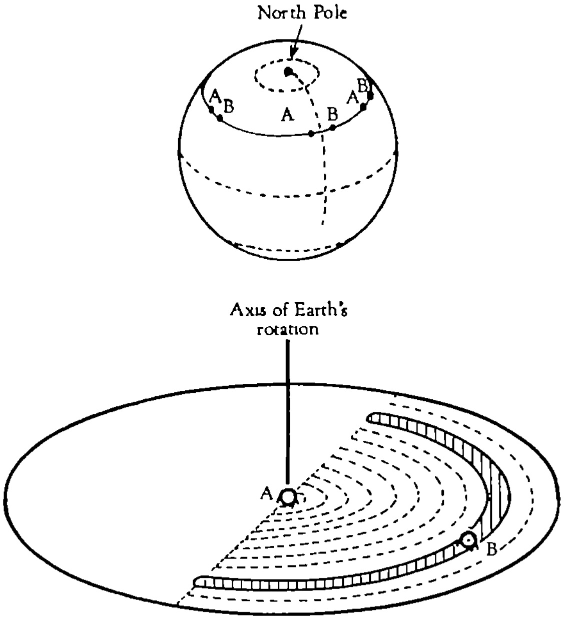

Fig. 1. Diagram to illustrate the change in relative positions of two aerials on an east–west axis during a period of 12 hr. and how observations with such an instrument provide one ring of the complete aperture

As with all methods of synthesis the signal-to-noise ratio obtained when a given area of sky is scanned in a given total observing time depends on the relative areas of the elementary aerials and the synthesized aperture; the new method has the considerable practical advantage over two-dimensional synthesis using transit observations that the observations over a particular period of time relate only to the small area of sky selected by the reception pattern of the elementary aerials. The system only requires the construction of a single railway track, and the total area of ground occupied is very small since it is contained within a narrow strip the length of which is equal to the diameter of the synthesized aperture.

The use of observations over a range of hour angle in order to provide differing projections of the interferometer axis on the sky was first applied by O'Brien[6], who synthesized a two-dimensional grating which he used for observing the distribution of radio brightness across the Sun. The method was afterwards applied to the observation of radio sources by Högbom[7], who

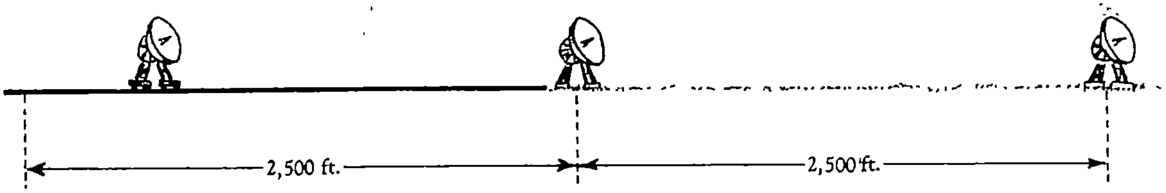

Fig. 2. Arrangement of the three paraboloids

used two small aerials at a wave-length of 3·7 m. to obtain a resolution of 2°.

The first attempt to provide an instrument of large resolving power by this method was made by Ryle and Miss Neville[8], using sections of the 178-Mc./s. interferometer to provide different spacings along an east–west line. Since, in this case, the individual sections of the aerial could not be moved in hour angle, observations were restricted to an area around the North Pole, 8° in diameter. This system provided a pencil beam response the width of which was 4·5 min. arc to half intensity, a resolution equivalent to that of a circular aperture 3,400 ft. in diameter. Observations were made for 12 hr. at each of the element spacings, and provided successive circular strips in the full aperture as illustrated in Fig. 1.

When the method is used for declinations away from the North Pole, provision must be made for tracking the individual aerials to observe the selected area of sky for 12 hr.; the synthesized aperture is now an ellipse, with the minor axis diminished by the sine of the declination.

The arrangement of the new Cambridge instrument is shown in Fig. 2; the signal from a rail-mounted aerial will be combined with those from two fixed ones arranged on an east–west axis, and the output from the two interferometers will be recorded separately. This arrangement compares favourably in cost with the use of a longer railway track and a single fixed aerial, and the observing time is halved.

Each of the three aerials will be an equatorially mounted paraboloid 60 ft. in diameter. As with the present instrument the signals will be recorded digitally on punched paper tape[9], so that they can be passed directly into *Edsac* for the synthesis computation. At high declinations the resolving power is equivalent to that of a circular aperture 5,000 ft. in diameter, and at declination 25° to an elliptical aperture 5,000 ft. × 2,000 ft. The sensitivity can conveniently be defined in terms of the equivalent aerial which would achieve the same signal-to-noise ratio when a given area of sky is observed in a given total time; it should be approximately the same as that of a paraboloid of diameter 750 ft. The overall performance, taking account of both resolving power and sensitivity, can be compared with that of a conventional paraboloid 1,250 ft. in diameter, and having a sufficiently accurate surface to allow it to operate on a frequency four times that used in the Cambridge instrument.

It is planned to use the instrument initially on a frequency of 408 Mc./s., with an expected resolution of 1·5 min. of arc. If the necessary phase stability can be achieved at 1,420 Mc./s., the resolution should be 25 sec. of arc.

It is hoped that work on the foundations will start within the next few months.

[1] Ryle, M., *Nature*, **180**, 110 (1957).
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HIGH-FREQUENCY, HIGH-POWER, PULSE SIGNALS FROM AN AUSTRALIAN TRANSMITTER

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BETWEEN September 1961 and February 1962, observations were carried out in various countries of an experimental pulse emission at 25·760 Mc./s. from a high-power transmitter in southern Victoria, Australia. The object was to seek anomalies of propagation which are to be expected when high-intensity irradiation occurs at the ionospheric *F* layer from an aerial with a low launching angle in the vertical plane. The peak pulse-power available was 50 kW. and the rhombic transmitting aerial, elevated 2·5 wave-lengths above the ground plane, had a gain with respect to a dipole computed to be greater than 17 db. with the peak of the main lobe in the vertical plane at an angle of approximately 6° to the Earth's surface. The azimuthal true bearing of the main lobe of the aerial was 312°, which meant that central south-east Asia, Europe and the United Kingdom were potential targets. 2 or 4 msec. pulses were used with a repetition period of either 40 or 80 msec. Radar back-scatter observations were first carried out in Melbourne, followed by observations of the forward transmitted pulse trains at locations in various parts of south-east Asia—mainly Singapore—and later in the United Kingdom. The relatively high frequency of transmission selected was intended to show up propagation anomalies during unstable or transitional conditions of the ionosphere. For a similar reason, operating schedules of 01.00–03.00 U.T. and 03.00–06.00 U.T. were adopted.

An aerial array of a new form was constructed for the reception of the back-scattered signal. This array will be described in a separate communication; but its essential property for the purpose of this present experiment was that it yielded some effective

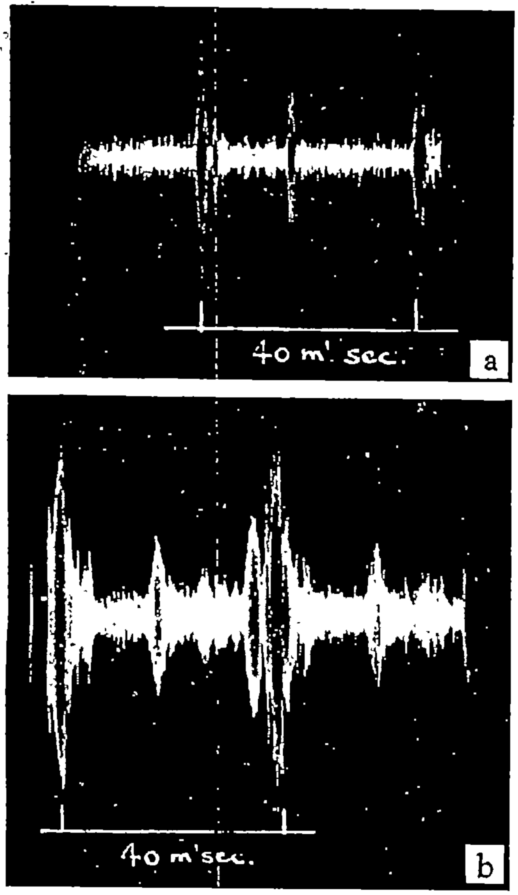

Fig. 1*a*, Radar back-scatter at Melbourne from north-west Australia, Multipath low—2 msec. pulse-length, 40 msec. repetition period. 01 30 U.T., October 19, 1961. *b*, Radar back-scatter at Melbourne showing multipath and other degradation—4 msec. pulse-length, 40 msec. repetition period. 02.15 U.T., October 19, 1961

pick-up at the lowest angles of down-coming radiation. The interesting results from Singapore on the forward pulse train were obtained by the use of rhombic aerials of high gain and high elevation for which the peak of the main lobe in the vertical plane occurred at an angle of approximately 7° to the Earth's surface.

Fig. 1*a* (01.30 U.T. on October 19, 1961) and Fig. 1*b* (02.15 U.T. on October 19, 1961) are typical of the normal radar back-scatter signals received at Melbourne on practically all the twice-weekly occasions of observation. A single-scattered pulse group of normal delay of the order 16–20 msec. indicates a target in north-western Australia. Frequently, during the latter part of an observation period for which this simple pattern had persisted, the delay period would increase to perhaps 25–30 msec.

On a large number of occasions, of which Fig. 2 is typical, double pulses appeared, often with multipath degeneration of the pulse shape—with a further delay for the additional echo of perhaps 5–10 msec. These are explained probably as two-hop back-scatter modes with target regions—depending on the delay time recorded and so on the angles of fire—in either Indonesia or northern Malaya.

When the incident pulse train was observed systematically at Singapore the anticipated simple pulse pattern with a 40 or 80 msec. repetition period was found to be the exception rather than normal. Fig. 3 (Singapore 01.00 U.T., November 16, 1961) gives an example of a single spurious echo of delay of the order of 20 msec. This was a fairly stable mode and could persist for most of a transmission schedule of duration 2 hr. Fig. 4, photographed 10 min. after the exposure of Fig. 3 on November 16, 1961, illustrates the well-defined type of second spurious echo which exhibits an additional delay of about 6 msec. While patterns of the type shown in Fig. 3 and Fig. 4 could be expected to occur frequently, there were occasions when extremely complicated echo displays would arise, and although these usually persisted for minutes only, they were reported by several independent observers to remain at times for periods up to 1 hr. Fig. 5 (Singapore 02.38 U.T. on January 9, 1962), which is derived from a long-exposure colour photograph at a time when the repetition period had been lengthened to 80 msec., includes unfortunately two sweeps of the time-base. The patterns can be differentiated by eye, however, and the existence of echoes throughout the repetition period of 80 msec. can be noted with a positively identifiable group of apparent delay of the order of 60 msec.

It is known that delays of the order of a few msec. can be experienced from signal components reflected from either side of the great circle propagation path[1]. Likewise signals scattered from regions of the Earth's surface illuminated by the minor lobes of the transmitting aerial could be expected to make contributions; but a delay of 16 msec., or more, as illustrated in Fig. 3, is difficult to explain. Although the evidence is not conclusive, it can be shown that a signal propagated from Victoria by the long great circle

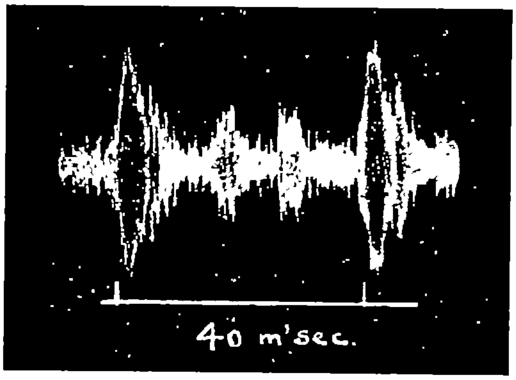

Fig. 2. Radar back-scatter at Melbourne showing scattering from both one- and two-hop target areas in South-East Asia.

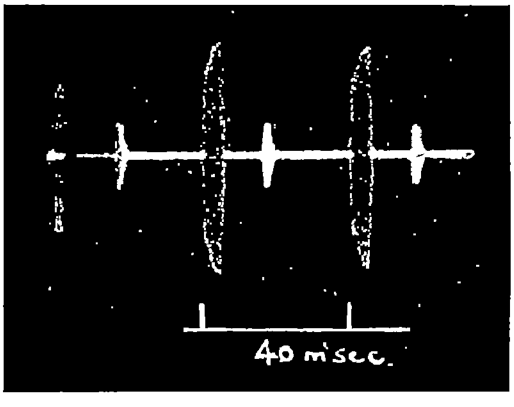

Fig. 3. Pulse train received at Singapore with a stable, delayed echo of about 16 msec. 01.00 U.T., November 16, 1961

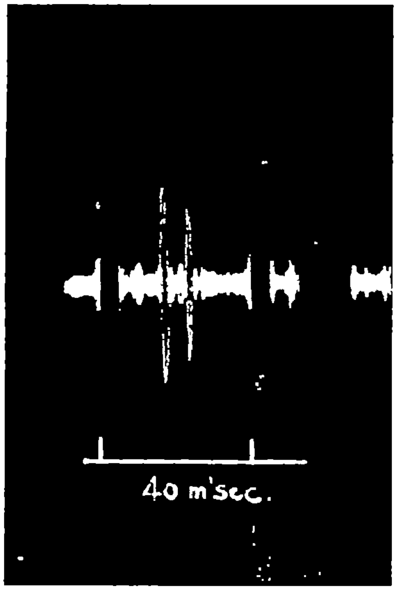

Fig. 4. Pulse train received at Singapore with a pair of delayed echoes. 01.10 U.T., November 16, 1961

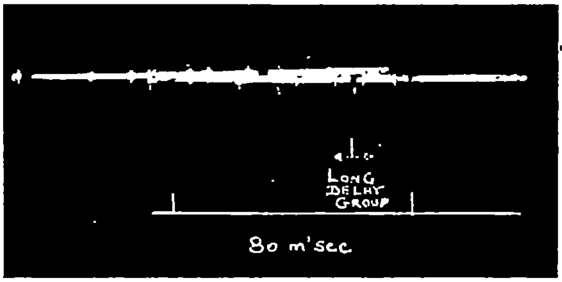

Fig. 5. Pulse train received at Singapore demonstrating complex propagation modes with very long delays. Repetition period 80 msec. 02.38 U.T. January 9, 1962

path could appear, by running through the 40-msec. time-base of the display, as a virtual 16-msec. delay. If this was the explanation, then the second delayed pulse of Fig. 4 could be considered to arise from the same mechanism of propagation but with a slightly higher-order mode structure, or even, as demonstrated by Shearman[2], from a mode pattern set up by the high angle, on bearing, secondary lobe of the transmitting aerial. In view of the high power of transmission, and the rather poor front-to-back ratio typical of a terminated rhombic, say, 12 db., there is a distinct possibility that long great circle transmission could have occurred. On the other hand, a careful examination of the corresponding oscillograph display when the pulse repetition interval was increased from 40 to 80 msec. appeared to confirm that an echo round the world in the desired direction of transmission was not the explanation of the pulse-time distribution experimentally obtained. Irradiation of the Earth's surface one hop to the rear of the transmitting aerial gives the possibility of scattered forward transmission along the short great circle path via Victoria to Singapore. This mechanism could account for an apparent delay of 16 msec. at Singapore.

No satisfactory explanation of the mechanisms represented by Fig. 5 can be advanced at this stage although, in this particular case, the existence of round-the-world echoes cannot be excluded. Such a transmission, as also that postulated for the long great circle case relating to Fig. 4, would have to occur over long-range paths in darkness for which the maximum usable frequency, as predicted traditionally, would be less than 25 Mc./s. even for 4,000-km. hops. In an attempt to confirm the presence of such modes, observations were carried out at the Radio Research Station, Slough, England, during February 1962, but no pulses were received.

The results demonstrate that if the *F* layer of the ionosphere can be illuminated by high-frequency radiation launched at a low angle with respect to the Earth's surface, complicated signal paths and mode patterns are established[3,4]. The overall effect of these various uncontrolled contributions is deleterious to high-frequency speech communication and almost intolerable for transmission of digital data. Such complexity suggests also that the distortion of speech thus transmitted, thought to be primarily a function of selective fading over the modulation band-width, might be due also to the partial destruction of the fundamental pitch frequency of voiced sounds by the spurious contributions of long (that is, greater than, say, 10 msec.) delay. The fact, however, that such paths can be established for long-range transmission at a frequency of 25 Mc./s. emphasizes that for low angles of fire and reception, established concepts for the prediction of maximum usable frequencies are unreliable. A new relationship between ionospheric critical frequency, maximum usable frequency and vertical angle of launching must be established for the case when this angle is less than 5°. Such a study has particular relevance to the problem of high-frequency communication during the present period of low sunspot activity and so of lowered critical frequencies.

If the complex pulse patterns exhibiting long delays as demonstrated can be shown to arise from ray contributions from the minor lobes of the transmitting and receiving aerials used—and these were typical of the best usually available for world-wide communications—the ineffectiveness of such aerials is obvious and the need for a more fundamental approach to the design of high-frequency aerial array is highlighted.

The final interesting conclusion arises from the fact that the present criteria of minimum spacing of aerials in space diversity along the transmission path are based on experience with existing types of aerials. It appears clear from Fig. 5 that the destruction of the correlation between the incident, radio-frequency energy contributions is not related, as now believed, merely to the imposition of the ionospheric fluctuations on the main (or any single) energy-carrying mode, but, on the other hand, to the effect of the uncorrelated, substantial energy contributions from other modes. The correlation coefficient, therefore, as at present understood, is a function of the aerial polar patterns (both transmitting and receiving) rather than of the ionospheric variability alone. Since the former are controllable, a new possibility appears to exist for the design of array groups for wide-aperture interferometry operating at high frequency.

The field work discussed here forms part of a larger programme of research directed towards the improvement of long-distance, high-frequency radio communication during the prevailing period of low sunspot activity. The essential mechanism to any such improvement is believed to be the selective initiation and reception of only the low angle of fire, long range, minimum hop, ionospheric *F* layer mode[5,6].

This presupposes launching and reception at vertical angles well below five degrees. The aerial design and propagation aspects of this work, together with the correlation experiments referred to above, are continuing at the University of Melbourne.

Extensive help for this world-wide project has been given by a number of organizations, and of these the contributions particularly of the following are acknowledged: Postmaster General's Department, Australia; Royal Australian Air Force; Cable and Wireless, Singapore; Department of Scientific and Industrial Research, Singapore and Slough, England; and the South-East Asia Treaty Organization. This programme of research at the University of Melbourne is supported in part by a grant from the Australian Radio Research Board.

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INITIATION OF CONTINENTAL DRIFT

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During the past few years, geophysicists have revived interest in the possibility that the continents may have drifted relative to the poles and relative to one another. The revival of interest is largely due to the results of work on palæomagnetism[1,2]; but it is significant that contributions have been made by workers in other fields such as oceanography[3], palæoclimatology[4,5] and crustal structure[6,7].

Recently, Runcorn[8] invoked the idea of a large-scale pattern of convection currents in the mantle to explain the present position of the continents. Following Urey[9], he supposes that the core of the Earth is growing and, in accord with the theoretical work of Chandrasekhar[10] and Vening Meinesz[28], as the core grows the higher harmonics for a convective motion in the mantle becomes more and more important. The continents are broken and moved by the horizontal motion of sub-crustal convection currents and come to rest over the descending currents, the rising limbs of the currents being near the centres of the ocean basins.

Independent support for this idea comes from the nature and position of the world-wide ridge-rift system. In general, the ocean basins are characterized by median ridges[11] and these often have rift valleys or fracture zones near their crests. The rifts are frequently seismically active[12,13] and measurements suggest they are characterized by high heat-flow[14]. In fact, they are features which might be expected over the rising limbs of convection currents[15]. According to Runcorn's hypothesis, there should be phases of continental break-up as each time the convecting pattern changes to a higher harmonic uprising currents must somewhere evolve under continents. Chandrasekhar[10] has shown that for a core of the present radius ($0{\cdot}55R$) the $n = 3,4,5$ harmonics are almost equally likely to be excited at marginal stability. We might therefore expect to find some places on the Earth's surface where new, rising convection currents are beginning to occur under or near to the continents. Fig. 1 shows a map of the world ridge–rift system, and it is immediately seen how well the distribution fits a large-scale convective pattern as visualized by Runcorn and supported by Chandrasekhar's calculations. It is also seen that there are at least two regions where the ridge–rift system intersects the continents; these are via the Gulf of Aden and Red Sea and via the Gulf of

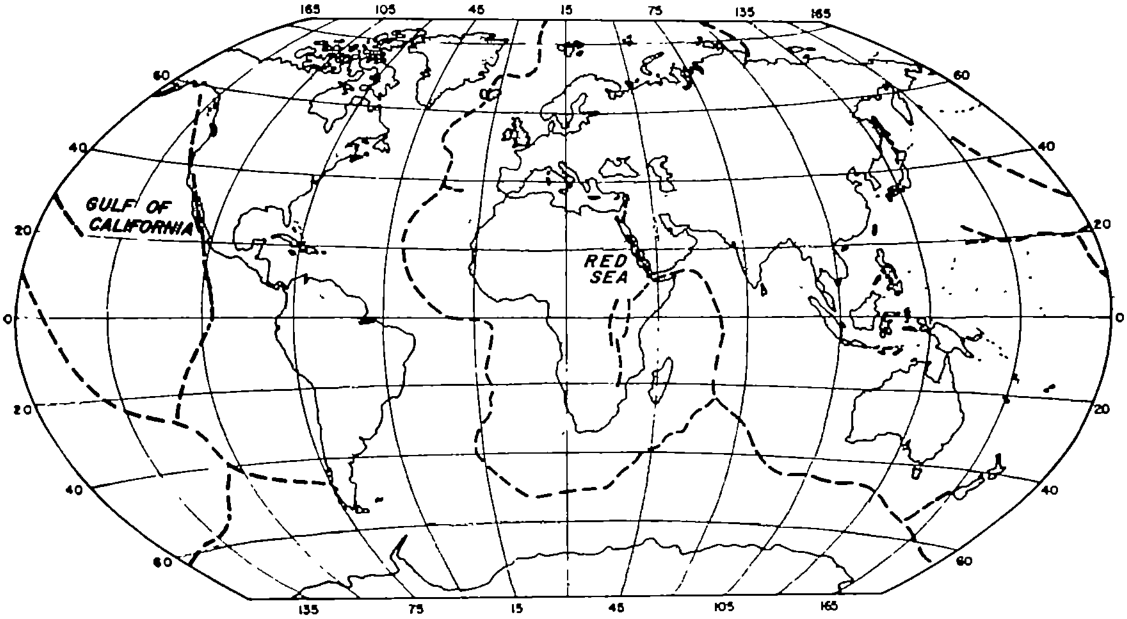

Fig. 1. Map showing the approximate location of the world ridge–rift system and associated features

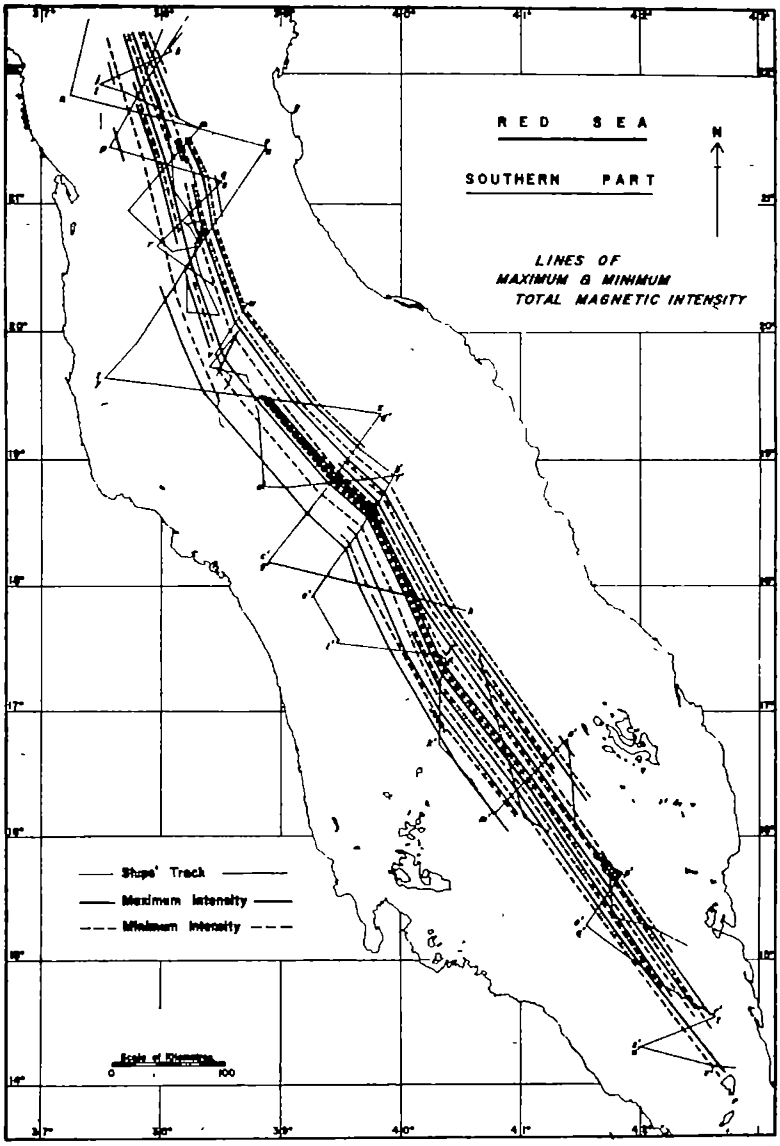

Fig. 2. Distribution of large magnetic anomalies in the southern part of the Red Sea

California. It is suggested that these two regions may give a clue as to how continental drift begins.

Recently, much geophysical work has been carried out in the Red Sea and Gulf of Aden[14-18]. A deep, axial trough under the centre of the Red Sea is characterized by large, positive Bouger gravity anomalies and these seem to be largest where the water is deepest. In 1957, I[19] interpreted this gravity maximum as being due to a basic mass, intruded through the downfaulted basement rocks, the large volume of intrusive material probably coming from beneath the Moho. More recent geophysical work supports this interpretation; for example, seismic refraction profiles by Lamont Geological Observatory, Columbia University[17], near the centre of the Red Sea show that a 7·1 km./s. layer is found at a shallow depth of 4·04 km. This is overlain by 2 km. of lava flows and sedimentary rocks, 0·45 km. of sediments and 1·5 km. of water. The crustal structure under the deep water therefore tends towards oceanic character and it seems that a strip of ocean reaching widths of up to 50–60 km. is in process of being formed in the centre of the Red Sea between the African and Arabian continents.

The areal extent of this recent oceanic crust may be mapped from the distribution of magnetic anomalies. Measurements of the total intensity of the Earth's magnetic field have been made by the Lamont Geological Observatory[17] and by Dr. T. Allan[20], lately of Cambridge. The anomalies over the deep, axial trough of the Red Sea have amplitudes of up to 1,200 γ ($1\ \gamma = 10^{-5}$ oersted) and gradients up to 200 γ/km. The large gradients make it difficult to contour the data, and lines of maximum and minimum total magnetic intensity have been plotted (Fig. 2) by correlating the features of the anomalies from profile to profile. It is seen that the trend of the anomalies is almost exactly parallel to the sinuous shore-lines of the Red Sea, suggesting that the continental crust has been torn apart leaving room for the intrusion of basic igneous rocks which give rise to the large magnetic anomalies.

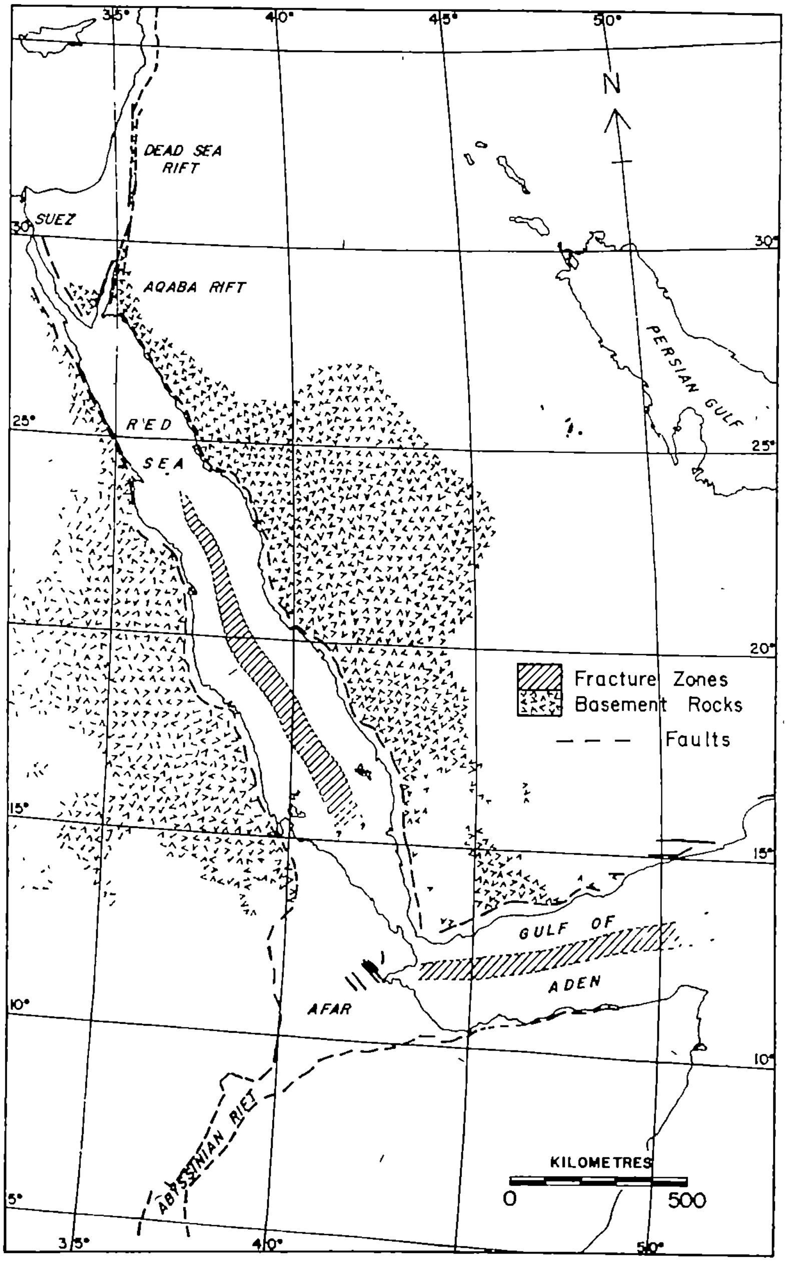

Fig. 3. Map of the Red Sea area showing the approximate location of the axial fracture zone, along which oceanic type crust seems to be forming

Large magnetic anomalies have also been observed along the centre of the Gulf of Aden[18,21] and these similarly trend parallel to the shore-lines. The possible extent of the fracture zone leading to the formation of the oceanic type crust is shown in Fig. 3, and it is seen that the tension crack opens up towards the south, suggesting a relative rotation of Arabia and Africa and the splitting apart of the continents. The Red Sea and Gulf of Aden form part of the East African Rift System, and it seems likely we now have geophysical evidence which might suggest that the Arabo-African continent is breaking up along the rift faults. The fact that the East African rift system forms part of the world ridge–rift system suggests that a new convection current may exist beneath the continental rifts giving a mechanism for their formation.

It appears likely that the Gulf of California has somewhat similar structure to the Red Sea and Gulf of Aden. Here, part of the world ridge–rift system (Fig. 1) intersects the North American continent via the East Pacific Rise. Geological evidence[22] indicates that the Baja Peninsula was once situated 480 km. to the south-east and formed part of the mainland; it has since rifted away by oblique tension across the San Andreas fault system. Gravity measurements[23] indicate that the southern part of the Gulf is in approximate isostatic equilibrium, and the deep water is underlain by heavy material. A seismic profile[24] in the axial deep at about 27·5° N. shows 0·4 km. of sediment and the Moho to be only about 6 km. beneath the sea floor or 8–10 km. below sea-level; the structure is therefore oceanic in character. Farther north, the

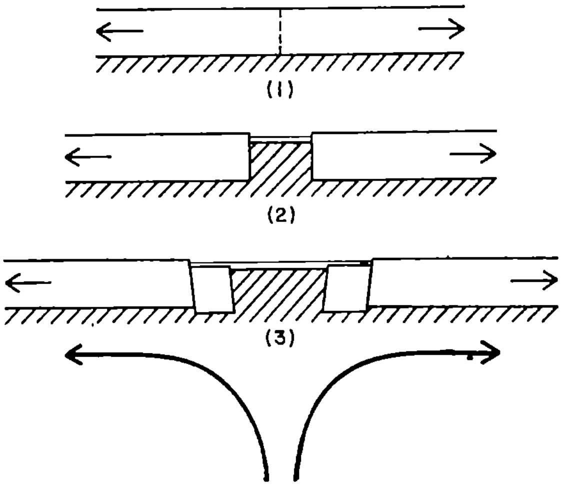

Fig. 4. A possible mechanism for the break-up of continental crust resulting in the formation of new oceanic type crust

structure of the Gulf is intermediate between continental and oceanic types with 5 km. of low-velocity sediments, 5 km. of rock with sialic velocity overlying material with velocity 7·5 km./s. Heat-flow measurements in the southern part of the Gulf[25] give values many times above normal. The Gulf is continued to the south by the East Pacific Rise which Menard[26] considers to be a very youthful part of the world ridge–rift system which is seismically active but as yet shows no median rift valley. Menard uses a rising convection current to explain the East Pacific Rise and it seems likely that the same current is causing a split of the Baja Peninsula from the mainland and the formation of oceanic structure along the rift zone.

Thus, we have two examples of what might be considered the earliest stages of continental drift, and it remains to consider a mechanism by which new oceanic crust may form. Fig. 4 is a diagrammatic representation of a possible mechanism based on the author's[19] mechanism of formation of the Red Sea structure. Tensional forces cause a crack in the Earth's crust, for example along what is now the centre of the Red Sea and Gulf of Aden (Stage 1). The tensional stresses were large enough to cause a separation of the continental crust and basic, igneous material is intruded into the tensional crack until hydrostatic equilibrium is obtained (Stage 2); the hydrostatic pressure of the magma is not sufficient for the intrusive material to reach the level of the continental crust, a consequence of its higher density. The filling of the crack causes subsurface movements which may result in faulting of the continental margins (Stage 3). The tensional stresses could be due to a rising convection current which is causing a separation of the continental crust with the formation of new oceanic crust in between. This gives a very simplified picture; the actual structure under the centre of the Red Sea is complicated and the axial, deep water is probably underlain by a series of wide, parallel fissures filled with basic, igneous rock[27].

In conclusion, it can be seen that a study of the world-wide ridge–rift system gives observational support to the theory of continental drift recently proposed by Runcorn. Besides satisfying a world-wide convection pattern, the presence of a possibly new current under the East Pacific Rise suggests a recently changing pattern as there a rising current is close to an Alpine fold-mountain belt; if convection is also used to explain fold mountains then the pattern must have changed within the past 3 × 10^7 years. Further, it is tentatively suggested that the regions where the ridge–rift system intersect the continents may show how continental drift is initiated with the formation of new oceanic crust depending on the degree of extension.

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RAINFALL INITIATION BY METEOR PARTICLES

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THE hypothesis that meteor particles could initiate rainfall was suggested by Bowen[1] in 1953. He investigated rainfall as a function of day of the year, and found peaks in mean rainfall records approximately 30 days after annually fixed meteor showers had occurred over the Earth. But neither the statistics in his first paper nor those in later and more sophisticated treatments[2–4] have convinced sceptical critics. On the other hand, no one has been able to show that he is wrong. New examinations[5] of rainfall records tend to show peaks on the dates where Bowen found them; but disagreement exists about the significance of the deviation.

No acceptable explanation of how meteor particles can initiate rainfall has been presented. Based on a theory about rainfall formation put forward earlier by Bergeron[6], Bowen indicated that the particles might work as ice-forming nuclei. The idea is that very many clouds do not yield precipitation because the small cloud droplets aggregate too slowly to form raindrops before the life-cycle of the cloud is terminated. In a cloud of supercooled droplets, however, an ice particle may grow rapidly. The vapour pressure is lower over ice than over water, and this causes transfer of water from the droplets to the ice particle. The upper layers of clouds often consist of supercooled droplets, and the ice particles may grow into snowflakes, which later may melt to form raindrops.

Closer investigation[7] has so far failed to support the hypothesis that meteor particles act as ice-forming nuclei. Neither the iron–nickel alloy nor the rock material of which meteors are predominantly composed, seems to have appreciable ice-forming tendencies.

There are other obstacles. Based on his world-wide investigations, Bowen states that the rainfall peaks lag about 30 days after the meteor showers, and that there is a spread of about ± 2 days between stations. Free fall is the only suggested mechanism of world-wide transport from the heights where the particles are suddenly decelerated by the atmosphere down to cloud heights. Calculations[8] show that this corresponds to a meteor particle diameter of about 10μ, with a range of about $0{\cdot}7\mu$.

Theoretical treatment of particle entry into the atmosphere shows that 10-μ particles are about the largest ones which can enter without catching fire and vaporizing. There is, however, no lower size limit. Therefore, an acceptable theory for meteor influence on rainfall must, through the mechanism involved, stipulate a lower limit very close to the 10-μ upper limit.

No such lower limit is suggested by the ice-forming nuclei theory.

However, the fact that meteors are made up of heavy material suggests another way of influencing droplet agglomeration. The 10-μ particle has a terminal velocity of fall greater than cloud droplets, and may therefore collide with them. The resulting droplet has a heavy core and therefore a greater fall velocity than the other droplets, and new collisions may occur. In this way water is accreted into one drop, and this is the first step towards rain formation.

It looks obvious that this mechanism must work best for the largest meteor particles so that a lower limit for the effect may be established. This question will now be examined in more detail.

A typical cloud droplet has a diameter in the range 10–20μ, and the meteor particle in question has a diameter about 10μ. The fall velocity of the meteor particle is higher than that of the droplet, and it should therefore catch up with the droplet. It is, however, very complicated to calculate the capture efficiency of the meteor particle. It is of a similar size to that of the droplet, and the influence of both bodies on the air stream must therefore be considered. No solution of the hydro-dynamical equations has yet been presented for this case, and only approximate calculations can be made.

Langmuir[9] has computed the collection efficiency of a fixed sphere for small spherical particles in the stream around it. He found the efficiency to depend on a parameter K, which may be modified for particles in Stokes's régime falling through the air to:

$$K = \frac{4 \cdot g \cdot \rho_2 \cdot r^2(\rho_1 \cdot R^2 - \rho_2 \cdot r^2)}{81 \cdot \eta^2 \cdot R}$$

where R and ρ_1 are radius and density of the fastest droplet or particle, r and ρ_2 those for the slowest, η is the viscosity of the air, and g is the acceleration of gravity.

The collision efficiency E_L was found to be:

$$E_L = 0 \text{ for } K \leqslant 1{\cdot}214,$$

$$\text{otherwise } E_L = \left[1 + \frac{3}{4}(\ln 2K)/(K - 1{\cdot}214)\right]^{-2}$$

The formulæ above have been criticized because the slowest droplet or particle in some respects was treated as a point. In order to overcome this deficiency Ludlam[10] applied a rough correction:

$$E = \left[E_L{}^{1/2} + \frac{r}{R}\right]^2$$

This formula has been used for computing the behaviour of a meteor particle entering a cloud composed of droplets. It is thus applied beyond the régime for which it was introduced; but for the purpose of this article the errors are probably acceptable.

In order to evaluate the hypothesis, the time and distance of fall from the moment the meteor particle enters the cloud until enough water is accreted to form a drop with a diameter of $\geqslant 60\mu$ were computed. The size 60μ is somewhat arbitrarily chosen. It is, however, felt that Stokes's formula can be used up to this size with acceptable errors, and that the meteor particle will have only a minor influence on the further growth of the drop, which already is well above the average cloud droplet in size.

Some details of the computation should be mentioned. The cloud droplets were assumed to be of uniform size, and the liquid water content of the cloud was set to $0{\cdot}3$ gm./m.^{3}, so that each droplet resided in a certain specific volume. E was computed from the formula presented here, and a collision was assumed to occur when, in a co-ordinate system fixed to the cloud droplets, the volume swept over by the particle times E was equal to the specific volume of the droplet. It was assumed that the collision resulted in a new droplet with weight and volume equal to the sum of those of the colliding bodies. The process was repeated with the new composite droplet taking the place of the particle. The computation was terminated when the droplet diameter was $\geqslant 60\mu$.

The calculations were carried out for a number of different configurations of particle sizes and droplet sizes, and for two specific weights of the particle, namely, 5 and 8. The viscosity of the air was set to $1{\cdot}53 \times 10^{-4}$ gm. sec.$^{-1}$ cm.$^{-1}$, a number appropriate for the upper troposphere. The calculations therefore apply to the top cloud-level.

The results are given in Fig. 1. The straight, heavy line in the lower part of each diagram (top and bottom) rising towards the right indicates the configurations where meteor particle and water droplet have the same velocity, and along this line no collisions should occur. Below this line the first collision occurs because the cloud droplet overtakes the particle; but the calculation is otherwise very similar to the method already described.

It should be noted that the diagrams in Fig. 1 can easily be applied to other liquid water contents,

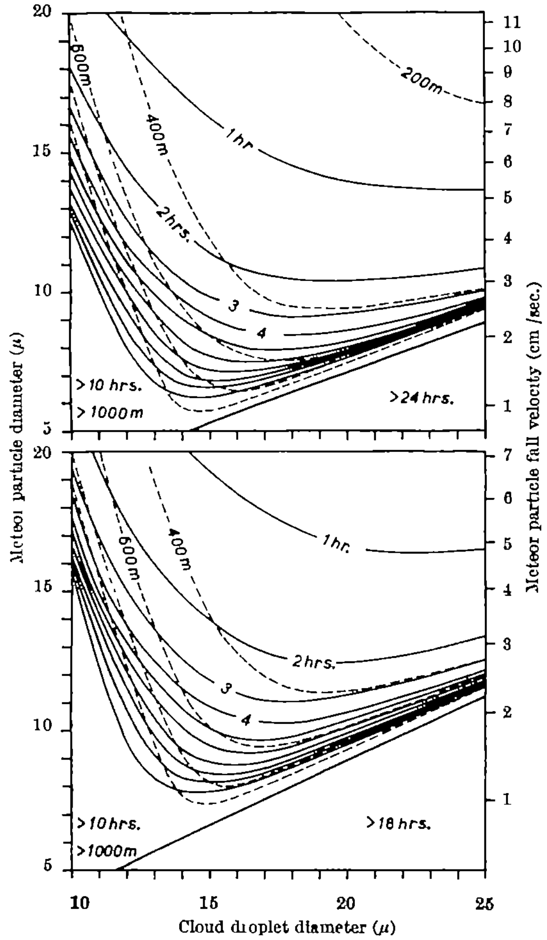

Fig. 1. Growth of a drop initiated by a meteor particle colliding with cloud droplets in a cloud with a liquid water content of 0·8 gm./m.^{3} The solid lines show the time of growth until a diameter of 60 microns is reached. The time-interval between the lines is 1 hour. The broken lines show the corresponding distance of fall; the interval between the lines is 200 metres. Along the straight, heavy line rising towards the right no growth occurs. The particles in the top diagram have a density 8 gm./m.^{3}; those in the bottom have a density 5 gm./cm.^{3}

because time and distance of fall are inversely proportional to the water content.

The diagrams in Fig. 1 indicate that large meteor particles among average-sized cloud droplets may grow by collision to 60μ size in a fairly short time, and that this time increases rapidly as the particle size decreases. It was mentioned earlier that due to the world-wide spreading of rainfall peaks, a variation in particle diameter of 0·7μ was tolerable. The calculations indicate that a decrease in particle size from 10μ to 9·3μ implies between 25 and 35 per cent increase in growth-time in clouds with droplet size in the range 15–20μ.

In a meteor shower with a broad-size spectrum such a sharp gradient in the rate of water accretion may well set a lower limit to the size of the particles with which the suggested mechanism will work well enough to be discernible in rainfall statistics. If, in addition, an upper limit of about 10μ is set by the vaporization during atmospheric entry, a narrow size-range remains for meteor particles capable of growing to raindrops.

The highest specific weight used for the particles in the computation is 8, corresponding fairly well to an iron–nickel composition. Particles composed of lighter material may be present in meteor showers. Comparing the two diagrams in Fig. 1 it is seen that the particles with specific weight 5 may act as rain nuclei at lower speed than the heavier ones. If the meteor showers differ in composition, it is possible that the time-lag of about 30 days differs somewhat from one shower to another.

It is also questionable if the time-lag necessarily must be the same all over the Earth. The fall velocity and the parameter K depend on the viscosity of the air, and because this quantity increases with temperature, the meteor effect may show a latitudinal variation and, at higher latitudes, a seasonal variation. A high cloud temperature requires larger particle size than a low cloud temperature for the same effect. This should be kept in mind when a statistical analysis of rainfall data is made.

The calculations terminated with a drop diameter of 60μ, which is far from the typical raindrop size of 1,000–2,000μ. As indicated in Fig. 1, the drop has already fallen some hundred metres, and in order to grow to raindrop size it must fall very many thousands of metres. A cloud of this depth will probably produce rain without any help. Simple scavenging is therefore scarcely the way meteor particles work.

There seem, however, to be more potentialities for influencing weather situations which in any case would produce rain. In order to discuss this problem, we may divide the development of a rain cloud into suitable stages. First comes the build-up of the cloud, when the small cloud droplets are formed. Then follows the ripening stage, during which aggregation and larger droplet formation take place. The third stage starts when updraught and condensation are fully developed and drop formation occurs rapidly owing to some kind of chain reaction, so that rain results.

Meteor collection will of course not have any influence whatsoever during the rainfall stage, because raindrops are formed in any event. But during the ripening stage the very thing is happening that meteor particles may initiate. They may therefore shorten this stage somewhat. The meteors may even work as ripening agent during the build-up stage, so that the total time from the cloud starts to form until rain starts to fall may be shortened considerably.

This hypothesis implies several possibilities for extra rain production, depending on the thermodynamical conditions for cloud formation. Where the clouds are formed in some daily cycle, and the resources of energy and moisture are not otherwise limited, the extra time added to the rainfall stage will increase the total amount of precipitation. Where the resources are limited in such a way that one cloud has a certain life-cycle during which it drains a certain area for energy and moisture, but at the same time suppresses cloud formation over a larger area by subsidence, the shortened life-time will give other clouds better opportunities to grow into rain clouds afterwards. More rain will fall because more cloud cycles are completed.

In a number of cases the cloud development stops without entering the rainfall stage. For clouds about to give precipitation, the extra push which meteor particles possibly give may bring the clouds into the rainfall stage. In such cases rain clouds are formed which would otherwise not have been formed. One may speculate on whether this can even cause the further development of weather systems which would otherwise subside.

The computations presented indicate that meteor particles may act as raindrop initiators in clouds by the simple process of collision with cloud droplets. The semi-theoretical formulæ used indicate that a lower size limit for this effect exists close to 10μ diameter. Combined with an upper size limit for particles occurring after a meteor shower of about 10μ, this may give a narrow size range for drop-forming particles. The hypothesis therefore supports Bowen's findings by statistical methods.

It is an open question if such an influence will increase the rainfall over a long period. Rainfall peaks on certain days may drain the moisture sources somewhat and cause reduced precipitation the following days. The mean rainfall curve for the world presented by Bowen really seems to suggest this. It is interesting to note that such an effect would tend to suppress the effect of possibly active nuclei after the first front of particles has arrived and make rainfall peaks sharper than the initiating meteor particle peaks.

The hypothesis outlined in this article lends itself to experimental tests, and it is to be hoped that such work will be taken up.

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CONTROL OF INTRODUCED DEER IN NEW ZEALAND

By M. J. DANIEL

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THE situation that exists to-day in New Zealand with regard to the many species of introduced mammals must surely be unique. Excluding the introduction in the fourteenth century of the Polynesian rat (*Rattus exulans*) and the Polynesian dog (*Canis familiaris*) by the Maori settlers, between 1779 and 1913 no less than 51 species of mammals were introduced[1]. Of these, only about 30 species became established[1]. These include 10 species of deer, which have no natural predators and, in contrast to many other parts of the world, do not seem to be limited by climatic factors. For these reasons the deer have attained pest proportions.

The red deer (*Cervus elaphus*), with more than a hundred liberations[2], is the most widespread over both islands and causes the greatest economic concern. Other species causing extensive damage to watershed protection forests and to pastureland are the Japanese deer (*Cervus nippon*) and the fallow deer (*Dama dama*) respectively.

During 1851–1930 the red deer was afforded partial or complete protection in most areas of the Dominion. As early as 1922, botanists and foresters were becoming gravely concerned at the rapid increase of the herds and of the damage being caused in watershed protection forests and planted pine forests[3]. Protection was altogether removed in 1930, and deer control was undertaken by professional hunters. Since then between 2–3 million deer have been shot by these hunters and by sportsmen[2].

The damage caused by deer in indigenous watershed protection forests, planted pine forests and in the vulnerable montane scrublands and sub-alpine tussock grasslands has been well documented by such botanists as Cockayne[4] and Zotov[5] and, more recently, by the forest ecologists carrying out the national forest survey[6–8]. This animal problem not only involves the several species of deer, however. Chamois (*Rupicapra rupicapra*), Himalayan thar (*Hemitragus jemlaicus*), pigs, goats, hares, rabbits and opossums are also very serious pests in some places. The last-named species, the Australian brush-tailed opossum (*Trichosurus vulpecula*), was introduced and liberated in many places during 1858–1921 for skin production, and it spread rapidly. It appears that by itself the opossum does not constitute a threat to the indigenous forests[9]. But when these canopy feeders are present in forests already containing numbers of ground browsers such as deer, goats and pigs, the resulting damage is very serious[8]. Some idea of the numbers of opossums in the forests can be obtained from the fact that 12 million were killed in the 10-year period 1951–61.

Although the large herds of the 1930's and 1940's have been reduced by hunting pressure, the deer problem in many parts of the country is still acute. It can be seen from Fig. 1 that the deer are widely distributed in New Zealand. The areas marked free of deer are either those most intensively farmed or otherwise unsuitable, such as the high glaciated region around Mt. Cook (3,860 m.).

The use of 1080 (sodium fluoroacetate) for domestic rodent control[10,11], the control of field rodents and coyotes in North America[12,13] and the control of rabbits in Australia and New Zealand is well documented[14–16]. This attempt by the New Zealand Forest Service to control deer with 1080 is a new use for this versatile poison, although Rudd and Genelly report[17] that about 160 mule deer were probably poisoned by 1080 laid in grain for squirrels in California.

In New Zealand as early as 1927 small trials were held to determine whether fallow deer could be controlled by using strychnine in salt-licks and in carrot. The results were inconclusive[1]. In 1956 it was found that red deer would accept branches of palatable trees sprayed with 1080 solution.

In 1957 a series of baiting trials was started on fallow deer[18], in the Caples Valley of South Island (Fig. 1). On either side the mountains rise to over 1,880 m., the dominant vegetation being *Nothofagus* forest. The valley floor has several grassy clearings which, being used by the deer, were ideal for such trials. More than 80 baiting trials were carried out in 14 months using root vegetables, fresh and dried fruits, hay, natural vegetation, salt-licks and lures. Although a statistical analysis of the trials cannot be made, it appeared that carrots and natural vegetation were the most acceptable. The majority of the baits were disturbed or eaten by opossums. The lures, such as oil of lime and aniseed were effective for the opossums but not for the deer.

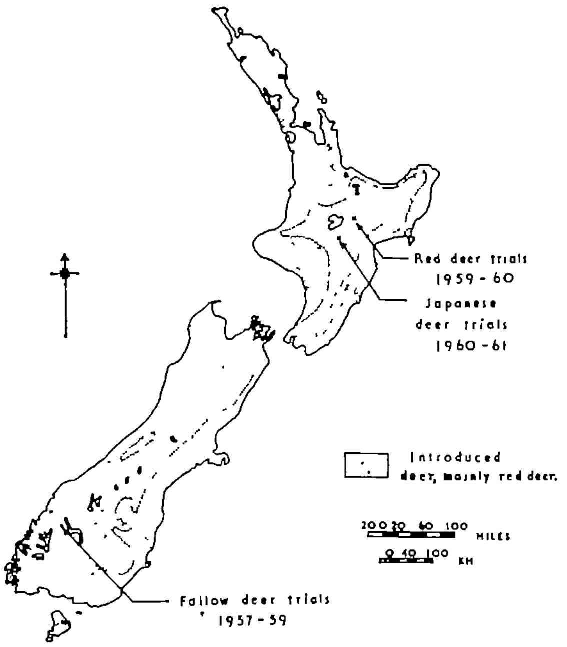

Fig. 1. Distribution of introduced deer in New Zealand and location of poison trials, 1957–61 (modified from Wodzicki, 1961)

Following these trials, three poison trials using 1080 in 1,100 kgm. of chopped raw carrot at a rate of 0·23 kgm. per 227–455 kgm. of bait were carried out. For effective distribution the bait was dropped by plane over the clearings. The total number of deer found dead was 247, representing kills of 60–90 per cent of the deer on the clearings at the time. None of the deer was found more than 280 m. from the poison line. The amount of bait in the rumen varied from a trace to more than 1·80 kgm. In the second poison trial, in which 70 deer were killed, the mean amount of bait eaten by the bucks was 1 kgm. and for the does only 0·5 kgm. Bleeding at the nostrils occurred in 33 per cent of the bucks and 10 per cent of the does. This difference is significant at the 2½ per cent level of probability. Taking the lethal dose for deer to be similar to that for sheep, 0·45 mgm. per 1 kgm. of body-weight, and the average weight for this species as 45–55 kgm., the amount of 1080 for a lethal dose would be 20–25 mgm. At the dosage used this amount would be contained in about 40 gm. of bait[19]. Some of the deer had consumed more than 30 lethal doses, causing severe bleeding at the nostrils and death within 2–3 hr. without convulsions. An examination of heart, liver and muscle tissue was made, and it was concluded that secondary poisoning from human consumption of deer meat poisoned with 1080 at the level used could not occur[19]. Other mammals poisoned in the trials included several red deer, rabbits, hares and opossums. No native birds were found dead.

These trials were followed by similar ones in the North Island on red and Japanese deer. Those on red deer[18] were held on the eastern boundary of Kaingaroa planted pine forest (Fig. 1). Poisoned carrots were distributed from a truck along several miles of road with scrub on one side and ponderosa pines on the other. A total of 61 red deer were poisoned in 6 trials. Success varied from 0 to 75 per cent in the trials. Disturbance by shooters and abundant spring growth lowered the expected kill. The trials on Japanese deer[20] were held along grassy river terraces and in the adjacent *Nothofagus* forest about 940 m. up on the central plateau of North Island (Fig. 1); 192 deer were poisoned on the grassy clearings and 42 in the forest. Probably many more were poisoned but not found. These represent about 80 per cent of the deer feeding on the areas at the time. This was the first indication that deer could be poisoned in indigenous forest.

From these trials considerable experience has been gained in handling and distributing large quantities of poisoned bait by plane. The knowledge that concentrations of deer on grassy clearings can be effectively and economically reduced is valuable. Further trials will have to be undertaken on goats, opossums and deer to determine whether a root bait or a pollard bait is the most effective for the different seasons in various localities. Also, knowledge of movements and concentrations of deer when attained will be of immense help in poisoning operations against deer in densely forested country. The question as to whether deer, goats, pigs and opossums can be effectively controlled by these methods, in many hundreds of square miles of mountainous and forested country, remains the problem of the future.

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OBITUARIES

Colonel P. A. Clayton

COLONEL PATRICK ANDREW CLAYTON died on March 17 at the age of sixty-five. He was educated at University College School and went on to the University of London. But, in 1915, his studies were interrupted by the First World War and, with the Royal Field Artillery, he saw service in Greece and Turkey. On demobilization, he joined the Survey of Egypt and his next few years were spent mapping the plateau of El-Tih in Central Sinai, which, rather unexpectedly, was found to attain a height of more than 5,000 ft. In 1927, he became surveyor to a Boundary Commission on the Egypto-Italian frontier and demarcated it by a line of 187 cairns (replaced in 1938 by concrete pillars) running south for 200 miles from the Mediterranean deep into the Sand Sea.

Later in that year he was associated in the contouring of the great Qattara Depression which his colleague G. F. Walpole had just shown to lie below sea-level and so to be a potential source of hydro-electric power. Next, Egypt's southern frontier claimed his attention and, in 1930–31, he extended the Nile Valley triangulation net for 350 miles to the extreme south-west corner of Egypt at 'Uweinat, observing 143 stations in 171 days. At 'Uweinat, he found and repaired a car which Major Bagnold's private expedition had abandoned and filled it with starving refugees, mostly women and children, who had already covered 200 waterless miles in a panic-stricken flight from the Italian bombing of Kufra Oasis. For their rescue King Fuad rewarded him with a gold medal *pour actes méritoires*. Among these activities Clayton found time for a bold reconnaissance of the west side of the Gilf Kebir, a great sandstone plateau the east side of which had first been sighted by Prince Kamal el-Din in 1926. A cave with rock paintings was found and, from the Sheb-Tarfawi area 100–150 miles west of Wadi Halfa, he brought back numerous querns, hand-axes and pottery sherds of neolithic and protodynastic age.

In 1932, Clayton took leave to accompany a private ground and air reconnaissance of the Gilf Kebir. His companions were the Hungarian Count Almasy, Sir Robert Clayton East Clayton (no relation) and Squadron-Leader Penderel. From the air various wadis were seen to dissect the plateau, and Almasy at once proclaimed that they had found "the lost oasis of Zarzura". In 1933, back with the Survey, Clayton set out from 'Ain Dalla and not only succeeded in reaching one of these wadis, Wadi 'Abd el-Malik, on the ground, but also actually made his way through the whole Sand Sea to Kufra. This passage was to prove of inestimable value to the Long Range Desert Group in the War to come.

That same year Orde Wingate had set out from Dakhla Oasis with a camel caravan to find Zarzura. After days of battling through a monotonous landscape of sand he at last sighted something dark on the western horizon which mirage exaggerated to a row of palms. But, as he got nearer, it dwindled again until it became a model *T* car, one of Clayton's, perched on a dune. There was a camp beyond but the mercurial Wingate never saw this. Instead, he turned straight back and made for Dakhla. His tracks, however, gave him away and that night the pursuing Clayton overtook him and made friends.

On the same expedition, scattered in the troughs of the Sand Sea, near the western frontier, Clayton picked up many tektites, weathered lumps of silica glass. This excited so much interest that a second trip was made to the site in company with Dr. L. J. Spencer and Dr. O. H. Little, the head of the Egyptian Geological Survey. Many museum specimens were brought in, some of which had been fashioned by Acheulean man into beautiful transparent hand-axes.

Italy's attack on Ethiopia in 1936 sounded a warning note, and both Clayton and Walpole were sent to commence what was to end up as a comprehensive survey of the 50,000 square miles of desert over which the Eighth Army and the Afrika Corps were to meet in battle. That, however, was accomplished by other hands. 'Egyptianization' overtook both Clayton and Walpole and they left the Survey in 1938—Clayton to join the Survey Department of Tanganyika.

In 1940 he was recalled from this to join Bagnold's Long Range Desert Group and was soon in his beloved Libyan Desert again, thrusting through the gap discovered in 1933 to raid the Italian communications between Jalo and Kufra. In January 1941, in command of *G* and *T* patrols of the Long Range Desert Group, he made contact with a small Free French force under Col. d'Ornano. On January 11 they raided the oasis of Murzuk, 1,100 miles from their base in Cairo. Three aircraft were destroyed on the ground; but two of the raiders were killed, one of them d'Ornano. A bloodless victory was scored next day at Traghen; but other posts proved too strong and had to be left alone. On January 31, Clayton with *T* patrol met a slightly stronger Italian motorized party at Gebel Sherif, 60 miles south of Kufra. The intervention of three Italian aircraft turned the tide of battle, and three of his cars were set on fire Clayton with two men were wounded and taken prisoner.

While a prisoner in Italy Clayton learnt of his promotion to Lieut.-Colonel with the award of a D.S.O. The Royal Geographical Society also honoured him with its Founder's Medal in 1941 "for surveys in the Libyan Desert and his application of this experience to desert warfare".

With the Italian collapse in 1943 Clayton escaped but was soon retaken. He spent the rest of the War in a German Stalag where his great skill as a draughtsman made him popular as a forger of passes for would-be escapers. After the War he was employed by the War Office in their Hirings Department, first as a serving officer and, after his retirement in 1953, as a civilian. He died on the day of his namesake, St. Patrick, March 17. His widow and a son survive him.

G. W. MURRAY

Mr. C. L. Hinton

CYRIL LANGLEY HINTON, formerly superintendent of research of the British Food Manufacturing Industries Research Association, died on March 15, at the age of seventy. He had retired from the Association's staff at the end of 1958 after nearly thirty-nine years of service.

Hinton was one of the three original members of the staff, the other two being the director, T. Macara, and L. E. Campbell. At that time the Association was connected primarily with the confectionery and jam trades, and his work on variations in the melting point of cocoa-butter, on the manufacture of fondant, and on the composition of fruits and jams will be long remembered. He became an authority on sugar analysis and was the author of the Department of Scientific and Industrial Research Food Investigation Special Report No. 48 on pectin and of a paper on the polyuronides in the *Annual Review of Biochemistry*, 1951. At various times he was chairman of the Technical Commission of the International Association of Confectionery Manufacturers, and therefore largely responsible for the work leading up to the publication of *Methods of Analysis* by that body. For many years he was a member of the Society for Analytical Chemistry's Sub-Committee for the determination of metallic impurities. He had a flair for foreign languages and became an acknowledged authority on international food legislation.

In his retirement C. L. Hinton found much to occupy his time and was author of the first number in Food and Agriculture Organization Food Additive Control Series *Food Additives Control in the United Kingdom*. He was up to the time of his death engaged on another work for the Food and Agriculture Organization on the utilization of sugar.

U.S. NATIONAL ACADEMY OF SCIENCES

New Officers

PROF. FREDERICK SEITZ, head of the Department of Physics in the University of Illinois, has been elected president of the U.S. National Academy of Sciences in succession to Dr. Detlev Bronk, president of the Rockefeller Institute, who has held office as president of the Academy for an unprecedented period—since 1950.

Two new members of Council of the Academy have also been elected: Dr. Roger Revelle, on leave from the University of California at San Diego to serve as science adviser to the Secretary of the Interior; and Dr. W. Barry Wood, jun., of the School of Medicine, Johns Hopkins University.

New Members

The following new members of the Academy have been elected:

Prof. L. H. Aller, professor of astronomy, University of Michigan;

Prof. W. A. Arnold, principal biologist, Oak Ridge National Laboratory;

Prof. V. C. Boekelheide, professor of chemistry, University of Oregon;

Prof. R. W. Briggs, professor of zoology, Indiana University;

Prof. H. Brooks, professor of applied physics, and dean, division of engineering and applied physics, Harvard University;

Prof. J. M. Buchanan, professor of biochemistry and head of the division, Massachusetts Institute of Technology;

Prof. G. F. Chew, professor of physics, University of California;

Prof. A. H. Coons, visiting professor of bacteriology and immunology, Harvard Medical School, and career investigator of the American Heart Association;

Prof. M. Doudoroff, professor of bacteriology, University of California, and professor, Miller Institute for Basic Research in Science;

Dr. A. E. Emerson, professor of zoology at the University of Chicago, and research associate, American Museum of Natural History and Chicago Natural History Museum;

Dr. S. E. Forbush, chairman, section of theoretical geophysics, Department of Terrestrial Magnetism, Carnegie Institution of Washington;

Dr. H. Friedmann, director, Los Angeles County Museum of History, Science, and Art;

Prof. R. M. Garrels, professor of geology, Harvard University;

Prof. D. A. Glaser, professor of physics, University of California, Berkeley;

Prof. D E. Green, co-director, Institute for Enzyme Research, and professor of enzyme chemistry, University of Wisconsin;

Prof. P. Herget, professor and director of the Observatory, University of Cincinnati;

Prof. H. L. James, professor of geology, University of Minnesota;

Prof. M. D. Kamen, professor of biochemistry and chairman of the Department of Biophysics, Brandeis University;

Prof. S. S. Kety, professor of psychiatry and psychiatrist-in-chief, Johns Hopkins University;

Prof. P. J. Kramer, James B. Duke professor of botany, Duke University;

Prof. Chia-Chiao Lin, professor of mathematics, Massachusetts Institute of Technology;

Prof. F. A. Long, professor of chemistry, Cornell University;

Prof. G. J. F. MacDonald, professor of geophysics, University of California, Los Angeles, and staff associate, Geophysical Laboratory, Carnegie Institution of Washington;

Prof. G. W. Mackey, professor of mathematics, Harvard University;

Prof. G. A. Miller, professor of psychology, Harvard University;

Prof. C. B. Morrey, jun , professor of mathematics, University of California, Berkeley;

Prof. A Pais, professor of physics, Institute for Advanced Study, Princeton;

Prof. W. H. Pickering, professor of electrical engineering, and director, Jet Propulsion Laboratory, California Institute of Technology;

Prof. I. Rouse, professor and chairman of the department of anthropology, Yale University;

Prof. E. L. Smith, professor of biochemistry and research professor of medicine, University of Utah;

Dr. P. Sporn, president and director, American Electric Power Co.;

Dr. T. D. Stewart, curator, division of physical anthropology, U.S. National Museum;

Prof. W. L. Straus, jun., professor of anatomy and physical anthropology, Johns Hopkins University;

Prof. S. Warren, professor of pathology, Harvard Medical School, and chief pathologist, New England Deaconess Hospital;

Prof. J. D. Watson, professor of biology, Harvard University.

Medal Awards

The Academy has awarded the following medals:

The *J. Lawrence Smith Medal* to Prof. Harold C. Urey, professor-at-large in the University of California in San Diego, "for outstanding achievement in the investigation of meteoric bodies".

The *Charles Doolittle Walcott Medal* to Dr. A A. Öpik, since 1947 Cambrian stratigrapher and palæontologist in the Federal Bureau of Mineral Resources, Canberra, "for outstanding contribution to the knowledge of Cambrian geology and palæontology".

The *Public Welfare Medal* to Dr. J. A. Shannon, director of the National Institutes of Health, Bethesda, Maryland, "for eminence in the application of science to the public welfare".

The *Jessie Stevenson Kovalenko Medal* to Prof. G. H. Whipple, professor of pathology and dean emeritus in the University of Rochester School of Medicine and Dentistry, "for contributions to medical knowledge".

The *Kimber Genetics Medal* has been awarded to Dr. M. Demerec, formerly director of the Department of Genetics of the Carnegie Institution of Washington, "in recognition of his many contributions to the understanding of the genetics of various plants, *Drosophila*, bacteria and viruses, and especially for his leadership in the investigation of unstable genes, the mutation process, genetics of micro-organisms and the genetic fine structure of the gene".

NEWS and VIEWS

Foreign Members of the Royal Society

The following have been elected foreign members of the Royal Society of London: Prof. D. Bovet, head of the Laboratory of Therapeutic Chemistry, Istituto Superiore Di Sanita, Rome, distinguished for his contributions to therapeutic chemistry; Dr. F. A. Lipmann, member of the Rockefeller Institute, New York, distinguished for his contributions to biochemistry, especially to enzymology and cell metabolism; Prof. J. R. Oppenheimer, director and professor of physics, Institute for Advanced Study, Princeton, distinguished for his contributions to modern quantum theory; Prof. V. Prelog, dean of the School of Chemistry, Swiss Federal Institute of Technology, Zurich, distinguished for his contributions to the development of modern stereochemistry.

Rothamsted Experimental Station:

Dr. G. W. Cooke

The Lawes Agricultural Trust Committee has appointed Dr. G. W. Cooke, head of the Chemistry Department at Rothamsted Experimental Station since 1956, deputy director of the Station in succession to the late Dr. A. Muir (see *Nature*, 194, 135; 1962). His work and writings have established him as the acknowledged authority on crop nutrition, a subject to which his recent book, *Fertilizers and Profitable Farming*, was a notable contribution. Cooke's training and interests give him an unusual ability to combine science with practice. The son of a Nottinghamshire farmer, he went from Loughborough Grammar School to the University College at Nottingham, where he graduated in chemistry in 1937. After a year in which he did biochemical research with Prof. J. M. Gulland at Nottingham, he obtained a research scholarship of the Ministry of Agriculture and Fisheries and with this went to work with Dr. E. M. Crowther at Rothamsted in 1938, where he was appointed to the staff in 1941. He first studied chemical methods of measuring the proportion of plant nutrients in soil that is available to plants, but during the War and afterwards, in collaboration with the Agricultural Advisory Services, he organized an extensive series of field experiments with phosphatic fertilizers. These had the dual purpose of finding alternatives to superphosphate when this was scarce, and of improving the use of fertilizers. Other experiments showed the benefits to be gained with some crops from placing the fertilizer near to the seed or roots. His more recent work has been concerned mainly with nitrogen fertilizers, comparing different forms and finding the best time to apply them. Along with these immediately practical problems, he has also done much to elucidate the form in which plant nutrients occur in soil and the changes they undergo there.

Chemistry at Glasgow :

Prof. D. W. J. Cruickshank

The University Court has recently founded a third chair in chemistry and named it after Joseph Black, who was the University's lecturer in chemistry during the period 1756–66. The first occupant of the chair, as from September 1, is to be Dr. D. W. J. Cruickshank, who, after taking a London degree with honours in engineering from Loughborough College in 1944, and then engaging in work for the Services, became research assistant in 1946 in the Chemistry Department, the University of Leeds. This directed his interests towards the potential applications of mathematics to chemistry and led to his spending three years at Cambridge, where he gained his B.A. in 1949, became a wrangler in Part 2 of the mathematical tripos and in 1950 gained distinction in Part 3. He then returned to Leeds as lecturer in chemistry and, in 1957, became reader in mathematical chemistry. He was awarded the degrees of Ph.D. at Leeds in 1952, and M.A. and Sc.D. at Cambridge in 1954 and 1961, respectively. Dr. Cruickshank's earlier work was in X-ray crystallography. In determining the structures of molecules from the X-ray diffraction patterns given by their crystals, the observational data, though of limited accuracy, are usually in large numerical excess over the structural parameters to be derived. Thus, mathematical and computational techniques can be of immense value in indicating best values for the parameters and in assessing their precision. In this field Cruickshank did pioneer work from the early days of the *Mark I* computer at Manchester, and his numerous papers have won him international reputation. His work on the thermal movement of molecules in the solid state is a beautiful example of combined X-ray and infra-red spectroscopic methods. Recently he has also turned his attention to the electron-diffraction method for studying molecular structure in the gaseous state, and more particularly to the quantum theory of chemical bonding.

European Launcher Development Association

In a written answer on April 16 the Minister of Aviation, Mr. P. Thorneycroft, stated that the Convention for the establishment of a European Launcher Development Association, drafted at the Lancaster House Conference last December, had now been signed by the Governments of Australia, Belgium, France, the Federal Republic of Germany, Italy, the Netherlands and the United Kingdom. The initial programme, which was now proceeding, provided for development of a launcher; using *Blue Streak* as the first stage and a French rocket as the second stage. The third stage would be developed under the leadership of the Federal Republic of Germany. The design, development and construction of the first series of satellite test-vehicles would be carried out under the leadership of the Italian Republic, the equipment for the ground guidance stations under the leadership of Belgium, and of the long-range telemetry links under the leadership of the Netherlands. Development firings of the first stage and of the complete three-stage launcher would be carried out at Woomera. In a written answer on April 17, Mr. D. Freeth stated that the Preparatory Commission for the European Space Research Organization had proposed that an inter-governmental conference should be held in May for signature of a Convention and other documents. It was not proposed that any of the establishments should be in the United Kingdom.

Nuclear Reactors for British Universities

In a written answer on April 17 the Parliamentary Secretary for Science, Mr. D. Freeth, said that one low-power nuclear reactor was being built for the use of the Royal College of Science and Technology, Glasgow, all the Scottish universities and Queen's University, Belfast. The Government had agreed in principle to make grants for the provision of two further reactors, one in Lancashire and one in the London area. A request for a second reactor at the University of London had been deferred and several universities had been told that there was no early prospect of grants being made. A grant of up to £255,000 had been made for the reactor for the Scottish universities and he hoped that the two further grants, totalling more than £500,000, would be announced later this year.

Air Pollution by Diesel Fumes

The Diesel Fumes Bill, which received a second reading in the House of Commons on April 6, was withdrawn after the Parliamentary Secretary to the Ministry of Transport, Mr. John Hay, assured the House that the Bill added little to the wide powers which the Ministry already possessed to deal with the problem under Section 64 of the Road Traffic Act. Already the Minister had power to prescribe densities of smoke and visible vapour and to prescribe that additives must be used in all Diesel engines. However, he suggested that Mr. R. Speir should propose an amendment to the Road Traffic Bill with the effect of Clause 3. This Clause allows Diesel-engined vehicles not already subject to special provisions about inspection to be tested at the roadside when reliable measurement is possible. Earlier, Mr. Hay referred to the very wide programme of research into air pollution which had been and was still being carried out by the Medical Research Council. Research work has not revealed any specific threat from Diesel fumes alone in the concentration in which they are found in city streets. Petrol engine exhausts are likely to be the main hazards, and the results of research in the United States in such fields are being watched as the problem there is far more acute. Mr. Hay repeated that extensive tests by the Department of Scientific and Industrial Research have established that a well-maintained and properly driven Diesel-engine vehicle does not normally emit any more than a slight puff of smoke. Neither fuel additives nor filter devices are as fruitful a field for reducing dark smoke as proper maintenance and adjustment of the engine. Mr. Hay said that during 1960–61 there were 1,875 prosecutions, and it was difficult to ensure that enforcement of the existing powers could be much improved although various possibilities were being examined. Furthermore, the law has recently been strengthened by a new regulation which from January 1, 1962, forbids the misuse by the driver of a Diesel-engined vehicle of the excess-fuel device, which in certain circumstances can provide extra power at the cost of black smoke. The regulation also requires control for the device on new vehicles to be such that it cannot be operated when the vehicle is moving and older-vehicles have to be modified in this respect by July 1.

The British Aerosol Manufacturers' Association

The first annual report of the British Aerosol Manufacturers' Association, covering the year ended December 31, 1961, records the provisional approval of a draft specification for metal containers for aerosol dispensers (Pp. 12. London: British Aerosol Manufacturers' Association, 1962). These specifications are to be considered by a technical committee of the British Standard Institution before circulation to industry for comment. The Executive Committee also recommended during the year that all aerosol containers filled by members should bear precautionary wordings. Detailed statistics of the aerosols filled by member firms during 1960 and 1961 have been collected and are listed. It is still open for discussion whether membership of the Association should be extended to include manufacturers of equipment and machinery.

Pollution by Agricultural Wastes

Although pollution of rivers is usually attributed to sewage or to discharges from factories, agricultural wastes cause trouble in many areas. This is emphasized in the seventh annual report of the Tweed River Purification Board, where it is pointed out that farm effluents, such as drainage from silage pits or piggeries, may be very strong and may overload a sewage works if they are discharged to a sewer (Pp. 46. Newtown St. Boswells: Tweed River Purification Board, 1961). The presence of traces of toxic insecticides or herbicides can also interfere with biological processes of sewage treatment. In an appendix to the report, information is given on the volume and composition of waste waters from a relatively new and rapidly expanding agricultural industry—poultry packing—obtained as the result of an investigation at a packing station in south-east Scotland. To give some idea of their polluting character, they are compared with those from a slaughter-house; on the basis of the demand for biochemical oxygen, the waste liquor from processing 12·5 turkeys or 32·3 chickens is equivalent to that from slaughtering one pig.

The Case Against Decimalization

Prof. A. C. Aitken, in a pamphlet entitled *The Case Against Decimalisation*, presents very skilfully the case against decimalization in general, starting with a lucid and cogent historical review (Pp. 22. Edinburgh and London: Oliver and Boyd, Ltd., 1962. 2*s*. 6*d*.). He is less persuasive, however, in arguing for the duodecimal system, adoption of which in coinage, with a pound of twelve shillings, he suggests. He claims that this is a much more efficient system than the decimal system and that its adoption offers substantial advantages on practical as well as on arithmetical grounds.

Committee on the Problem of Noise

The Committee set up in April 1960 by the Minister for Science under the chairmanship of Sir Alan Wilson has now fixed May 15 as its closing date for receiving written evidence concerning the nature, sources and effects of the problem of noise, and further measures which can be taken to mitigate it. Organizations and persons who intend to submit written evidence should send it to the secretary of the Committee, Mr. R. S. Matthews, at the Ministry of Health, Savile Row, London, W.1.

The Ciba Foundation

The report of the Ciba Foundation for 1961 records the holding of seven general international symposia on very varied subjects as well as a colloquium on the detection and assay of hormones by immunochemical means, and the fourteenth meeting in the series of colloquia on endocrinology. Four study groups were arranged, dealing with progesterone and the defence mechanism of pregnancy; biological activity of the leucocyte; the mechanism of action of water-soluble vitamins; and curare and curare-like agents; while seven discussion meetings were also arranged. At the three Dunant Coterie dinners the subjects of discussion included the Yeti 'scalp', medical education and research in Africa and Asia, the barrier function of the small intestinal mucous membrane, and some implications of the use of food additives. In the winter a series of film sessions, besides prize-winning medical films from abroad, French films on tropical medicine and an international selection of recent medical films, were shown. The thirteenth Ciba Foundation lecture, delivered by Prof. E. Katchalski, dealt with synthetic macromolecules as protein models. Three Anglo-French bursaries were awarded by the British Selection Committee and two by the French Selection Committee. Besides lists of publications in print or in preparation and accessions to the library, full details of the symposia and colloquia are appended to the report.

Scottish Field Studies Association

It is regrettable that the strong student support given to the Scottish Field Studies Association has not been matched by similar financial support. The annual report describes the fine work the Association is doing in Scotland, and makes it clear that no real progress can be made until the Association has a Field Centre of its own and enough money to make tidy administration possible (*The First Ten Years*. By B. W. Ribbons. Annual Report, 1961. Glasgow: The Scottish Field Studies Association). The modest sum required would not seem to be beyond the resources of industrial and charitable organizations in Scotland. Details of the courses available in 1962 and how help can be given to the Association are obtainable from the Secretary, 179 West Regent Street, Glasgow, C.2.

Sir John Hammond: a Pioneer in Modern Farm Animal Breeding

The February issue of *Animal Production* (4, Part 1; 1962) is devoted to the jubilee of Sir John Hammond, the pioneer of the modern outlook on the breeding of farm animals in Britain and a world leader in the physiology of fertility. Elizabeth O. Cockburn has contributed a most interesting account of his career and there is a characteristic photograph of him arriving with his bicycle for the dinner in his honour. Steeped in agriculture and of an old-established veterinary family in the eastern counties, he intended to qualify and become a third generation in the profession, but not having done Latin at school, the need to study it for the entrance examination led almost fortuitously to a change in programme and he entered the then new Agricultural Department of Cambridge, with results that have so fortunately influenced much of the quality of farm livestock. To-day, Hammond's pupils and collaborators are found in all or most of the countries with strong livestock interests. The achievement in 1958 of the breeding of ten million cattle by artificial insemination under the auspices of the British Milk Marketing Board can be traced to his pioneer activities. No less remarkable is the wide range of physiology explored and developed from Cambridge in the course of researches on the reproductive system, following, of course, on the foundations laid by the late F. H. A. Marshall. A selected bibliography accompanies this biographical note. The *Journal of Reproduction and Fertility* (3, No. 1; 1962) is concurrently marking Hammond's contribution to the study of reproductive physiology.

Irish Journal of Agricultural Research

The first number of the *Irish Journal of Agricultural Research*, issued by An Foras Talúntais (Agricultural Institute), Dublin, which became available in 1961 (1, No. 1; October 1961. Pp. 82. Published twice yearly. Annual subscription, 20*s*.; 3 dollars. Single number, 10*s*.; 1.50 dollars), provides a means whereby research workers in all branches of agricultural science including soils, plants and animals, engineering, economics and sociology can publish the results of their researches and will appeal primarily to scientific workers in agriculture. The first number contains papers covering a wide field ranging from soil analysis, cereal root eelworm, tomato culture, management of bogland, supplementary feeding of lambs and marketing of agricultural output.

The British Journal of Social and Clinical Psychology

The British Journal of Social and Clinical Psychology is to be devoted to social psychology and abnormal and clinical psychology (1, Part 1; February 1962. Pp. 80. Edited by Michael Argyle and Jack Tizard. Published three times per annum. Subscription price per volume, 50*s*.; 8.50 dollars. Separate parts, 20*s*.; 3.50 dollars. London and New York: Cambridge University Press). Papers on industrial, gerontological and sociological matters, such as delinquency, are to be included so long as they deal with psychological questions. This seems an excellent programme, and the first issue sets a good example which it is to be hoped will be followed by those to come. There are two papers on schizophrenia, one on personality questionnaires, another on space concepts in the mentally subnormal. Others are on institutionalism in mental hospitals, maternal attitudes to child socialization, and the attitudes of teachers to education. There is also a study of performance of tests of memory and learning in elderly patients. There are to be book reviews, and the books reviewed in this issue are given adequate notices. In the past, journals of this nature have tended to be either entirely clinical or else devoted to pure psychology, so that papers on the application of such things as psychological tests on abnormal patients have been pushed into odd corners. Now, with this new journal, they will be certain of reaching those who are interested in them, and the writers will not feel that they are being published on sufferance by editors who would prefer to devote their space to different matters. Everyone interested in psychology and psychiatry will welcome this journal and hope that it will have a long and successful career.

Association of Applied Biologists

The following honorary officers were elected for 1962–63 at the recent annual general meeting: *President*, Prof. H. G. H. Kearns; *Vice-President*, Dr. J. S. Kennedy; *President-Elect*, Prof. A. H. Bunting; *Treasurer*, Dr. F. Raw; *General Secretary*, Dr. F. T. Last (Glasshouse Crops Research Institute, Rustington, Littlehampton, Sussex); *Programme Secretary*, M. J. Way; *Editor*, R. W. Marsh; *Assistant Editor*, Dr. I. Thomas. New members of Council elected were: Prof. J. Colhoun, Dr. S. H. Crowdy, J. E. E. Jenkins, R. M. Lister and A. H. Strickland.

National Vegetable Research Station Association Scholarship

A HEINZ scholarship tenable at the National Vegetable Research Station, Wellesbourne, Warwick, was announced in *Nature* of April 28, p. 339. Another scholarship, which, apart from the name, is identical, is also tenable at the Research Station. This scholarship has been established by the National Vegetable Research Station Association (a body of growers and others interested in horticulture). Further information can be obtained from the Secretary of the Station. Closing date for applications is May 31.

University News: Cambridge

THE following have been elected to Fellowships in Churchill College:

Title A: Dr. D. B. A. Epstein, Trinity College, College lecturer and director of studies in mathematics; Dr. C. P. Wroth, Emmanuel College, University lecturer in engineering, College lecturer in engineering; G. R. Nicoll, University lecturer in engineering, College lecturer in engineering.

Title F (*Overseas Fellowships*): Dr. R. W. Clough, professor of civil engineering in the University of California (for 1963–64); Dr. J. A. K. Quartey, lecturer in chemistry in the University of Ghana (for 1963–65); Dr. G. Feinberg, assistant professor of physics in Columbia University (for 1963–64); Dr. R. Blankenbecler, assistant professor of physics in Princeton University (for 1963–64); Dr. J. Eells, jun., associate professor of mathematics in Columbia University (for 1963–64).

Announcements

MR. JOHN HEREWARD PITCHFORD, chairman and managing director of Ricardo and Co., Engineers, Ltd., has been elected president of the Institution of Mechanical Engineers in succession to Sir Kenneth Hague. Mr. Pitchford was elected a vice-president of the Institution in 1959, and he is also chairman of its General Purposes Committee.

DR. R. E. PARKER, lecturer in organic chemistry in the University of Southampton, has been appointed secretary and registrar of the Royal Institute of Chemistry, in succession to Dr. H. J. T. Ellingham.

THE 1962 Standards Laboratory Conference will be held at the Boulder Laboratories of the National Bureau of Standards, Colorado, during August 8–10. Further information can be obtained from Alfred E. Hess, Circuit Standards Division, National Bureau of Standards, Boulder, Colorado.

THE thirty-seventh annual conference of the Electrical Association for Women will be held in Buxton, Derbyshire, during May 14–16. Further information can be obtained from the conference secretary, Miss G. Shaw-Mackenzie, Electrical Association for Women, 25 Foubert's Place, London, W.1.

A SUMMER school, sponsored by the North Atlantic Treaty Organization, will be held in the University of Ghent during July 23–August 4. The topics to be treated concern the optical properties of semiconductors and insulators. Further information can be obtained from Prof. W. Dekeyser, Laboratorium voor Kristallografie en Studie van Vaste Stoffen, Rozier 6, Ghent.

Two postgraduate courses on "X-Ray Diffraction Techniques" and "Recent Advances in Non-destructive Testing" will be held in the Department of Metallurgy of the College of Advanced Technology, Birmingham, during July 9–13 and July 16–20, respectively. Further information can be obtained from Dr. I. G. Slater, Department of Metallurgy, College of Advanced Technology, Gosta Green, Birmingham 4.

A POSTGRADUATE course on "Nuclear Power" will be held in the Department of Mechanical Engineering, Imperial College of Science and Technology, commencing in autumn 1962 and lasting one year. The course will comprise lectures, practical work and a special project, and is intended for students holding a good honours degree in mechanical, civil, electrical or chemical engineering, or applied scientists who have graduated in physics or metallurgy. Further information can be obtained from the Registrar, Imperial College, London, S.W.7.

AN international symposium on "Food Regulations in Relation to International Trade", which will follow the first international congress of food science and technology, is to be held in London on September 24. Subjects under discussion will include: the present chaotic position; food-additive regulations; food-labelling regulations. Further information can be obtained from the the Honorary Secretary, The Food Group, 14 Belgrave Square, London, S.W.1.

THE 1962 national symposium of the Institute of Radio Engineers' Professional Group on Microwave Theory and Techniques will be held in Boulder, Colorado, during May 22–24. The programme will include ten technical sessions: microwave frontiers, theory, wave-guide properties; theory, wave-guide discontinuities; filters; high power; measurements; components; limiters; parametric amplifiers; future of microwave and solid state. Further information can be obtained from Mrs. Barbara Patterson, 1962 PGMTT National Symposium, Boulder Laboratories, National Bureau of Standards, Boulder, Colorado.

A SUMMER course on "Biological Servomechanisms", organized by the Massachusetts Institute of Technology, will be held at the Institute during July 9–20. The course will be divided into three sections: the general theory of dynamic feedback systems, linear and non-linear; the problem of modelling biological systems and interpreting their behaviour in terms of the general theory of dynamic systems; experimental sessions to demonstrate the behaviour of biological systems. Further information can be obtained from the Director of the Summer Session, Room 7–103, Massachusetts Institute of Technology, Cambridge, Massachusetts.

ERRATA. The title of the communication "Absorption-Elution Grouping of Modifications and Development of Bloodstains" by R. A. Outteridge (*Nature*, April 28, p. 385) should read: "Absorption-Elution Grouping of Bloodstains Modifications and Development".

IN the article entitled "Release of Hydrocyanic Acid from Crushed Tissues of All Stages in the Life-cycle of Species of the Zygaeninae (Lepidoptera)", by David A. Jones, John Parsons and the Hon. Miriam Rothschild, appearing in the January 6 issue of *Nature*, it is stated on p. 53, right-hand column, line 16, that "Blowfly larvæ . . . (were) found to be free of rhodanese activity". The authors state that the blowfly (*Calliphora vomitoria* L.) is, in fact, positive for rhodanese; unfortunately, the authors' proof did not reach *Nature* office in time for this correction to be made.

SHORTAGE OF ENGINEERS IN THE UNITED STATES

THE U.S. Engineering Manpower Commission reported that, for the fourth consecutive year, college freshman enrolments in engineering in the autumn of 1961 declined compared with the year before (*Interim. Sci. and Tech.*, February, 1962). The percentage of engineers in the total college population is also declining. In 1957, engineering students numbered 10·8 per cent of the total freshman class; in four years, this has dropped to well below 7 per cent. The result, according to the Commission, is that "the nation will have to make do with fewer engineering graduates for the next four to six years". Engineering graduates have fallen from 52,700 in 1950 to 37,800 in 1960. It now appears that, by 1965, the annual total will sink further to some 32,000.

In contrast, a report from the National Science Foundation indicates that the demand for engineers continues to soar. The American economy requires a little less than a million engineers today : by 1970 this will rise to nearly 1·5 million. To meet this, the annual output of new engineers over the next decade must be 81,000, or more than twice as many as anticipated.

Why is the engineering manpower supply not keeping pace with the demand? Are increased science enrolments the reason for the slump? Carl Frey, of the Engineering Manpower Commission, states that engineering enrolment actually rose a little—2·5 per cent—in those schools offering both science and engineering, while science enrolments in those schools were up by 24.4 per cent. The 'glamour' sciences are probably drawing away some students who might otherwise have elected to read engineering, but "it hardly seems possible that this alone accounts for the drop in the relative standing of engineering freshmen". The Engineering Manpower Commission report concludes that, "the need is not to take from science and give to engineering, but to increase engineering enrolments while maintaining science at its present level of growth". During the 1960's the Engineering Manpower Commission anticipates that engineering graduates will number about 35,000 annually, while graduates in the sciences, according to estimates by the Natural Science Foundation, will number some 80,000 annually.

If the sciences do not account fully for the slump in engineering, what does? The real cause, according to M. H. Trytten, director of the National Academy of Science, Office of Scientific Personnel, is the inadequate preparation of sufficient young people for careers in engineering: about half the high school students in the United States attend schools which are too small to provide proper training for college work in engineering and science. Trytten believes that the National Science Foundation forecast of the demand for engineers is too conservative, because neither the demands of the space programme nor the need for engineers in the American-sponsored programmes abroad is included in the forecast. "The only way out of the shortage is through stronger programmes of public school preparation 'down into the grades'."

New educational programmes are bringing improvements in the teaching of public school science and mathematics. But Trytten and others, including Prof. J. Conant, insist that these must reach deep into areas of educational neglect such as those now served only by the small rural school and those whose productivity of scientists and engineers is low, particularly in the South. In times when the demands were less severe, these deficiencies were less apparent. With science and technology in such dominant positions in society, there is deep concern that the United States is neglecting the education of one-half of its potential students.

GEOLOGY IN THE U.S.S.R.

ACADEMICIAN D. V. NALIVKIN discusses in the October issue of *Priroda* the future prospects of geological work in the Soviet Union (*Priroda*, 10, 67; 1961). He notes the existence of a very large number of geologists carrying out prospecting work for finding new deposits of economic minerals and ores and emphasizes the enormous work done in geological cartography in the production of petrographic, stratigraphic, palæontological, geophysical, metallogenic and other types of maps. For example, palæographical maps will be collected in two large atlases which will be exhibited at the International Geological Congress in India in 1964. Another important feature of Soviet geology is the establishment of fundamental boreholes, aimed at elucidating important geological successions. The author notes four important problems facing the modern Soviet geologist, namely: (1) synthesis of minerals in laboratories and on the industrial scale; (2) problems of tectonics (here he mentions the establishment of a new branch of science—"astrogeology"—which studies the influence of cosmic factors on terrestrial events); (3) absolute age of sedimentary and igneous rocks, which provides a new chronological scale in geology; and (4) the problem of the so-called 'underground rocket', a boring apparatus of a new type, which will make possible boring to a depth of 12 km. for the study of the deeper zones of the Earth and for extended prospecting.

Geological age. A new method of determining the age of rocks and meteorites is briefly described by G. F. Komovsky (*Priroda*, 5, 90; 1961). It is based on the measurement of the relative heights of the peaks of thermoluminescence of rocks heated to 400° C. This method, however, despite its simplicity, can provide only an approximate date.

Geological folds. Field tectonics and experimental studies lead V. V. Belousov (*Priroda*, 6, 14; 1961) to postulate the following types of crustal folds: (1) block folds, produced directly by vertical movement of the crust; (2) folds of compression—folds produced in the incompetent (plastic) strata, forming either domes or rolls and due to the attempt to re-establish equilibrium lost during movement of the crust; (3) deep-seated folds, usually occurring in metamorphic or ultra-metamorphic rocks; (4) folds of general compression resulting in widespread linear folds. The general mechanisms of folding are discussed in the article.

Earth's mantle. The project of the study of the Earth's mantle, accepted by the International Union of Geodesy and Geophysics in the summer of 1960, is briefly discussed by V. A. Magnitsky (*Priroda*, 6, 46; 1961). Naturally, this study will be applied only to the upper part of the mantle. A deep boring through the Mohorovičić discontinuity is projected, together with associated seismological, magnitometric, gravimetric and thermal flow studies.

Iron and manganese in ocean deposits. One of the scientific results of the Soviet research vessel *Vityaz* is the mapping of the concentration of iron–manganese concretions in the deposits of the Pacific Ocean, a brief account of which is given by N. L. Zenkevich and N. S. Skornyakova (*Priroda*, 2, 47; 1961). The average amount of these concretions in the area of their concentration is 8,600 tons/km.^{2}. Submarine photographs reveal a very high proportion of the oceanic bottom rich in concretions. These results agree with the findings of H. W. Menard and C. J. Shipek (*Nature*, 182, 1156; 1958).

Applied geochemistry. Geochemical landscapes, geochemical maps, dispersion aureoles and other problems of applied geochemistry are discussed by A. I. Perel'man (*Priroda*, 9, 98; 1961) and by A. A. Saukov and A. I. Perel'man (*Priroda*, 10, 59; 1961).

Transformation of a desert. An article by V. N. Kumin (*Priroda*, 9, 61; 1961) gives a succinct account of the important irrigation scheme encompassed in the construction of the large Karakum Canal, which carries the waters of the River Amu-Daria over 100,000 hectares of fertile desert lands. Various irrigational and biological problems are discussed.

Palæoclimatological maps. G. I. Bushinsky (*Priroda*, 8, 54; 1961) describes palæoclimatological maps constructed on the basis of detailed analysis of lithogenesis by N. M. Strakov, who subdivided lithogenesis into four main types: humid, arid, glacial and volcanological-sedimentary. These types are mapped out on the world map as definite zones for the successive geological periods.

Stromatoliths as indicators of Polar wanderings. A. G. Vologdin (*Priroda*, 11, 102; 1961) reports that, in collaboration with Chinese geologists, he was able to study in detail stromatoliths (banded algal limestones) of Sinian age (1,200–600 million years) in the region of Zisyan Mountain, 110 miles east-north-west of Peking. It was discovered that these platy formations exhibited on their upper surface convexities which were assumed to be due to the heliotropism of the algae. Previous measurement of the orientation of the convexities of stromatoliths in this region and in the Enisey range showed a displacement of the pole by 40° during the whole of the Sinian period. This method is considered to be adjunct to the usual palæomagnetic methods in the study of polar migration.

S. I. TOMKEIEFF

EXPORT MARKETING

THE reasons why export marketing has not aroused the same enthusiasm in Britain as in, for example, Germany, the United States, or the U.S.S.R. are discussed in a recent issue of *Planning* which has been prepared from material supplied by N. A. H. Stacey, economic adviser of the General Electric Co.*.

The relative decline in British exports has often been attributed to the post-war resurgence of Western Germany and Japan, but the report suggests that the length of time which has now elapsed since the end of the Second World War considerably reduces the strength of this contention. Apart from this, two explanations are generally advanced. The first is that Britain has failed to adapt the commodity structure of its exports to changing world needs, and the second that it has failed to adapt its traditional export market pattern to new developments in world trade, in particular to the rapid increase in trade between highly industrialized nations since the War.

Where do the deeper faults lie, and how can they be remedied ? The broadsheet concentrates on what is believed to be one of the major reasons for Britain's lack of success in the world's export markets, namely, the British manufacturer's use of obsolete marketing techniques resulting from a neglect of market research.

The neglect of the marketing function by British industry must be attributed, paradoxically, to the long, unbroken success of British manufacturing industry. Since Britain was the supplier for more than a century and a half of immense quantities of manufactured goods to the nations of the world, the notion that the process of manufacturing was the foundation of sound business gained an unwarranted sanctity. The corollary to this was the belief that distribution, even if not unproductive, was not as valuable as manufacturing.

Such an attitude was justified while Britain was the sole mature industrial nation, as it was until the 1880's. Now it has to share this position with a dozen or more industrial nations. The increased uniformity in the technical performance and efficiency of the goods made by reputable manufacturers in many of the industrial countries has meant that the effort needed for making a sale has assumed much greater importance.

The most important part of successful export effort in the competitive conditions of to-day is sound knowledge of the overseas markets. Familiarity with buyers' requirements, with the needs of the country, the preference of its population, and knowledge of its recent economic and social development are the determinants of import requirements both to-day and to-morrow. It is part of intelligent marketing to discern requirements and to anticipate needs. Sound marketing is based on the assiduous collection of information to answer two questions. These are: (1) In what (new) markets can the manufacturer sell his existing range of products (with or without modification) or extend his sales ? (2) What new products should be made for which there is a demand in his existing markets ?

The neglect of marketing, based on systematic marketing research overseas, is a dominant cause of Britain's present-day export predicament. To find out customers' preferences, to define the characteristics of the market, to estimate the volume of sale, to obtain information on products and prices of the competing manufacturers, to check on the efficiency of publicity, to secure economic information on likely developments in the future, these uses of marketing research have not been properly considered by many British manufacturers.

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* *Planning*, Vol. 28, No. 459 (February 19, 1962) Problems of Export Marketing. Pp. 19–38. (London: Political and Economic Planning, 1962.) 3*s*.

Why is this so ? The explanation is to be found in Britain's past successes. Until recently, certain traditional markets were considered a prerogative of British industry, which led to institutional thinking instead of entrepreneurial action in overseas trading. For German, French or American firms evaluation of overseas markets in the post-war period was a necessity if they were to compete successfully with the already well-entrenched British manufacturers. Foreign manufacturers had to gain markets rather than find them—with the result that their marketing methods had an aggressiveness about them lacking in those of British markets.

The rest of this admirable broadsheet gives practical advice on how companies can develop vigorous marketing policies.

OPERATIONAL RESEARCH AND THE HEALTH SERVICES

FOR some time the Nuffield Provincial Hospitals Trust has been alive to the potentialities of the application of scientific method and modern management techniques to the planning and administration of the National Health Service. In recent years it has particularly encouraged the development of operational research studies in the socio-medical field. The full scope of the work being carried out is not well known, and during December 14–15, 1960, the Trustees arranged a symposium of interested persons for a general review of progress in the field*.

The conference was attended by most of the leading authorities on operational research in the fields of hospital and public health as well as representatives of the Ministry of Health, the Department of Health for Scotland and the Ministry of Health, Northern Ireland. At the same time, most bodies at present in a position to sponsor such research were represented.

The papers presented to the symposium were commissioned with the object of setting out the opportunities and the realities. The plan consisted of portraying the main issues as seen by a medical administrator, and of contrasting these with known operational research applications in the field and, as an astringent epilogue, of presenting accounts of two recent studies actually carried out.

Among the points brought out by the chairman, Prof. M. G. Kendall, in his concluding remarks were that there are widespread opportunities for the useful application of operational research techniques in studies of the National Health Service; such an application can be made at various levels of administration, and the number of operational research studies should be increased as soon as possible. Such studies could profitably examine not only the possibility of the National Health Service as it now exists but also the possibility of modification. The field to be covered is so vast that it would be difficult to specify an order of priority of importance in topics to be studied, but it should prove possible to draw up a classification. Recognizing that research on these matters may proceed in a number of locations, under a number of different administrations, there should be some arrangement to encourage inter-communication between the research units concerned. In particular, there should be periodic conferences of interested persons, and some form of centralized information service, to ensure that the nature and existence of present-day research is brought to the notice of all those who are likely to engage in it and use it.

The desirability of encouraging interest among hospital and local authorities in the possibilities of operational research in the National Health Service field should be recognized, and can be brought about by conferences, lectures or case-studies and direct encouragement from Central Government authorities. Recognizing the shortage of expert operational research personnel, Prof. Kendall suggested that there should be encouragement in universities and elsewhere for the formation of operational research units, the members of which could be made available for consultation or could provide leadership to authorities engaging in operational research studies.

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* Nuffield Provincial Hospitals Trust. *Towards a Measure of Medical Care—Operational Research in the Health Services—a Symposium.* Contributors: J. O. F. Davies, J. Brotherston, N. Bailey, G. Forsyth and R. Logan. Pp. xi+91. (London: Oxford University Press. Published for the Nuffield Provincial Hospitals Trust, 1962.) 5s. net.

CALCUTTA SCHOOL OF TROPICAL MEDICINE

IN the annual report of the Calcutta School of Tropical Medicine and the Carmichael Hospital for Tropical Diseases, 1959–60*, the director, Dr. R. N. Chaudhuri, describes some of the more important observations made during that period.

A new technique, devised for experimental studies on amœbiasis, was successfully utilized in guinea pigs to produce a condition simulating chronic and recurring amœbiasis in man; this permitted direct studies on the evolution of the disease-process, and *in vivo* evaluation of amœbicidal drugs, without killing the animals. Human experiments have shown that asthmatic symptoms with high eosinophilia may result from ingestion of embryonated eggs of dog ascarids. This supports previous observations based on animal experiments and suggests a probable parasitic role in the ætiology of tropical eosinophilia.

Four types of protein malnutrition were categorized and follow-up studies showed the probability of one passing into another, indicating that the clinical pattern in them all resulted from the same ætiological process, namely, protein cum caloric deficiency, aggravated or precipitated by infection. Plant proteins, though not quite so good as milk, have been found useful in deficiency-states, and a pulse–cereal–green vegetable gruel sweetened with jaggery has produced encouraging results. It has the advantage of simple preparation at low cost and may considerably replace milk. The supply of vegetable proteins by a "supplementary feeding" programme to infants and young children in the low socio-economic group of the population may hold the promise of a prophylactic against this condition. Protein-rich biscuits were also prepared from protein hydrolysate of pulses and fish-protein hydrolysate. Clinico-pathological and experimental observations were also made on the nature and extent of hepatic involvement in diabetes mellitus.

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* Annual Report of the Calcutta School of Tropical Medicine, and the Carmichael Hospital for Tropical Diseases, 1959–60. Pp. 131. (Calcutta: Calcutta School of Tropical Medicine and the Carmichael Hospital for Tropical Diseases, 1961.)

As had been previously shown, the percentage of *Staphylococci* resistant to prevalent antibiotics remained high, namely, 70 per cent, though they were uniformly sensitive to erythromycin. Resistance of *Escherichia coli*, from cases of urinary infection, to sulphonamides, streptomycin and tetracycline was found to be on the increase.

Work on the survival of smallpox virus in a non-pathogenic form in the brain of infant mice is in progress with the view of finding the LD_{50} in different passage-levels of the variola virus. Studies are still being carried out to determine the normal antibody titre in random population samples, and especially among young children, against variola virus.

Leucoderma skins receiving ultra-violet radiation, compared with a non-irradiated control, on histological examination showed an increase of melanocytes with proliferation of dendrites and abundance of melanin. To substantiate previous observations that counter-irritant drugs, commonly applied locally, are apt to produce hard erythematous and reactionary patches, ultimately resisting any form of treatment, a number of patients with leucoderma were treated with psoralen ointment in a soothing base. The results have been encouraging, and further work is in progress.

Histamine reaction in cases of non-lepromatous leprosy showed significant quantitative difference when compared with that on the normal skin. Studies on serum protein in the two polar types of leprosy revealed an increase of γ-globulin fraction in sera of lepromatous cases. Formation of leishmanial granuloma was demonstrated in the spleen of drug-resistant kala-azar. Biochemical observations of interest were made on the liver of animals infected with *Trypanosoma evansi*.

A new species of *Dioscorea* yams, obtained from the Darjeeling area, was found to contain as much as 4 per cent diosgenin. The glycoside content of *Digitalis* was found to reach its maximum when the plant was about 1½ years old. Three compounds of indigenous drugs were prepared for clinical trial, namely, arborine hydrochloride, aegelin and psoralen. A volatile oil derived from the seeds of *Nigella sativa* was shown to have a mosquitocidal property; its chemical composition was determined. Four new compounds were synthesized for possible use as insecticides and insect repellants.

A number of new drugs was also subjected to clinical trial to evaluate their therapeutic efficacy, particularly against amœbiasis, leishmaniasis, helminthiasis, and skin diseases, including superficial mycosis and leprosy.

CANCER SERVICES IN EUROPE

A PAPER prepared by Dr. A. Chaklin, chief of the Cancer Division, World Health Organization, Headquarters, Geneva, and Dr. J. Mosbech, Office of Medical Statistics, Danish National Health Service, served as a background document for the technical discussions held during the eleventh session of the World Health Organization Regional Committee for Europe at Luxembourg in September 1961. The main points have been brought together in an article in the *WHO Chronicle* (16, No. 3; March 1962). While the ætiology of most forms of cancer is still unknown, many of them can be effectively treated. For this, well-organized services are essential. There are considerable differences in the organization of cancer control in the various countries of Europe. In the Scandinavian countries cancer falls within the province of the family doctor and the general hospital. In the United Kingdom, also, there is no separate speciality for malignant disease, all treatment being carried out on a regional basis within the ordinary facilities of the National Health Service. There are, however, certain hospitals and clinics reserved for cancer treatment, and a number of homes for the care of incurable cases.

The situation in the U.S.S.R. and the countries of eastern Europe is quite different. A network of cancer services with a high degree of independence, staffed by doctors especially trained in the detection, treatment and prevention of the disease, was established by Government decree in the U.S.S.R. after the Second World War.

This network consists of cancer stations, cancer dispensaries, and hospital cancer departments. At the cancer stations, the public is offered advice and education for the prevention and early detection of malignant disease, while mass screenings are organized, cancer cases registered and patients put in touch with expert facilities. Every doctor is required to refer any patient with an obviously or possibly malignant lesion to one of these stations. The cancer dispensaries, concentrated in the larger towns, are highly specialized and well-staffed institutions the functions of which include not only the application of the surgical and other treatment required but also the assembly and the analysis of data, the training of medical and allied staff, the education of the public and general collaboration with public health authorities in cancer control. The hospital cancer departments concentrate mainly on the purely medical aspects of cancer treatment at a specialized level.

Thus, although in both the U.S.S.R. and in the United Kingdom it is the general practitioner or his equivalent who makes the first contact with a case of malignant disease, the subsequent procedure is very different; in the former country the patient will be treated by a specialized service while, in the latter, he is most likely to be dealt with in the local general hospital with appropriate facilities. The differences between the two services may eventually become less because of the trend in many countries towards the centralization of hospital services. As knowledge increases, the collaboration of many specialists becomes necessary and the cost of equipment mounts; these factors favour the concentration of cancer patients in a few hospitals at regional, or even at national level.

Efficient cancer services cannot be planned or operated without valid statistics, which must relate to morbidity as well as mortality. Thus, some form of cancer registration is essential. A registration or notification system, particularly if combined with an analysis of treatment and survival statistics, is of the greatest value in providing not only an index of therapeutic efficiency but also important material for epidemiological research. In a small, closely knit country such as Denmark or the Netherlands, a single national registry for all forms of malignant disease is possible. In others it may be necessary to work from several national registers; or there may be separate national registers dealing with particular types of tumour such as bone sarcoma. It is from such statistical information as that concerned with the rapid increase of morbidity and mortality from lung cancer in men that ætiological conclusions—and preventive action—may develop.

The prevention of cancer has found its most obvious application in many countries in the attack on causative occupational and industrial factors. Many different carcinogens are encountered in the chemical industry, mining and other occupations; and certain components of polluted air in cities and food additives are known or suspected to be carcinogenic. Ionizing radiation is another potential source of malignancy. Measures to prevent the pollution of air, particularly by benzpyrene, and to limit the hazards of radiation are now established elements of public health service practice in many countries.

Another approach to prevention is through the mass screening of the population to detect precancerous lesions. In most countries of western Europe, such screening is carried out by particular industries or professional groups on a private basis. In eastern European countries and the U.S.S.R., however, more and more extensive examinations of both working and non-working populations are performed each year; this has resulted in the detection of precancerous lesions—particularly of the uterine cervix, breast, skin and stomach—and the early recognition of actual neoplasms. Experience in the U.S.S.R. has demonstrated the importance of planning such surveys carefully. Examinations of persons more than 30–35 years of age are conducted by teams of specialists, and other diseases are looked for simultaneously. Regular radiographic examinations of the chest, and sometimes of the upper gastro-intestinal tract, is also a feature.

In some countries the approach is through examination of all patients attending factory or hospital out-patients clinics for whatever reason. The mass gynæcological examination of women in factories and hospitals is also valuable in connexion with tumours of the cervix and pelvis. In some areas, cytological diagnosis is being practised on a large scale, and this method holds great promise.

One of the greatest problems in obtaining good results from cancer treatment is that of seeing patients at a sufficiently early stage of the disease. Only too often delays constitute a formidable handicap to success. The time-interval between the earliest symptoms and radical treatment may be several months, even for the common cancers of the breast and stomach. In many cases cerebral tumours do not come to operation for a year or 18 months after the first headache. Early diagnosis is achieved partly by educating the public (and the general practitioner) to be aware of, and willing to report, possible symptoms; and partly by providing suitable facilities for diagnosis. A number of European countries has special diagnostic centres, appropriately equipped, attached to hospitals; and in the U.S.S.R. there are the designated cancer dispensaries or polyclinics. A widespread application of the technique of cytological diagnosis may perhaps prove a valuable aid to early diagnosis. This has already shown itself, in some parts of the world, to be of first importance in detecting early cancer of the cervix by means of a simple vaginal smear.

A general development of mass screening and early diagnostic measures depends on sound education of the general public in the issues at stake; yet this is often neglected. Controlled surveys have shown that public attitudes can be changed by campaigns, particularly those directed against specific tumours, the most important effect often being to overcome a feeling of hopelessness about cancer treatment in general. In the U.S.S.R. and in eastern European countries such education is a responsibility of the Ministry of Health; there are institutes of health education, and mass examinations are carried out in factories, all propaganda media being harnessed for the purpose. In other countries this task is left to national societies dealing either with cancer in general or with particular lesions. Cancer of the lung is one of these, and many unofficial bodies are now aiming at the prevention of smoking in school-children and young people. It is interesting to note that, while a leading British authority stresses the risks of imparting too much clinical information to laymen, the view in the U.S.S.R. is that clear and detailed information on every aspect of the problem is the best way of avoiding cancerophobia.

In most countries there is no special legislation governing cancer diagnosis and treatment facilities. In some, where the problem is handled at ministerial level, appropriate regulations exist, for example, in Czechoslovakia and France, where notification is compulsory. Whether special legislation is required is mainly determined by the adequacy of the existing general health legislative framework. In all cases, the objects must include efficient prevention, diagnosis, treatment, after-care and rehabilitation irrespective of economic status; the accumulation of reliable statistics; and the provision for cancer research.

COMPOSITION OF 'PEARLS'

By Prof K. VOZOFF, R. M. ELLIS* and Prof. G. D. GARLAND
Department of Physics, University of Alberta

RECORDS of micropulsations of the Earth's magnetic field at times exhibit 'pearls' which appear to result from mixing two sinusoidally varying magnetic fields having slightly different frequencies and roughly equivalent amplitudes. Typically these frequencies lie in the range 0·2–2 c.p.s. and may differ from one another by 0·01–0·1 c.p.s. An identical wave form could be obtained through amplitude modulation of a single oscillator.

In the course of our magnetotelluric investigations a recording containing about 1 hr. of impure pearls was analysed. The recording of the two horizontal electric components and the three magnetic components was made between 1430Z and 1520Z, on September 7, 1961, at a location 20 miles north of Edmonton, Alberta. The average zero crossing period of the pulsations was about 1·4–1·5 sec. Their modulating envelope appeared to be a sinusoid of about 26 sec. period which was itself being modulated. There was a pronounced tendency for the entire pattern to repeat itself at periods of about 145 sec., or after 10 'pearls'. The entire pattern was superposed on much slower variations. Polarization in the horizon-

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* Petroleum Research Fund Fellow.

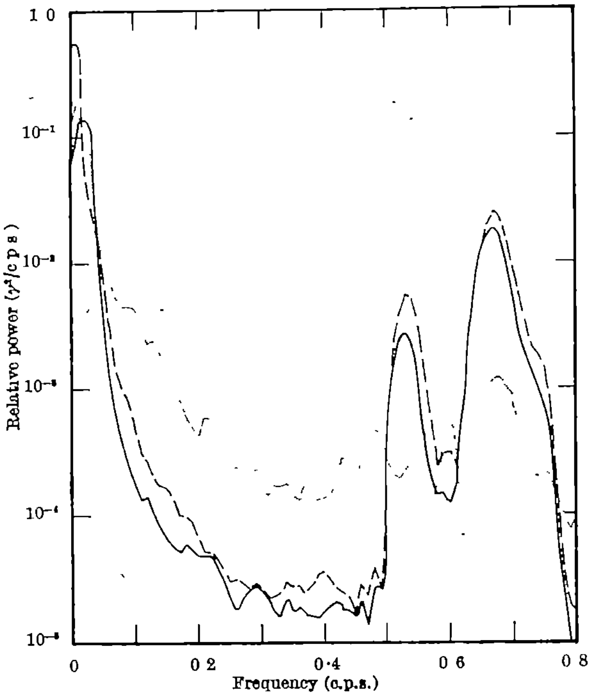

Fig. 1. Compound spectra. —, H_x; — —, H_y; . . H_z

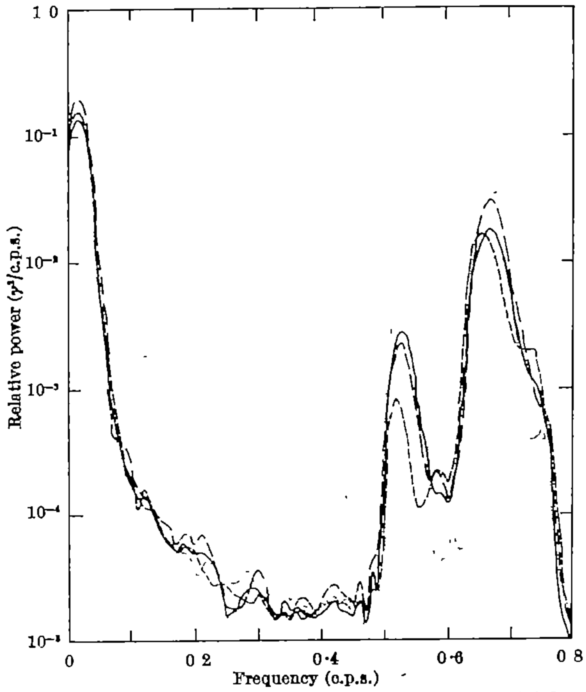

Fig. 2. Partial H_x spectra. . . ., First third; —·—, second third; — — —, last third; —, entire record

tal plane was at times elliptical, at other times linear. The directions of linear polarization varied through 135°. Maximum peak-to-peak amplitude was about 0·5γ.

The character of the signals underwent a gradual transition during the recording interval. Consequently, after the average spectra were computed for each component, the records were split in thirds and re-analysed.

It is apparent from either of the average horizontal spectra (Fig. 1) that the majority of energy is concentrated in three bands: two, relatively narrow, centred at 0·53 c.p.s. and 0·67 c.p.s. respectively, and the much broader low-frequency band. In these average spectra the amplitude of each band appears to be about the same in the X as in the Y direction. The vertical (Z) component must obviously be treated separately. The beat period which one would expect from mixing 0·53 c.p.s. and 0·67 c.p.s. is not an obvious feature of the records except in the first third, because the 0·53 band is generally somewhat smaller in amplitude. Most of the character is due to structure within the 0·67 c.p.s. band.

When the records are re-analysed by thirds (Figs. 2 and 3), it becomes evident that there occurred drastic changes in the directional orientation and amplitudes of the 0·53 c.p.s. components. Average amplitudes in the other two peaks vary by less than 50 per cent through the hour. Regardless of the picture one uses to explain the fields, it would appear that the sources of the 0·53 c.p.s. and 0·67 c.p.s. peaks must be independent of one another.

We noted that our two major peaks lay very close to a possible pair of modes predicted by MacDonald[1] for this magnetic latitude (60° N.). For this reason we obtained as many other observations for the period as we could. On only two of those we obtained could pulsations in this frequency range be seen. These were recorded at Austin, Texas (*EERL*, University of Texas) and at Reykjevik, Iceland, in one of Prof. Benioff's observatories. The charts were not suitable for analysis at these frequencies. However, the Texas record did show very small sinusoidal fluctuations of about 0·5 c.p.s., superposed on much larger, long period oscillations. The Iceland chart showed pronounced 0·3 c.p.s. pulsations with a weak recurrence tendency of roughly the same period as seen on the Alberta records.

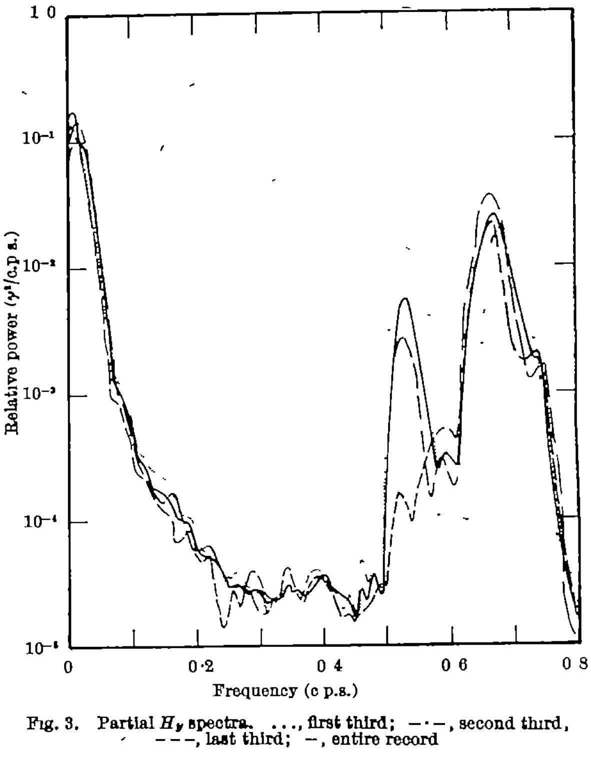

Fig. 3. Partial H_y spectra. . . ., first third; —·—, second third, — — —, last third; —, entire record

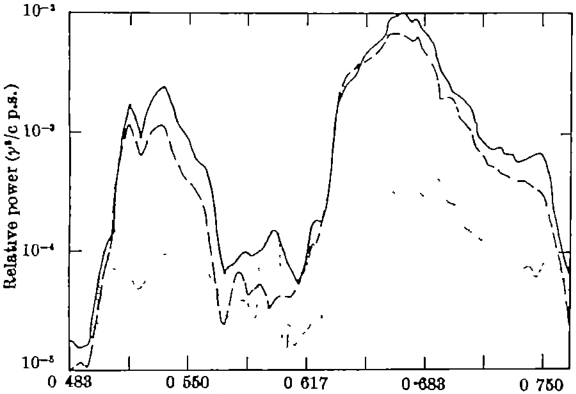

Fig. 4. Expanded spectra. —, H_y, – –, H_x; . . . ., H_z

The frequencies observed on our records 0·53 c.p.s. (1·9 sec. period) and 0·67 c.p.s. (1·49 sec. period) would correspond to the third and fourth trapped modes in MacDonald's high-density model at this latitude (60° N.). The 0·5 c.p.s. pulsation on the Texas record would correspond to the fourth trapped mode in the same model at that latitude (40° N.). The 0·3 c.p.s. pulsation on the Iceland record does not appear to be a permissible mode of the model at that latitude (67° N.). It should be noted that it was mid-afternoon in Iceland but early morning in western North America when the observations were made.

Re-analysis of the Alberta tapes with greater frequency resolution (Fig. 4) partially resolved the 0·53 c.p.s. band into a pair of bands at 0·517 and 0·536 c.p.s. Slight shifts in the location of this peak in Figs. 2 and 3 suggest that this may be due to a frequency change about two-thirds of the way through the record, rather than to splitting. Individual peaks were not apparent within the 0·67 c.p.s. band, but the spectra, particularly in Z, were suggestive of several closely spaced bands which also shifted slightly with time. Re-synthesis shows that most of the observed characteristics of the records can be accounted for by the calculated spectra. An exception is the 145-sec. recurrence pattern, which must almost certainly appear in the spectra as persistent structure within the 0·67 c.p.s. band.

Physically, the recurrence pattern is suggestive of an amplitude modulation. This might be explained by a disturbance travelling in the free mode, and exciting the trapped modes each time it enters (or leaves) the velocity channel. The calculated first free-mode period in this model is 185 sec.

We conclude that the changes, in time, of the relative amplitudes and orientations of the two major bands imply that the source oscillations can be excited independently. If we assume that any filtering which occurs between source and observer is unable to distinguish between frequencies so close together, we must conclude that relative amplitude and orientation changes arise at the sources. There is a suggestion that the oscillations are of the trapped V-type predicted by MacDonald[1], possibly excited by the free-mode disturbances of the same type.

This work was aided by grants from the Petroleum Research Fund, the American Chemical Society and the National Research Council of Canada. We thank the groups that made records available to us, as well as the Pacific Naval Laboratory, Miss G. Baril and particularly to Mr. M. D. Burke for considerable assistance in this programme.

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[1] MacDonald, G. J. F., *J. Geophys. Res.*, **66**, No. 11, 3639 (1961).

VIBRATIONAL SPECTRUM OF A TWO-DIMENSIONAL DISORDERED LATTICE

By M. D. BACON and DR. P. DEAN

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A PROBLEM fundamental to any investigation of the lattice vibrations of disordered atomic systems is that of the determination of the frequency spectrum. Investigations into various aspects—the thermodynamic effects of vibrations, optical and infra-red absorption, electron–phonon interactions—these and others depend on at least some knowledge of the spectrum. The history of research into the spectra of disordered systems has reached the stage at which exact information is now available for certain classes of one-dimensional models[1–3]. However, two- and three-dimensional systems have received little serious attention. In this article we present the spectrum for a disordered two-dimensional lattice which we believe is the first to be obtained for a disordered system other than the linear chain. Although the spectrum is based on calculations on small sections (16 × 16) of lattice, it shows points of considerable interest when compared with the one-dimensional results, and we can draw some conclusions which are probably of wide validity.

Our model of a disordered lattice is one in which the sites of a square lattice are occupied randomly, with equal probability, by one or other of two kinds of atom, light (L) and heavy (H). The atoms interact with nearest neighbour central and non-central forces as in the monatomic and periodic analogues of the system, used by other authors[4]. Such a model does not, of course, represent any particular physical system. It is essentially a mathematical model which nevertheless is extremely valuable for the insight it provides into more realistic systems.

The method of calculation of the density of normal mode frequencies is a modification of one outlined by Dean and Martin[5]. Following the text and notation of that paper we see that: (1) the frequency problem transforms to the eigenvalue problem of a symmetric matrix $S - \omega^2 I$ of block triple-diagonal form; (2) a process based on the 'negative eigenvalue theorem' is given for the calculation of the matrix eigenvalue spectrum. The theorem states that the number of negative eigenvalues of $S - \omega^2 I$ is equal to the sum of the numbers of negative eigenvalues of matrices $U_1 = A_1 - \omega^2 I_{l_1}$, $U_j = A_j - \omega^2 I_{l_j} - B_j^T U_{j-1}^{-1} B_j$ ($j = 2, 3, \ldots N$) where A_j and B_{j+1} are, respectively, the jth diagonal and off-diagonal blocks of S, and I_{l_j} is the unit matrix of the same order as

A_j. In this communication we use a particularization of this result. We decompose our dynamical matrix $S - \omega^2 I$ into just two U-matrices, the first of which is of unit order and is thus the top left-hand element ($=a_{11}$) of $S - \omega^2 I$. U_2 is a matrix of order one less than that of S. The essential point in this reduction is that the number of negative eigenvalues of $S - \omega^2 I$ can be obtained from the number in U_2 and the sign of a_{11}. If now U_2 is regarded as a matrix to be decomposed (into U_1' and U_2') in precisely the same way as $S - \omega^2 I$ the process can be repeated until, finally, the number of negative eigenvalues is simply the number of negative terms in the n unit matrices $U_1 = a_{11}$, U_1', U_1'', . . . , n being the order of S. This procedure of counting negative eigenvalues of $S - \omega^2 I$ yields the proportion of squared frequencies smaller than ω^2—in other words, the integrated spectral function $M(\omega^2)$. The derivative of this function, $D(\omega^2) = dM(\omega^2)/d\omega^2$, is the usual density or spectrum of squared frequencies.

Computations (using the *ACE* of the National Physical Laboratory) were performed on sections of lattice of size 16 × 16; thus the dynamical matrix was of order 256. Toroidal boundary conditions were imposed on each section in one direction and, for mathematical convenience, rigid walls in the other. $M(\omega^2)$ was calculated throughout the whole range of the spectrum at intervals in ω^2 of 1/8. The mass ratio chosen was 4 : 1 and the ratio of central to non-central force constant taken to be unity. (In the notation of Dean and Martin[5] the parameters of the system are $k = k' = 1$; $m_H = 4$; $m_L = 1$.)

Curve (*a*) of Fig. 1 is the integrated spectrum of squared frequencies obtained for one particular disordered lattice section. Results for other sections show precisely the same features and hence curve (*a*) can be taken as typical. Curve (*b*) is the integrated spectrum for the infinite monatomic lattice of heavy (*H*) atoms[6]. Curve (*c*) is the result for the infinite diatomic periodic ('chessboard') array:

$$[D(\omega^2) = (2\omega^2-5)(4+4y)^{-1/2}K(k),$$

$$k^2 = (3+2y-y^2)/(4+4y),$$

$$-1 \leqslant y = (5/2-\omega^2)^2 - 13/4 \leqslant 3,\ M(\omega^2) = \int_0^{\omega^2} D(\omega^2)d\omega^2,$$

where $K(k)$ is the complete elliptic integral of the first kind. The programme used for the main calculations produced results which followed very closely the analytical curves (*b*) and (*c*) when applied to small sections of the relevant lattices.

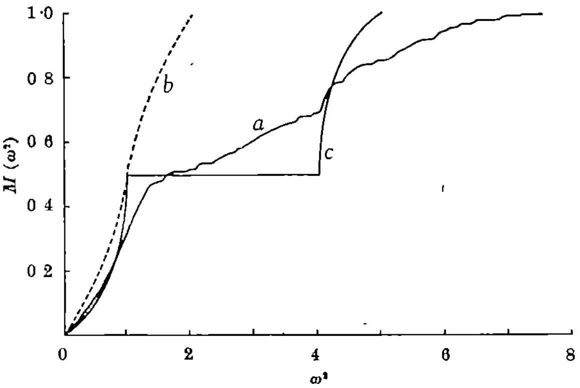

Fig. 1. Integrated spectra for two-dimensional lattices. *a*, the disordered lattice; *b*, the infinite *H*-monatomic lattice; *c*, the infinite diatomic alternating array

The form of the integrated spectrum for the disordered two-dimensional lattice is significant when compared with the known results for the linear chain. The spectrum of the linear system has two distinct parts: in a region corresponding approximately to the band of the *H*-monatomic lattice it is smooth and continuous; at higher frequencies it becomes extremely complex and has been shown to be essentially a weighted sum of the frequencies of numerous highly localized 'defect' modes which are associated with various kinds of short-range chain structures[2]. Curve (*a*) suggests that the situation is similar for the two-dimensional disordered systems examined in this article. The step-like nature of the function $M(\omega^2)$ in the region $\omega^2 > 1{\cdot}5$ approximately, shows the density of frequencies to have numerous peaks of different intensities. We conjecture that these peaks correspond to localized modes which are associated with particular kinds of small-scale lattice structure. If this conjecture is valid, and proof (or disproof) should soon be forthcoming, then the evidence for the spectra of disordered three-dimensional lattices being of the same kind will be overwhelming. It should then be possible to determine the main features of the spectra of realistic alloy systems from a study of the highly localized modes which can occur in these systems.

The work described here was carried out as part of the research programme of the National Physical Laboratory and this article is published by permission of the Director of the Laboratory.

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FORMATION OF SPHERULITES IN POLYETHYLENE

By W. BANKS, J. N. HAY*, A. SHARPLES and G. THOMSON

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REFERENCE has frequently been made (for example, ref. 1) to the ease with which polyethylene crystallizes to form spherulites which can be readily observed in the optical microscope (that is, of dimensions in the region of 10^{-2}–10^{-1} cm.), and indeed one of the first papers to suggest that spherulite formation might be a general phenomenon in high polymers was concerned with a study of polyethylene[2]. Most of the previous work has involved thin

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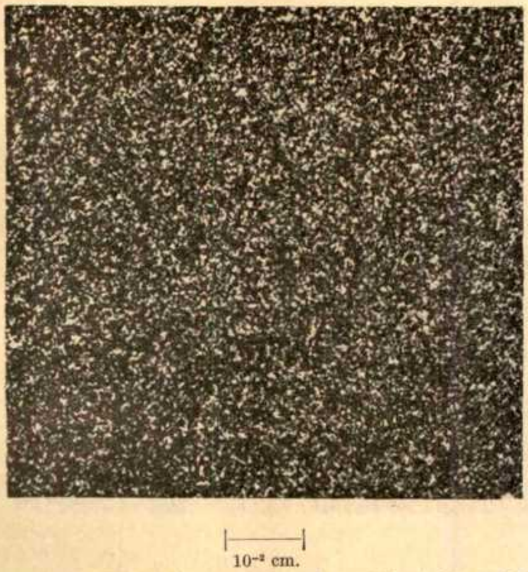

Fig. 1. Ziegler-type polyethylene ($M.I. = 0{\cdot}22$) heated to 200° C. for 5 min. and crystallized at 115° C.

films of polymer crystallized between glass surfaces, although on occasions sections from larger samples have been examined[3], and their appearance has supported the view that the behaviour of thin films is characteristic of that of the bulk material. Recent work in this laboratory, however, has indicated that although resolvable spherulites may be formed under certain conditions, the characteristic structure of a wide variety of crystallized polyethylene samples is not obviously spherulitic.

The samples studied included various types of low and high pressure polyethylenes, copolymers with butene and propylene, a polymethylene and fifteen fractions of molecular weights ranging from 3,000 to 500,000, obtained from two Phillips-type low-pressure products. In general, after heating between cover-slips at various temperatures up to as high as 300° C., the birefringent structures formed on subsequent cooling contained no resolvable spherulites, and were of the appearance shown in Fig. 1. The fact that the samples were invariably birefringent indicated that oriented regions were present of dimensions at least of the order of the wave-length of light, and it is reasonable to suppose that the structures observed contained distinct entities, possibly even spherulites, but of a size less than that capable of being resolved by the microscope. In the present case, careful observations during crystallization established that growing centres separated by distances greater than 2×10^{-4} cm. would have been detected; this is two orders less than the dimensions quoted for typical spherulites. Exceptions occurred to this general behaviour, and, for example, with a Phillips-type homopolymer, spherulites were occasionally detectable in the unresolvable background (Fig. 2). Also three of the fractions examined (molecular weights 9,800, 18,700 and 52,000) gave resolvable nuclei on slow growth, and in some areas the nucleation density was low enough to enable spherulites of about 10^{-2} cm. diameter to be formed (Fig. 3). Even in such cases, however, spherulite formation was not uniform throughout the sample.

The possibility of spurious nucleation at the glass surfaces was ruled out as a result of experiments using mica, and also from observations on sections cut from samples crystallized in bulk. In addition, the persistence of nuclei from previous crystallizations[4] seemed unlikely for several reasons, among which was the fact that in kinetic studies[5] all previous history was found to be removed at less than 1° C. above the melting point. Finally, it was shown that unresolvable structures resulted whatever the rate of crystallization, when the time scale was changed from a fraction of a second to several days.

Although resolvable spherulites similar to those previously recorded[1,2] were not observed on normal melting and cooling, it was found that they could be produced by a variety of methods involving heat and

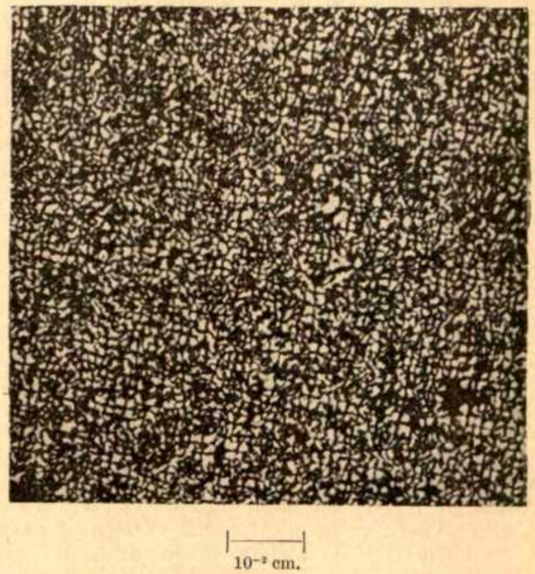

Fig. 2. Phillips type polyethylene ($M.I. = 0{\cdot}67$) heated to 170° C. for 5 min. and crystallized at 123° C.

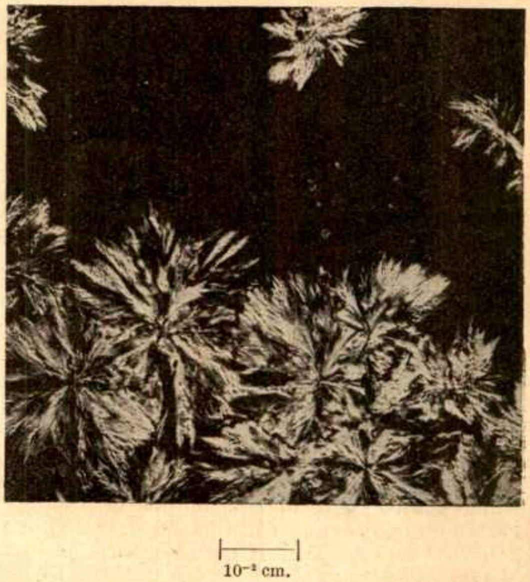

Fig. 3. Polyethylene fraction ($\bar{M}_v = 18{,}700$) heated to 200° C. for 5 min. and crystallized at 128° C. for 6 hr.

solvents, of which the following is an example. The Ziegler-type polyethylene used to give Fig. 1 was heated at 320° C. between cover-slips for 10 sec. and cooled, after which it produced spherulites of diameters up to 10^{-2} cm. without difficulty (Fig. 4). Similar treatments produced similar results for all samples examined, and it is considered likely that the polyethylene spherulites which have previously been reported resulted from treatments which produced states not characteristic of the original material.

In view of the fact that spherulite formation is the basis of existing theories of polymer crystallization[6], it is of considerable importance to establish the conditions under which they are formed. In addition, as the above experiments suggest that spherulite size can be modified by suitable treatments, presumably involving a reduction in the numbers of nucleating centres, it is also of practical interest to determine the nature of the process, as spherulite size is known to be correlated with optical and mechanical properties[7]. In the experiment reported above, the conditions used to produce resolvable spherulites were also conducive to thermal degradation. However, the unchanged melting point of the sample suggested that no significant change in molecular weight had occurred. Also, work to be reported in detail elsewhere indicated that homogeneous thermal degradation of the bulk material is unlikely to be the reason for the observed effects, as, for example, in the case of the treated Ziegler polyethylene (Fig. 4), the original appearance could be restored simply by dissolving and removing the solvent *in situ*. Subsequent melting and cooling produced structures similar to that given in Fig. 1. In addition, various thermal treatments of the larger samples failed to convert them to states capable of producing large spherulites.

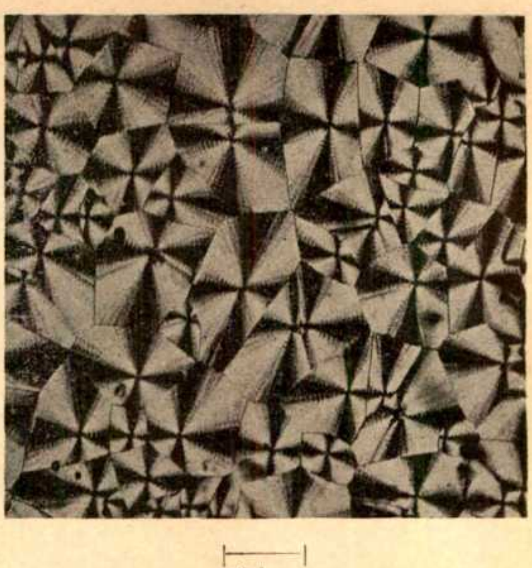

Fig. 4. Ziegler-type polyethylene ($M.I. = 0{\cdot}22$) heated to 320° C. for 10 sec. and crystallized at 110° C.

Although no detailed explanation is at present available to account for all the observations, it would seem unlikely that homogeneous modification of the whole material by any specific chemical reaction is involved. This receives support from experiments with other chemically different polymers, such as polyesters and polyamides, which, although normally appearing again in the form of unresolvable birefringent structures, can also be converted by suitable thermal treatment to forms where large spherulites can be observed. It is more probable that growth occurs from heterogeneities, and that the effective number of these centres is reduced during treatment either by chemical modification or by some physical process such as aggregation.

We are grateful to B.P., Ltd., for financial assistance in this project.

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VASCULAR ACTION OF ACTEINA: ACTIVE CONSTITUENT OF *ACTAEA RACEMOSA* L.

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THE hypotensive action of the liquid extract of the rhizome and roots of *Actaea racemosa* L. is well known[1,2]. We have investigated the effect of various fractions of this extract on arterial pressure when injected intravenously in unanæsthetized rabbits.

Following our experimental results, that is purifying the most active fractions, Panizzi *et al.* (personal communication) have obtained certain crystalline substances with different melting-points.

The results so obtained (Table 1) suggest that the hypotensive effect of the extract of rhizome and roots of *Actaea racemosa* L. is due to the resinous part, insoluble in water and mainly to that constituent of it, called 'acteina', which is soluble in chloroform.

Pharmacological investigations have shown that acteina does not provoke acute toxicity (Table 2) or stimulate any local effects; it does not affect the heart or spontaneous movement in animals.

The hypotensive effect of acteina on unanæsthetized rabbits or on urethanized (1 gm./kgm.) cats is shown even with doses of 1 mgm./kgm. and reaches its maximum with doses of 10 mgm./kgm.; an increase in such doses does not provoke further decrease.

On the other hand, this effect, even with doses of 5 mgm./kgm., is not constant in unanæsthetized or chloralosed (1 gm./kgm.) dogs. There is no effect on a conscious human being—either normal or hypertensive subjects.

In man, acteina provides an increase in peripheral blood flow even at 0·5 mgm./kgm. Such a dose is effective also in subjects affected by peripheral arterial disease (Fig. 1).

Experiments on the vasal action of acteina have shown that: (1) It does not affect the periphery or the ganglionic synapses of the somatic nervous system. (2) When injected intravenously in the rabbit, it decreases the vasomotor reflexes caused by clamping and unclamping the common carotid arteries. (3) When injected in the thyroid artery in dogs, it has no effect on the chemoreceptors of the carotid sinus. (4) When injected by means of a catheter in the right atrial cavity, it has a hypotensive effect in rabbits even with doses (4 μgm./kgm.) which are inefficient when injected in the marginal vein of the ear. (5) When injected in the common carotid artery, it has a hypotensive effect in the cat even with doses (30 μgm./kgm.) which produce no effect when injected in the jugular vein. (6) When injected in the peripheral circulation in the dog it provokes peripheral dilatation.

Table 1. EFFECT OF THE LIQUID EXTRACT OF *Actaea racemosa* L. AND OF THE VARIOUS FRACTIONS OF IT ON THE CAROTID BLOOD PRESSURE OF UNANÆSTHETIZED RABBITS

Substances intravenously administered at a dose of 1 mgm./kgm.	Decrease of the pressure values (*m* ± *S.E.*) per cent
Fluid extract	10±2·2
→Hydrosoluble fraction	0
→Not hydrosoluble fraction	10±3·1
Soluble in ethyl acetate ←	9±1·8
Soluble in ethyl ether ←	8±1·9
Soluble in chloroform ←	18±3·7
→Purified (crystalline) with m.p. 256° C.	15±3·0
→Nitrogenous basic	0
→Crystallized by ethyl acetate with m.p. 200° C.	5±0·8
→Crystalline, not soluble in chloroform, with m.p. 256° C.	0
→Crystalline, soluble in chloroform with m.p. 222° C.	15±2·7
Purified (crystalline) with m.p. 276° C.: acteina ←	20±3·5

Table 2. MINIMUM LETHAL DOSES OF ACTEINA*

Animal used	Route of administration	Doses (mgm./kgm.) Single	Doses (mgm./kgm.) Administered daily for 30 days
Rabbit	Gastric	—	>6
	Hypodermic	—	>3
	Intravenous	>70	—
Mouse	Intraperitoneal	>500	>10
Rat	Gastric	>1,000	—
Dog	Hypodermic	—	>6

* On groups of 10 animals.

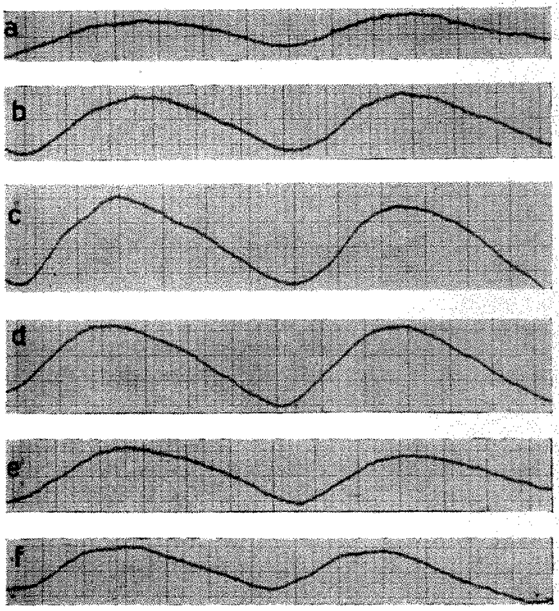

Fig. 1. Photoplethysmographic records of a man, forty-nine years old, affected by peripheral artery disease, treated with 1 mgm./kgm. of acteina. *a*, Basal record; *b*, *c*, *d*, *e*, *f*, records 30 min., 1, 2, 3 hr. after injection. Calibration: 1 cm.=0·5 mV.

Furthermore, it must be emphasized that in almost 70 per cent of cases, the interruption of the vagus nerve on the neck inverts the hypotensive effect due to acteina; in such cases even the hypertensive reflexes of the carotid sinuses become normal again.

Therefore we conclude that acteina affects the function of the vagus, having a hypotensive effect in unanæsthetized rabbits and anæsthetized cats.

Its effect is discontinuous so far as its hypotensive effect on the unanæsthetized dog is concerned, even if it clearly shows a peripheral vasomotor effect.

It probably acts on the vasomotor centres, provoking inhibition of some of them and excitation of others, through either direct or reflected central mechanism.

In man this drug has no hypotensive effect, though its peripheral vasodilatory effect is evident.

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FURTHER INVESTIGATIONS OF THE CARBOHYDRATE METABOLISM OF COCK SPERMATOZOA

By DR. P. E. LAKE*, DR. F. W. LORENZ, and WINIFRED D. REIMAN

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LORENZ[1] reported that fructose accumulated in a phosphate buffer medium whenever cock (*Gallus domesticus*) spermatozoa were incubated in the presence of glucose or mannose; the reverse reaction could not be clearly demonstrated. We have examined this observation further in an attempt to elucidate the metabolic pathway for the conversion, using paper chromatographic and autoradiographic techniques.

Semen, free from transparent fluid[2], was collected from New Hampshire cocks and quickly diluted with 3 parts of a buffered avian Ringer phosphate, *p*H 7·25, containing 4 mgm./ml. of either glucose, fructose or sorbitol. On separate occasions 1 μc. of either uniformly labelled D-[^{14}C]-glucose or D-[^{14}C]-fructose was added to the final mixtures containing the same sugar. In each experiment initial aliquots were taken and the remainder incubated for 1 or 3 hr. at 37° C. either aerobically or semi-anaerobically as described previously[1]. After incubation the diluted semen

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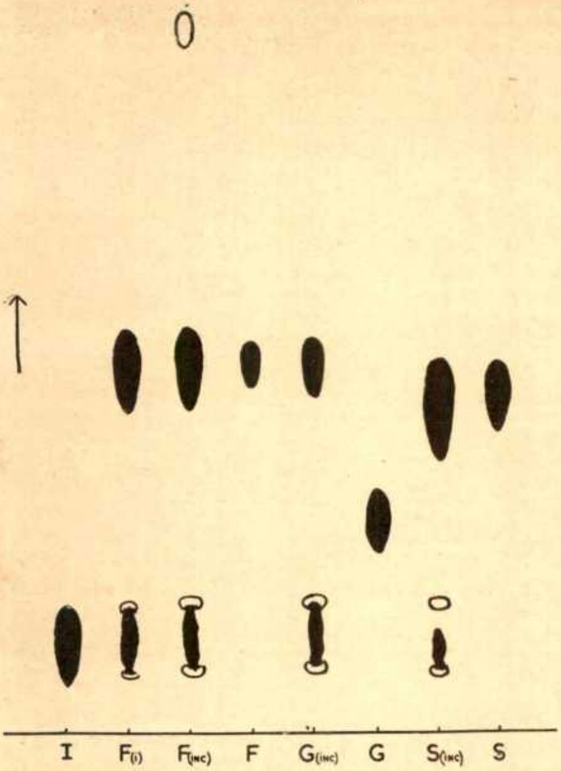

Fig. 1. Chromatogram of extracts of cock semen incubated aerobically for 3 hr. at 37° C. separately with glucose, fructose and sorbitol. Descending (run off end of paper), 48 hr., 27° C. Silver nitrate-alcoholic sodium hydroxide location reagent. Solid spots stained brown or black after treatment with alcoholic sodium hydroxide solution. Outlined spots remained as white areas after this treatment; the foremost spot was unidentified, but the two slow-running spots proved to be fructose-6-phosphate, glucose-6-phosphate and other phosphorylated compounds. These latter were present in unincubated semen and increased in amount during incubation (though more irregularly than suggested here) whether or not the semen was incubated with added carbohydrate. $F_{(i)}$, semen with fructose before incubation; F(inc), semen after incubation with fructose; G(inc), semen after incubation with glucose; S(inc), semen after incubation with sorbitol; *I*, *F*, *G* and *S*, authentic marker spots of inositol, fructose, glucose and sorbitol respectively

samples were deproteinized, using barium hydroxide and zinc sulphate followed by freezing and thawing. The supernatant fluids were passed consecutively through ion-exchange resins 'Dowex 50' (H^+ form) and 'Amberlite *CG* 45', type 2 (OH^- form); the resultant effluents and washings were evaporated to dryness on a rotary evaporator. The residues were dissolved in small volumes of distilled water (0·3 ml. per ml. original semen). In addition to the incubated semen samples, fresh uncontaminated seminal plasma[2] and spermatozoan fractions were prepared and treated in the same manner. Aliquots of 20–60 μl. were spotted on Whatman No. 1 paper and subjected to unidimensional, descending chromatography in one of three solvent systems, phenol-concentrated ammonia–water (80 gm.: 0·5 ml.: 20 ml.), ethyl acetate–pyridine–water (60 ml.: 25 ml.: 20 ml.) and *n*-butanol–acetic acid–water (60 ml.: 15 ml.: 25 ml.)[3]. Autoradiograms were then developed during two to four days with the appropriate X-ray film[4]. Authentic markers of fructose, glucose, sorbitol, inositol, fructose-6-phosphate, glucose-6-phosphate, and uniformly labelled D-[^{14}C]-glucose and D-[^{14}C]-fructose were used rather than relying on R_F values.

The specific ketose location reagent naphthoresorcinol–acetone–H_3PO_4 (ref. 5) was used to verify the formation of fructose. It was found that glucose sometimes reacted slowly with naphthoresorcinol over a few days at room temperature to give a blue colour; fructose formed a pink-brown colour immediately on heating.

The results of the chromatographic and autoradiographic studies showed that extracts of fresh spermatozoa and uncontaminated seminal fluid contained no detectable reducing sugars, but inositol was found in whole semen or seminal plasma. The sugar alcohol had previously been found and estimated (10 mgm. per 100 ml.) in cock seminal plasma (Lake, unpublished observations). Fructose always accumulated under the conditions of the experiments when cock semen was incubated with glucose, thus confirming previous observations on cock spermatozoa[1]; glucose disappeared completely from the medium after 3 hr. at 37° C. Conversely, after semen was incubated with fructose, glucose was never detected either on autoradiograms or ordinary chromatograms. Glucose and fructose were found to separate best in the phenol-concentrated ammonia–water system; a typical chromatogram is illustrated in Fig. 1.

A preliminary examination of possible pathways for the formation of fructose from glucose by cock spermatozoa was made. It was considered possible, but remotely so, that glucose was being phosphorylated, and, by phosphohexoisomerase, converted first to fructose-6-phosphate and then by phosphatase to free fructose. Such a pathway was suggested by Mann[6] for fructose formation in bull seminal vesicles. However, as no fructose could be detected after spermatozoa had been incubated for 2 hr. with fructose-6-phosphate (sodium salt; 4 mgm. per ml.) this pathway was ruled out.

King and Mann[7] presented evidence for the existence of sorbitol dehydrogenase (a ketose reductase) in ram spermatozoa which catalyses the interconversion of fructose and sorbitol; indications of a slower reduction of glucose to sorbitol were also obtained. King, Isherwood and Mann[8] also reported the presence of a small amount of sorbitol in cock semen. Hers[9] showed that glucose was irreversibly converted to fructose by ram seminal vesicle tissue: two enzymes, an aldose reductase and a ketose reductase (sorbitol dehydrogenase), were involved in a two-step process with sorbitol as the intermediary compound. If these observations could be extrapolated to cock spermatozoa they would suggest a pathway for the conversion of glucose to fructose: (*a*) reduction of glucose to sorbitol, which should be irreversible according to our observations and taking place, possibly, under conditions different from those obtaining in the ram[7]; (*b*) oxidation of sorbitol to fructose catalysed by sorbitol dehydrogenase. The latter oxidation proceeds in intact ram spermatozoa[7,10] despite an apparently unfavourable equilibrium, presumably because of a favourable ratio between oxidized and reduced diphosphopyridine nucleotide. Hers[9] suggested that the reaction glucose → sorbitol → fructose was irreversible in ram seminal vesicle tissue because triphosphopyridine nucleotide occurs almost exclusively in the reduced form and diphosphopyridine nucleotide largely in the oxidized form. Similar distributions of oxidized and reduced forms of these co-factors were found in several animal tissues by Glock and McLean[11].

With our chromatographic techniques we were unable to separate, clearly, sorbitol and fructose. Any involvement of sorbitol in the interconversion of glucose and fructose by fowl spermatozoa was therefore difficult to study. Developing the chromatograms of incubated extracts with a location reagent for polyhydric alcohols[12,13] and comparing with others developed with the silver nitrate reagent yielded ambiguous results which, however, were not inconsistent with the involvement of sorbitol. Cock semen was incubated aerobically with sorbitol and traces of fructose accumulated as determined by the resorcinol test[1]. Although the accumulation of this rapidly metabolized ketose sugar, even in traces, suggests that a route for conversion of sorbitol to fructose does exist, it is considered, at present, that the feeble activity observed is inadequate to account for the large accumulation of fructose in semen incubated with glucose.

In conclusion, the conversion of glucose to fructose by cock spermatozoa has been demonstrated to be an irreversible process. The pathway does not involve glucose-6-phosphate isomerization; a pathway involving sorbitol is possible, but remains to be convincingly demonstrated.

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INHIBITION BY PHENOTHIAZINIC COMPOUNDS OF THE EFFECT OF 2,4-DINITROPHENOL ON THE RESPIRATION OF HEART MUSCLE SARCOSOMES

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DURING an investigation of the effect of antihistaminic drugs on the oxidative phosphorylation of guinea pig heart sarcosomes it was found that phenothiazinic compounds inhibited the stimulation of respiration displayed by 2,4-dinitrophenol on mitochondria.

Heart muscle sarcosomes were prepared by a modification of the method of Clelland and Slater[1] previously used by us[2,3]. After homogenization and washing the heart in a medium containing 0·33 *M* sucrose and 1×10^{-3} *M* ethylenediamine tetraacetic acid, *p*H 7·4, the sarcosomes were suspended in the same medium in the proportion of 0·5 : 1 ml. of medium per heart used. The average protein content in the original suspension of heart mitochondria was 14·39 mgm. per ml. The reaction medium contained 0·33 *M* sucrose, 0·02 *M* phosphate buffer *p*H 7·4, and 0·01 *M* potassium chloride.

Respiration was measured using oxygen electrode described by Voss and Bacila[4]. It was made with a platinum electrode of the stationary type combined with a magnetic stirrer and a potassium chloride bridge. The magnetic stirrer is able to mix the reagents promptly in a closed mixing chamber with a capacity of 2·0 c.c. The inconvenience of the open cuvettes is thus completely avoided, as well as the contamination of the platinum electrode, which is coated with a cellulose tubing paper. Oxidative phosphorylation was measured according to the method of Chance and Williams[5] and the phosphorus/oxygen ratios determined as adenosine diphosphate/oxygen ratios.

The effect of phenothiazinic compounds on the normal properties of guinea pig heart sarcosomes were examined with the following: *promethazine*—10-(2-dimethylaminopropyl)phenothiazine; *chlorpromazine*—2-chloro-10-(3-dimethylaminopropyl)-phenothiazine; *neozine* — lævo-methoxy-3(dimethylamino-3′methyl-2)′-propyl-10-phenothiazine; *perphenazine* — 1-(2-hydroxyethyl)-4-[3-(2-chloro-10-phenothiazinyl)propyl] piperazine.

The main characteristic of these compounds is the presence of a phenothiazinic nucleus, besides the fact that they all have a very marked pharmacological activity some of them being of attaraxic activity and others displaying an anti-histaminic action.

The effect of one of these drugs (perphenazine) on the respiration and oxidative phosphorylation of heart muscle sarcosomes is shown in Table 1. It can be seen that the respiratory control coefficients, calculated as the ratio between the rate of respiration during the active state of respiration with adenosine diphosphate (ADP) and the control respiration after

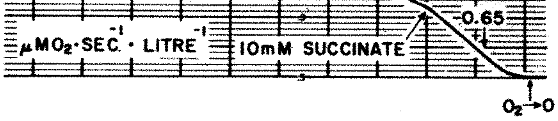

Fig. 1. Effect of promethazine on the stimulus of respiration by 2,4-dinitrophenol of guinea pig heart sarcosomes. To the aerobic mitochondria, 10 m*M* of α-ketoglutarate were added and the respiration-rate recorded (0·17 μ*M* oxygen $\text{sec.}^{-1}\ \text{l.}^{-1}$). The addition of $2 \cdot 2 \times 10^{-5}$ *M* of 2,4-dinitrophenol increased the respiration-rate (0·50 μ*M* oxygen $\text{sec.}^{-1}\ \text{l.}^{-1}$). This was followed by the addition of 5×10^{-4} *M* of promethazine; it caused a slight increase in the respiration-rate which later levelled off to the initial rate of 0·17 μ*M* oxygen $\text{sec.}^{-1}\ \text{l.}^{-1}$. The addition of 10 m*M* succinate increased the rate of respiration to 0·65 μ*M* oxygen $\text{sec.}^{-1}\ \text{l.}^{-1}$

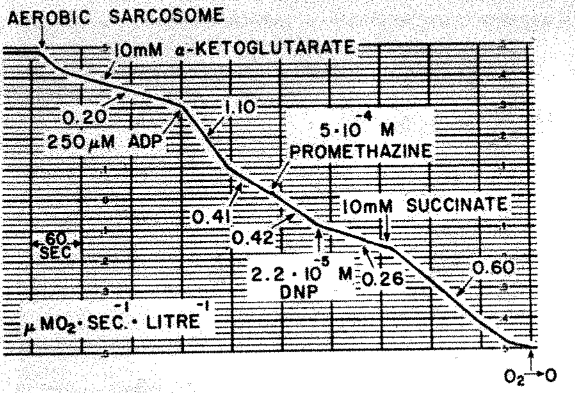

Fig. 2. Effect of promethazine on the oxidative phosphorylation and 2,4-dinitrophenol action on guinea pig heart sarcosomes. To the aerobic mitochondria, 10 m*M* of α-ketoglutarate were added and the respiration-rate recorded (0·20 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$). The addition of 250 µ*M* ADP increased the rate of respiration during the active state of respiration to 1 × 10 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$, and when the acceptor of inorganic phosphate was used up there was a levelling off of the respiration-rate to 0·41 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$. The respiratory control coefficient (ratio between the respiration-rate during the active state and the rate of the controlled respiration after ADP) was equal to 2·68 and the ADP/O ratio was equal to 2·8. This was followed by the addition of 5 × 10^{-4} *M* promethazine and then by 2·2 × 10^{-5} *M* 2,4-dinitrophenol, which did not stimulate respiration. Then the further addition of 10 m*M* succinate increased the rate of respiration to 0·60 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$

ADP, is inhibited by increasing amounts of perphenazine. Furthermore, the rates of respiration during the active state decrease in the presence of increasing amounts of perphenazine.

Table 1. Effect of Perphenazine on Oxidative Phosphorylation of Heart Muscle Sarcosomes

Perphenazine (pre-incubation)	Respiration-rate with α-keto-glutarate (10 m*M*)	Respiration-rate during ADP (active state)	Controlled respiration-rate after ADP	Respiratory control cofficient
No addition	0·27	0·62	0·27	2·3
6·2 × 10^{-5} *M*	0·24	0·62	0·24	2·5
1·2 × 10^{-4} *M*	0·17	0·50	0·20	2·5
3·5 × 10^{-4} *M*	0·21	0·47	0·26	1·8
4·9 × 10^{-4} *M*	0·19	0·29	0·19	1·5
6·2 × 10^{-4} *M*	0·19	0·19	0·00	Completely uncoupled

System contains: 1·9 ml. of aerobic medium, 0·1 ml. of sarcosome suspension, 10 m*M* of α-ketoglutarate, and suitable amounts of ADP and perphenazine. The figures for the rates of α-ketoglutarate respiration, the active state respiration during ADP, and the control respiration after ADP are given in µ*M* oxygen sec.$^{-1}$ l.$^{-1}$.

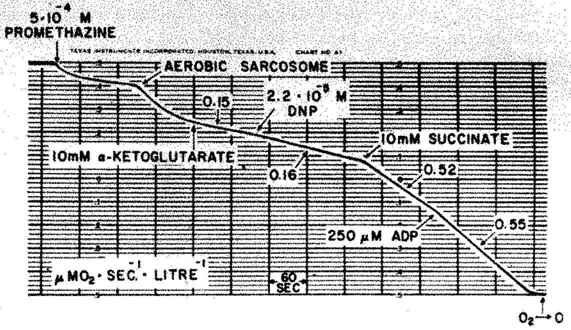

Fig. 3. Effect of the pre-incubation of sarcosomes with promethazine on the action of 2,4-dinitrophenol on respiration and oxidative phosphorylation. Sarcosomes were pre-incubated with 5 × 10^{-4} *M* promethazine for about 90 sec. Then, 10 m*M* α-ketoglutarate were added and the respiration-rate recorded (0·15 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$). The addition of 2·2 × 10^{-5} *M* of 2,4-dinitrophenol did not stimulate respiration. Then 10 m*M* succinate were added and the rate of respiration was increased to 0·52 µ*M* oxygen sec.$^{-1}$ l.$^{-1}$. Further addition of 250 µ*M* ADP did not stimulate respiration to a significant rate either

Promethazine, as well as the other phenothiazinic compounds, besides inhibiting oxidative phosphorylation of heart sarcosomes, show a very interesting effect by inhibiting the stimulation of respiration displayed by 2,4-dinitrophenol when added to a suspension of mitochondria. In the presence of suitable concentrations of promethazine, the effect of 2,4-dinitrophenol in stimulating the respiration of mitochondria fails to appear. This effect seems to be parallel to the inhibition of oxidative phosphorylation; inhibition of oxidative phosphorylation also occurs in the presence of promethazine. On the other hand, in the presence of succinate, the promethazine-treated sarcosome can respire again, even when the DNP effect and the oxidative phosphorylation are inhibited. Figs. 1, 2 and 3 illustrate this effect.

Analysis of these data indicates that phenothiazinic compounds have a very marked effect on the properties of heart sarcosomes. It was also possible to show a parallelism between the DNP effect in stimulating the respiration and the oxidative phosphorylation of the sarcosome, being both of them inhibited by these compounds. Succinate, however, can restore the respiratory activity of the mitochondria to which a phenothiazinic compound was already added.

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LATEX AGGLUTINATION TEST WITH *BRUCELLA* ANTIGEN AND ANTISERUM

By (the late) Prof. L. FLECK and Z. EVENCHIK

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SINGER and Plotz[1] introduced the application of latex suspension to the serological diagnosis of rheumatoid arthritis[1]; later this method was used in other serological tests[2-4].

Hirschberg and Yarbrough sensitized red blood cells or collodion particles with various antigens derived from *Brucellae*. Successful results were obtained with extracts of *Brucellae* in trichloroacetic acid or in phenol using collodion suspensions, but not with red blood cells sensitized with the same antigens. These findings have been confirmed by us; however, the preparation of collodion is very troublesome. We have therefore replaced it by latex, which is commercially available.

The following technique has been developed: A smooth culture of *Brucella abortus*, strain 6195 or strain 544 (strain 6195 obtained from the Veterinary Institute, Ministry of Agriculture, and the strain 544 from the Veterinary Institute in Rotterdam), grown for 48 hr. at 37° C. on potato agar in bottles, was suspended in 5 ml. of saline and centrifuged at 1,500 r.p.m. for 15 min. The sediment was washed once with saline, centrifuged again and re-suspended in distilled water. The bacteria were killed by heating for 1 hr. at 70° C., whereon the suspension was ultrasonically vibrated (Raytheon, 10 kc.) for 60–120 min. The suspension was then again centrifuged for 10–15 min. at 18,000 r.p.m., and the light opalescent supernatant was used as the sensitizing antigen. The antigen could be stored for 4–6 weeks at 4° C. and the latex suspension sensitized with it for at least 4 weeks.

Sensitization of latex particles. 'Bacto-latex 0·81' (Difco) diluted 1 : 20 in 0·85 per cent saline (*p*H 6·8–7·2) was mixed with an equal volume of the sensitizing *Brucella* antigen. It was found that a solution of 1 : 5 to 1 : 10 of the antigen was optimal for the sensitization. Following an incubation of 30 min. at 37° C., the mixture was centrifuged for 30 min. at 3,000 r.p.m. the supernatant discarded, the sediment washed twice with saline and finally re-suspended in the original volume of the diluted latex suspension. This sensitized latex suspension was checked against known positive and negative sera.

Titration of anti-Brucella sera. To 0·5 ml. of inactivated serum (1 : 50–1 : 6,400), one drop of the sensitized latex suspension was added, and the tubes shaken. After an incubation of 45 min. at 37° C. the tubes were centrifuged for 15 min. at about 1,000 r.p.m., after which the reading could be carried out immediately.

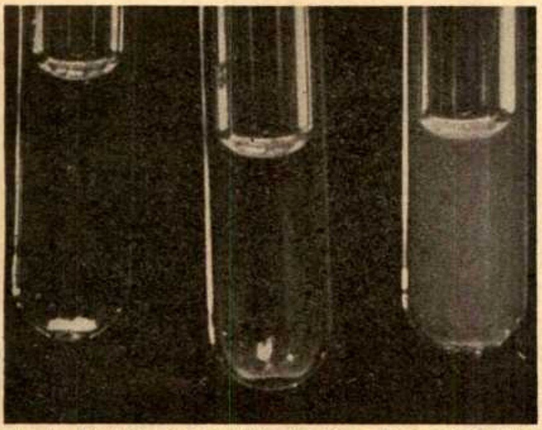

Fig. 1. Positive and negative reactions of sensitized latex suspension agglutination

Fig. 1 shows positive and negative reactions; the highest dilution of serum still showing clear agglutination represents the titre of the serum. When comparing the sensibility and the specificity between the sensitized latex suspension test and *Brucella* bacterial agglutination, no discrepancies were observed.

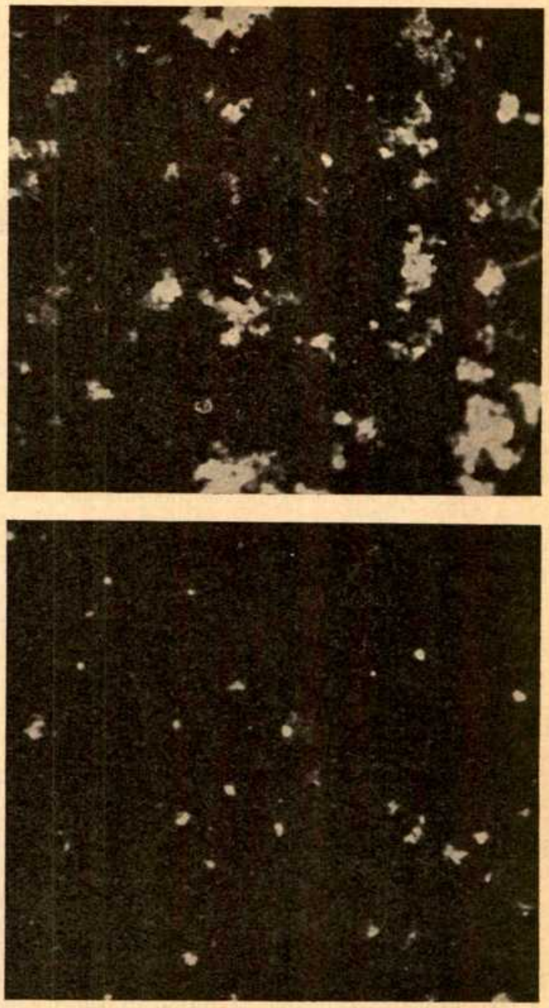

Fig 2. Top, specific fluorescence of agglutinated sensitized latex suspension (×400); bottom, fluorescence of non-agglutinated sensitized latex suspension in non-specific inhibition (×400)

Detection of small quantities of Brucella antigen by inhibition of latex agglutination. Inhibition of the latex agglutination as described here can be demonstrated with material in which *Brucella* antigen is present (Table 1).

If *Brucella* antigen is present in any clinical material tested, the inhibition index of the antigen in the material is calculated by dividing the titre of immune serum in the control test by the titre obtained when a given dilution of the tested material is added to the system. It can be seen in the case shown in Table 1 that a quantity of antigen present in 0·5 ml. of a dilution of 1 : 1,000 was still detectable. A

Table 1. INHIBITION OF LATEX AGGLUTINATION BY *Brucella* ANTIGEN

	Dilution of serum								
	50	100	200	400	800	1,600	3,200	6,400	
Control: 0·5 ml. immune serum 0·5 ml. saline 2 drops SLS	+++	+++	+++	+++	+++	++	+	−	
Test: 0·5 ml. immune serum 0·5 ml. inhibiting antigen 2 drops SLS									Inhibition index
Dilution of antigen 1 : 10	+++	+	−	−	−	−	−	−	32
1 : 100	+++	+++	+++	+	−	−	−	−	8
1 : 1,000	+++	+++	+++	+++	++	−	−	−	4
1 : 5,000	+++	+++	+++	+++	+++	+++	+	−	1

SLS, sensitized latex suspension

parallel precipitation test with the same antigen showed that at least a twenty-fold concentration of antigen was required for a precipitation to occur.

The application of this inhibition test is limited by the presence of many non-specific inhibitors in biological materials tested for the presence of the antigen (for example, urine). In order to distinguish between specific and non-specific inhibition of sensitized latex suspension agglutination, the fluorescent antibody technique was used. This technique was based on the 'direct' method of Coons and Kaplan[6]. Anti-*Brucella* conjugated globulin of immunized goat (agglutination titre 1 : 6,400) was used in a tube test. In a control agglutination test 0·2 ml. of a 1 per cent labelled globulin was added to an equal volume of sensitized latex suspension. The mixture was incubated 2 hr. at 37° C. and overnight at 4° C. The tubes were centrifuged for 10 min. at about 3,000 r.p.m., the sediment was washed in saline and re-suspended in a drop of phosphate buffer (*p*H 7·2–7·4). A loopful of this suspension was smeared on a slide, mounted in buffered glycerine and examined under the fluorescence microscope. Specific fluorescence of agglomerated sensitized latex suspension is shown in Fig. 2, top. In the inhibition test, labelled globulin was first incubated for 1 hr. at 37° C. with the dilutions of the antigen investigated, then mixed with sensitized latex suspension and treated as above.

In the case of specific inhibition of agglutination, the latex particles were not agglutinated and showed no fluorescence, since all antibody was bound by the inhibiting antigen. In the case of non-specific inhibition, there was no agglutination of latex particles, but they showed fluorescence (Fig. 2, bottom); the non-specific inhibitors prevent only the agglomeration of sensitized latex suspension but do not affect the adsorption of specific antibody to them.

We have been able, by introducing the sensitized latex suspension test, to investigate the antigenic fractions of *Brucella*.

The inhibition test as described above permits the detection of minute amounts of *Brucella* antigen in biological materials, while the fluorescent antibody method can be used to distinguish between specific and non-specific inhibition.

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EARLY HEPATIC DYSFUNCTION PRECEDING BILIARY OBSTRUCTION IN SHEEP INTOXICATED WITH SPORIDESMIN

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THE mechanism of bile excretion by the hepatic cell and related physiological phenomena have been investigated by many workers. For example[1], the hepatotoxins ethionine, carbon tetrachloride and α-naphthylisothiocyanate behave differently with regard to their effects on liver cell necrosis, bile flow and proliferation of the bile ductular epithelium. Excretion of bile pigments, alkaline phosphatase and water by the hepatic cells of rabbits intoxicated with icterogenin has been followed by Heikel *et al.*[2]. Cameron and Hasan[3], in studying the effects of common bile duct ligation in rats, suggested that liver cell function is disturbed before the bile can exert its toxic action. They concluded, however, that experimental proof for this suggestion was difficult to obtain because the available techniques were inadequate. The purpose of this article is to direct attention to the possibility of using sporidesmin, a metabolic product of the fungus *Pithomyces chartarum* (Commonwealth Mycological Institute Herbarium No. 74473; Ellis[4]; Synge and White[5]), to provide experimental data on the mechanism of bile excretion and also on the events leading to biliary obstruction.

In the present experiments sporidesmin was administered orally to 56 sheep at a dose-level of 1·0 mgm./kgm. body-weight. At 2, 4, 7, 10, 14, 21 and 28 days after administration of sporidesmin, groups of 8 sheep were killed. In addition, 18 animals that had not received sporidesmin were used as controls. Serum samples were taken from all remaining sheep on the days shown in Fig. 1. The bulked gall bladder bile from each group was collected under toluene then lyophilized. Liver tissue was placed in 4 per cent (w/v) formaldehyde in isotonic saline for histological examination, was frozen and sectioned. Sections were examined for the presence of fat after staining with oil red *O* and Mayer's hæmalum. The bulk of the remaining liver was cut into thin strips and immersed in boiling ethanol within 15 min. of death. Fat was extracted from the liver tissue[6], steroids determined by the Sperry and Webb[7] procedure and lipid phosphorus by the method of Fiske and Subbarow[8] as modified by King[9]. Glutamic oxalacetic transaminase[10] and bilirubin[11] levels were determined on all serum samples. Results are summarized in Fig. 1. The solid content of gall bladder bile and the incidence of colourless bile is shown in Table 1.

Fat staining could not be found in liver sections of the control animals. However, livers of animals

Table 1. CHANGES IN GALL BLADDER BILE FOUND IN SPORIDESMIN-INTOXICATED SHEEP

Days after dosing	Vol. of bulked bile in each group of 8 sheep (ml.)	Dry weight of bulked bile (mgm./ml.)	No. of cases of colourless bile
0	84	110	0
2	107	55	0
4	68	39	0
7	108	41	1
10	86	44	4
14	81	31	3
21	147	30	6

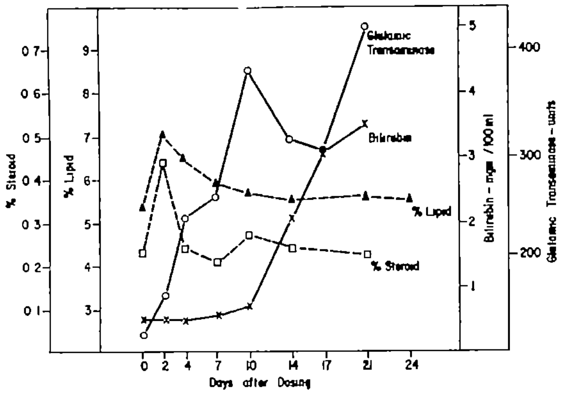

Fig. 1. Changes in liver lipids, serum glutamic oxalacetic transaminase and serum bilirubin after intoxication of sheep with sporidesmin

△, Total liver lipid; □, liver steroids; ○, serum glutamic oxalacetic transaminase; ×, serum bilirubin. Values for control animals were closely similar to those given for day 0

killed two and four days after intoxication showed marked fat staining which was confined to the peripheral areas of the lobules. Sections from animals killed 10 days after dosing showed little or no fat staining. Livers of subsequent groups showed stainable fat in some instances, but by contrast with those of the earlier groups the distribution was mainly centrilobular. The changes revealed by the histological observations coincided with changes in the amount of extracted lipid. In addition, changes in the texture of the extracted lipid were noted. Whereas in the normal livers this lipid was brittle, two days after dosing the lipid was invariably waxy. Four days after dosing the liver lipids were also generally of a waxy texture, but in the groups killed afterwards the texture of the liver lipids was mainly friable and similar to that of normal animals. As the lipid phosphorus content of the liver remained nearly constant throughout the experimental period changes in the lipid composition must occur in the non-phospholipid fractions. Part of the increase in lipid content after two days is attributable to the steroid fraction (Fig. 1).

The increase in serum glutamic oxalacetic transaminase between days four and seven provides further evidence of hepatic cell derangement, for such increases are widely believed[12] to be associated with tissue damage and the attendant leakage of cell contents into the circulating fluids. The changes observed in gall bladder bile (Table 1) are consistent with changes in hepatic cell function, but alternatively might be accounted for by the inflammatory changes which were found in the gall bladder and in the extra-hepatic bile ducts.

The early changes just described were followed after 10 days by elevated serum bilirubin, phospholipid, and 'cholesterol' levels as well as photosensitivity. These changes were related to the biliary obstruction observed on post-mortem examination.

Thus, it seems likely that in sporidesmin intoxication of sheep (similar results have also been obtained in rabbits) injury to the hepatic cell precedes biliary obstruction.

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MORPHOLOGICAL INFLUENCE OF EXUDATES OF MYCORRHIZAL AND NON-MYCORRHIZAL FUNGI ON EXCISED ROOT CULTURES OF *PINUS SYLVESTRIS* L.

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SLANKIS[1] examined the effects of exudates from some mycorrhizal fungi on the development of excised *Pinus sylvestris* L. root cultures, and found them to be influential on the elongation and in production of dichotomous lateral roots. From these and other results he suggested a hypothesis for the morphogenesis of mycorrhizal roots[2]. These observations were of importance in understanding the influence of mycorrhizal fungi on their hosts, but it remained unknown whether this effect was confined to mycorrhizal fungi alone. The formation by soil micro-organisms of products which influence higher plants is widespread, and it seemed probable that non-mycorrhizal fungi would influence the growth of excised pine roots. A summary of work on this problem is presented here.

Exudates of 54 fungi were tested; most were obtained by direct isolation from *P. sylvestris* root tips or dichotomous roots, or from sporophores collected in coniferous plantations. The method of excised root culture was essentially that of Slankis[3], except that ferric chloride in the nutrient was replaced by iron ethylenediamine tetraacetic acid. Development occurred at laboratory temperatures, and records made of increase in length and lateral root formation. Fungi were grown on the same nutrient medium and filtrates tested when good mycelial growth had occurred. The mycelium was removed by Seitz filtration and 5 ml. of the filtrate added aseptically to each of 15 or 20 single root replicates on inoculation; 10 ml. of the same filtrate were added to each of five single replicates of older root cultures with well-developed lateral root systems.

Of all filtrates tested only four (7 per cent), *Boletus granulatus* (L.) Fr., *Boletus subtomentosus* (Schaeff.)

Table 1. TAXONOMIC SUMMARY OF THE INFLUENCE OF FUNGAL CULTURE FILTRATES ON DEVELOPMENT OF EXCISED *Pinus sylvestris* ROOTS

	Phycomycetes	Fungi Imperfecti	Basidiomycetes			
			Mycorrhizal	Non-mycorrhizal	Status unknown	Total
No. tested	7	24	7	13	2	22
No. inducing dichotomies	1 (14 per cent)	1 (4 per cent)	4 (57 per cent)	6 (46 per cent)	1	11 (50 per cent)
No. stimulating elongation	2 (28 per cent)	1 (4 per cent)	2 (26 per cent)	2 (15 per cent)	1	5 (23 per cent)
No. stimulating lateral root formation	0	0	1	1	0	2

Fr., *Mucor spinosus* van Tiegh., and *Trichocladium asperum* Harz were without any apparent effect on initial elongation of the excised radicles. Stimulation of elongation was recorded with ten (19 per cent) isolates, *Amanita muscaria* (L.) Fr., *Boletus bovinus* (L.) Fr., *Clitocybe aurantiaca* (Wolf) Studer., *Fusarium oxysporum* Schlecht. ex. Fr., *Marasmius androsaceus* (L.) Fr., *Mortierella baineri* Const., *Mucor ramannianus* Moller, two isolates of *Mycelium radicis atrovirens* and *Peniophora sanguinea* (Fr.) Bres. Filtrates of all other fungi (74 per cent) caused some reduction, slight in the case of *Mycena galericulata* (Scop.) Fr. and *Zygorrhynchus* sp., to the severe inhibition by filtrates of *Botrytis cinerea* Pers. ex Fr., *Fomes annosus* (Fr.) Cooke and *Trichoderma viride* Pers. ex Fr. Retardation of elongation was frequently accompanied by a brown or black discoloration of the roots.

The pattern of lateral root formation roughly followed that of elongation. Only two filtrates, those of *A. muscaria* and *P. sanguinea*, stimulated lateral root production; most other filtrates caused a reduction, with no formation at all with those of *Calcarisporium arbuscula* Preuss, *Cylindrocarpon radicicola* Wollenw., *Gliocladium* sp., and *Phallus impudicus* L. A number of fungi was found to produce substances in culture which induced the formation of dichotomous roots, such roots being comparable with those found associated with mycorrhizal fungi under natural conditions. Of the mycorrhizal fungi examined, differential response by root cultures to the exudate of a particular species was observed. No dichotomous roots developed in cultures containing filtrates of *A. muscaria*, *B. granulatus* and *B. subtomentosus*, although profuse development of lateral roots occurred in cultures containing the *B. granulatus* exudate. Mycorrhizal fungi found to induce dichotomy were, in order of decreasing influence, *B. variegatus* (Swartz) Fr., *B. badius* Fr., *B. luteus* (L.) Fr., and *B. bovinus*. Dichotomous roots, however, were also found in cultures containing filtrates of fungi other than those known to form mycorrhizal associations, that is, *Gliocladium deliquescens* Sopp, *M. spinosus*, *Peniophora gigantea* Mass., *Pholiota mutabilis* (Schaeff.) Fr., and *Tricholoma rutilans* (Schaeff.) Fr.

Cultured mycelium pieces of *B. variegatus*, *Collybia putida* (Fr. Kuhner and Maire, *Lycoperdon pyriforme* (Schaeff.) Pers., and *P. impudicus* were also added to well-developed root cultures. In each instance, fungal growth was markedly superior to that in nutrient medium alone, indicating a transfer of some beneficial root metabolite to the fungus. Growth of each isolate was extensive over the root surface, and finally killed the root. Dichotomous roots were produced in each culture; they were brown in colour, except those induced by *P. impudicus*, which were purple-mauve. This was additional evidence that colours of mycorrhizal roots are determined by the fungal components[4].

An examination of the data was made to determine the relationship between growth influences and ecological groups[5] of the fungi involved. Soil saprophytes, which included both specialized and unspecialized types, were almost invariably inhibitory, but exudates of one isolate induced dichotomous root production. A similar situation existed with exudates of the group of wood-destroying fungi, although a higher proportion of exudates induced dichotomies. Variable influence was also found with exudates of the mycorrhizal group of fungi, with stimulation of both elongation and lateral root formation being limited to a single isolate, and of elongation only to two others. The remaining four isolates caused reduction in varying degrees, as was previously found with *B. variegatus*[1]. Formation of dichotomous roots was not found to be characteristic of the group as a whole, but rather a property of particular individuals. A consideration of the taxonomic groups involved, however, afforded a greater correlation. No connexion was found between the degree of stimulation of elongation and lateral root formation, but this was noted in association with the production of dichotomous roots; these results are summarized in Table 1. Dichotomies were induced by exudates from one isolate of seven Phycomycetes, one of twenty-four Fungi Imperfecti, and eleven of twenty-two Basidiomycetes. It would seem that the ability to occasion production of dichotomous roots in cultures of excised roots of *P. sylvestris* was almost a characteristic of the Basidiomycete group, the number inducing dichotomies being divided equally between mycorrhizal and non-mycorrhizal isolates.

The results observed support the suggestion[6] that the study of mycorrhiza should be regarded only as a detailed part of the wider subject of root and fungus interaction. Although the great majority of fungi tested (92 per cent) produced exudates which were influential in culture, assessment of the relative importance of such exudates, if they occur naturally, to the entire plant is both difficult and largely hypothetical. The effects of fungal secretions are likely to be most pronounced at the region of the highest level of metabolic activity[7], so it is probable that only those capable of entering into the close cortical relationship would be of far-reaching significance. In addition, it was apparent from the results obtained for the seven known mycorrhizal fungi that physiologically the isolates were very different, and it seems certain that different species of mycorrhizal fungi, as well as strains of the species[8], are unequally effective in their contributions towards growth of the host plant[4].

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STRONGLY CAROTENOID-DEFICIENT *CHROMATIUM*-STRAIN *D* CELLS WITH 'NORMAL' BACTERIOCHLOROPHYLL ABSORPTION PEAKS IN THE 800–850 mμ REGION

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IN the course of work on the pigment-protein complexes of purple bacteria, continuing earlier work[1,2], we endeavoured to cultivate carotenoid-deficient cells of *Chromatium*, strain *D*, by the method devised by Goodwin and Osman[3,4], using diphenylamine (DPA).

The absorption spectrum of the green cells, thus obtained, determined with a Beckman *DU* spectrophotometer by the method of Shibata[5], shows their strong deficiency in carotenoids by very great reduction of the absorption in the 450–550 mμ region of the spectrum (Fig. 1). However, the bacteriochlorophyll absorption maximum at 850 mμ is of the same order of magnitude, and in some cases even higher than that found in normally grown cells of *Chromatium*, strain *D*. The same holds for the pigment-protein complexes, present in extracts of both types, prepared by grinding (Fig. 2). In all cases the spectrum shows the shape which is usual for *Chromatium*, strain *D*, namely, with an 850 mμ maximum about 1·2–1·5 times as high as the 800 mμ maximum. The spectra of the green cells and their extracts tend to show a somewhat decreased 890 mμ absorption as compared with normally grown cells.

The occurrence of the 850 mμ maximum in DPA-treated cultures in the same way as present in normally grown cells of this strain is at variance with the observations of Bergeron and Fuller[6], who report a strong reduction of the 850 and 890 mμ absorption peaks, accompanying the decrease of absorption in the carotenoid part of the spectrum, owing to the DPA treatment. Although the concentration of DPA applied by Bergeron and Fuller[6] is higher than 12 mgm./l., at which our cells were grown, the absorption in the carotenoid part of the spectrum is equally low in both cases. We have not been successful, so far, in growing *Chromatium*, strain *D*, at DPA concentrations above 12 mgm./l. In attempts of this sort, starting from either 'red' or 'green' cultures, the medium in the inoculated bottles remains clear, the cells of the inoculum become bleached and drop to the bottom. Like Fuller (personal information), we used a pale brown preparation of DPA ('purum'). Three times recrystallization of the substance from ethanol–water mixtures yielded a white product. Using this, however, the maximum concentration at which the bacteria still multiply was not increased; it rather tended to some decrease. It seems possible that the differences in the spectra of carotenoid-deficient bacteria obtained by Bergeron and Fuller[6] and in our experiments are connected with the fact that those investigators were able to cultivate the bacteria at much higher concentrations of DPA, at which the cells may develop an absorption spectrum with lowered 890 and 850 mμ peaks. Moreover, the possibility remains of a difference in the bacteria: what is called '*Chromatium*, strain *D*' may not be fully identical in both cases.

Anyhow, the observations presented in this article demonstrate that there is not necessarily any relation between disappearance of carotenoids and disappearance of the 850 mμ bacteriochlorophyll maximum in *Chromatium*, strain *D*. As for the 890 mμ peak, we fairly consistently found this weaker in carotenoid-deficient bacteria than in those normal.

Furthermore, it occurs to us that the coincidence of disappearance of carotenoids and increased sensitivity to photo-oxidation, reported by Cohen-Bazire and Stanier[7] and Fuller and Anderson[8], and now under investigation in our laboratory, need not necessarily be explained as a direct protection of bacteriochlorophyll by carotenoids against photo-oxidation. It may as well mean that DPA inhibits the biochemical formation of carotenoids, and separately produces an enzymatic structure in the chromatophores which renders them more susceptible to

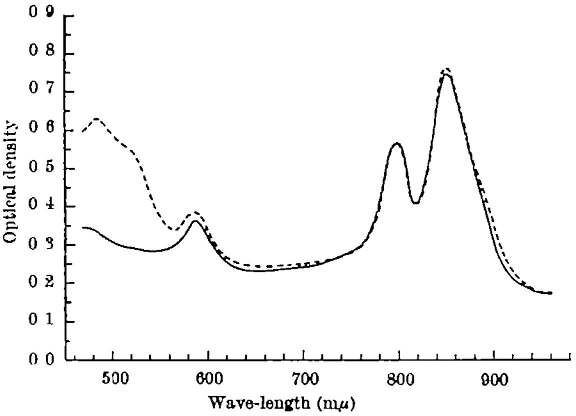

Fig. 1. Absorption spectra of *Chromatium*, strain *D*, grown in the absence and in the presence of DPA (12 mgm./l.). The 3-day-old cultures were suspended in phosphate buffer, *p*H 7·8. A sheet of filter paper was inserted into the beam to minimize the effect of scattering of the cells on the spectrum
– – –, Normal cells; ——, carotenoid-deficient cells

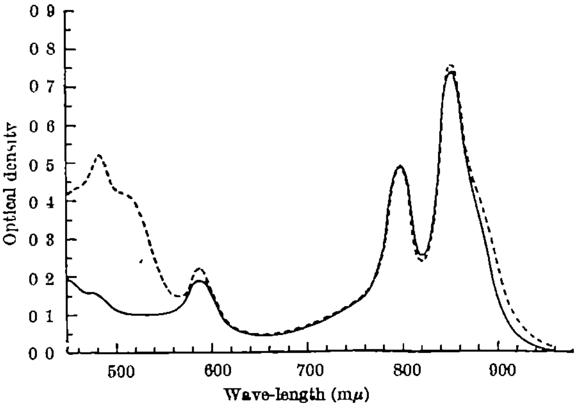

Fig. 2. Absorption spectra of aqueous extracts prepared from normal and carotenoid-deficient cells of *Chromatium*, strain *D*, by grinding
– – –, extract of normal cells; ——, extract of carotenoid-deficient cells

photo-oxidation. This change in structure may be reflected, for example, in the decrease of the 890 mμ complex. At very high concentrations of DPA, the biochemical structure of the chromatophores may be so much different from normal that the formation of the 850 mμ complex is also affected.

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We thank Dr. Fuller for discussions.

Note added in proof. The same holds for DPA *pro analyse*; in more recent experiments we found that, using a heavy inoculum, cultures still could be grown in solutions containing up to 20 mgm. DPA/l., without difference in the 800 and 850 mμ absorption bands

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IRRADIATION EFFECTS ON STRAND SEPARATION OF DEOXYRIBONUCLEIC ACID

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A NUMBER of effects of ultra-violet and ionizing radiation on deoxyribonucleic acid (DNA) have been investigated from the point of view of their possible relevance to the damage produced by these radiations in living cells. Ultra-violet (254 mμ) light is mainly absorbed by the purine and pyrimidine residues and reversible hydration is considered to be the main mechanism of its action[1,2]. Recently, it has been shown that dimerization of thymine occurs when aqueous solutions of these compounds are irradiated with ultra-violet light[3,4]. Whether this effect occurs in living cells remains uncertain although Wacker *et al.* claim to have isolated thymine dimers from irradiated bacteria[5]. Ionizing radiation, on the other hand, is capable of producing several effects such as single-stranded and double-stranded breaks of the double helix structure and cross-linking[6]. Doty *et al.*[7] have shown that separation of the two strands in a DNA molecule can be achieved by heating and that recombination of the separated strands occurs when heated DNA solutions are cooled slowly. This separation mechanism is presumably very important for the replication of DNA and thus for proliferation of cells. We have investigated whether this strand separation and recombination are sensitive to irradiation.

The experiments were carried out with calf thymus DNA isolated according to the method described by Kay *et al.*[8]. Analytical data of the DNA preparation are: RNA, 0·5 per cent; protein less than 1 per cent; E_{260}/E_{280}, 1·83. Ultra-violet irradiations were performed with an unfiltered low-pressure ultra-violet source (Philips *TUV*) at 6 cm. from the lamp. The dose under these conditions was 93 erg./mm.^{2}/sec. 4-ml. samples of DNA solutions (20–25 γ/ml.; $E^{1\text{ cm.}}_{260}$ mμ, 0·380) were irradiated in Petri dishes (diam. 5·6 cm.). X-irradiations were performed with a General Electric Maxitron, 250 kV., 30 m.amp, half-value layer 2·0 mm. copper, dose-rate 250 r./min.

The DNA preparations were stored in 0·14 *M* sodium chloride (*p*H 6·5) at –10° C. at a concentration of 1·5 mgm./ml. Before use, samples were thawed and diluted with 0·14 *M* sodium chloride to a concentration of 20 or 25 γ/ml. After incubation of the DNA solutions for 20 min. in a water-bath at 89° or 95° C. the samples were transferred to cuvettes. Reading of the optical densities was started 1 min. after the samples were taken out of the bath and continued for 20 min., during which the temperature decreased to 25° C.

Two series of experiments were carried out: (*a*) those in which ultra-violet irradiation of DNA solutions was applied before heating of the samples; (*b*) those in which ultra-violet irradiation was applied between two heating periods.

(*a*) *Ultra-violet irradiation before heating.* The results of a typical experiment are shown in Table 1.

(*b*) *Ultra-violet irradiation between two heating periods.* In these experiments the DNA solutions (25 γ/ml.) were heated and cooled several times. After the second incubation period the Δ and hyperchromic shift (*H.S.*) values are increased as compared with the corresponding values obtained after the first incubation. After the third incubation, however, these values remain almost constant as compared with the second incubation. Therefore ultra-violet radiation was applied between the second and third incubation period.

Table 1. EFFECT OF ULTRA-VIOLET IRRADIATION ON DNA IN SOLUTIONS

Time of irradiation (min.)	E_1	E_{20}	Δ	*H.S.*
0	399	348	51	118
1	395	345	50	114
2	386	340	46	105
5	374	335	39	93
10	367	332	35	86
20	365	332	33	84

E_1, extinction $\times 10^3$ of a DNA solution at 260 mμ measured 1 min. after termination of the incubation; E_{20}, extinction $\times 10^3$ of a DNA solution at 260 mμ measured 20 min. after termination of the incubation; E_0, extinction $\times 10^3$ of a DNA solution at 260 mμ before incubation; Δ, $E_1 - E_{20}$; *H.S.* (hyperchromic shift), $E_1 - E_0$.

Table 2. EFFECT OF ULTRA-VIOLET IRRADIATION ON DNA IN SOLUTIONS

	Δ		H.S.	
	2–1	3–2	2–1	3–2
No irradiation	4 ± 1	1 ± 0·6	19 ± 7	6 ± 2
5 min. ultra-violet	4 ± 2	– 11 ± 3	20 ± 8	– 3 ± 2
10 min. ultra-violet	4 ± 1	– 7 ± 2	17 ± 4	– 3 ± 2

The figures represent the differences ($\times 10^3$) in extinction values: 2–1, differences between first and second incubation period, 3–2, differences between third and second incubation period.

The results summarized in Tables 1 and 2 demonstrate that ultra-violet irradiation under the different experimental conditions causes a decrease of the Δ and *H.S.* values. Even after repeated heating and cooling of DNA ultra-violet irradiation disturbs the process of strand separation and recombination. Moroson and Alexander[9] also reported an effect of ultra-violet irradiation on a hyperchromic effect in DNA. Their experiments were carried out under completely different conditions.

The effects of ultra-violet light observed disappear partially by another heating after the irradiation, especially when low doses of ultra-violet light (1–5 min.) were used. In these cases 40–50 per cent of the ultra-violet effects can be restored; heating after irradiation with relatively high doses of ultra-violet light (10–20 min.) leads only to 20–30 per cent restoration of the irradiated effects.

In experiments performed with X-irradiation, in which doses of 50–10,000 r. were applied, we did not observe a definite effect on the Δ and *H.S.* values.

Our results show that separation and recombination of the DNA double helix structure occurs less easily after ultra-violet irradiation and the effects are partially reversible. It is unlikely that under our experimental conditions appreciable rupture of hydrogen bonds occurs, because no change in extinction values could be observed after ultra-violet irradiation only. The effect observed can be explained in different ways. Dimerization of nucleotides which is known to occur might prevent proper uncoiling of the double helix and separation of the strands and this could account for the irreversible part of the irradiation effect. It is also possible that ultra-violet irradiation causes a shift towards the keto-form of the hydroxyl groups of the bases which might result in a strengthening of the hydrogen bonds and this could account for the reversible part of the irradiation effect.

It remains uncertain whether the observations described have any relation to the action of ultra-violet light on living cells.

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FUNCTION OF ASCORBIC ACID IN THE CONVERSION OF PROLINE TO COLLAGEN HYDROXYPROLINE

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THE synthesis of collagen, one of the most abundant animal proteins, is intimately associated with specific hydroxylation mechanisms. Thus, collagen hydroxyproline arises from proline rather than from free hydroxyproline[1,2], and lysine rather than hydroxylysine is the major precursor of collagen hydroxylysine[3,4]. Collagen synthesis therefore differs from that of other proteins, the amino-acid residues of which are derived from the corresponding free amino-acids. Collagen formation is reduced in ascorbic acid deficiency, and administration of ascorbic acid to deficient guinea pigs leads to rapid collagen synthesis[5,6]. In contrast, there seems to be no evidence that synthesis of other proteins requires ascorbic acid. The mechanism by which ascorbic acid increases synthesis of collagen is not yet known. It has been proposed that a proline-containing pre-collagen accumulates in ascorbic acid deficiency, and that ascorbic acid functions in the hydroxylation of such peptide-bound proline[6]. Failure to find peptide intermediates[5,7] has led to the suggestion that there is a separate pool of 'active hydroxyproline' derived from proline[7]. That ascorbic acid may be required for the maintenance of collagen has also been considered[8]. Mitoma and Smith[9] concluded that hydroxylation of proline is not affected by ascorbic acid deficiency, and suggested that ascorbic acid influences maturation of fibroblasts.

In an attempt to evaluate these possibilities we have examined incorporation of proline into collagen of granulomas induced in normal and ascorbic acid-deficient guinea pigs by injection of carrageenin[6]. When minces of granulomas from deficient animals were incubated with proline labelled with carbon-14, the specific activity of the collagen hydroxyproline was much lower than that of non-deficient controls. Although this suggested that conversion of proline to hydroxyproline is reduced in ascorbic acid deficiency, this result could be explained by a decreased rate of collagen synthesis. To obtain information about both hydroxylation of proline and synthesis of collagen, we have carried out experiments with tritiated proline. The use of tritiated proline would provide a new parameter for following hydroxylation if formation of tritiated water was stoichiometrically related to formation of hydroxyproline. We incubated minces of granulomas obtained from normal and scorbutic animals with media containing 3,4-ditritiated L-proline. The mixtures were then frozen and lyophilized, and the water was trapped. Collagen was isolated from the residue by extraction with hot trichloroacetic acid followed by precipitation with tannic acid. The collagen isolated from deficient and control granulomas had approximately the same amino-acid composition as that of purified gelatin. To check further the purity of the collagen, use was made of the fact that this protein lacks tryptophan. When ^{14}C-tryptophan was incubated with the granuloma, radioactivity was incorporated into non-collagen proteins but not into collagen.

When tritiated proline was incubated with granuloma obtained from deficient guinea pigs, the specific activity of the collagen proline afterwards isolated was moderately reduced as compared with the normal. In contrast, there was striking reduction of incorporation of proline into collagen hydroxyproline. Similar results were obtained with ^{14}C-proline (Table 1).

As indicated in Fig. 1 the formation of tritiated water was markedly reduced in experiments with deficient granulomas. The initial lag period is similar to that found for incorporation of proline into collagen, and may be due to the time required for proline to enter the tissue. That formation of tritiated water is a valid measure of hydroxylation was shown by the fact that the tritium incorporated into hydroxyproline and that found in the water

Table 1. INCORPORATION OF PROLINE INTO THE COLLAGEN PROLINE AND HYDROXYPROLINE OF GRANULOMAS FROM NORMAL AND SCORBUTIC GUINEA PIGS*

Isotope	Exp. No.	Incubation period (min.)	Specific activity (d.p.m./μmole) Normal Proline	Normal Hydroxyproline	Deficient Proline	Deficient Hydroxyproline
^{3}H	1	30	6,150	2,400	4,580	<100
		60	13,300	5,070	9,300	150
		120	21,300	8,700	17,800	360
^{14}C	2	30	790	695	787	<10
^{14}C	3	30	745	680	530	70

* Granuloma mince (2 gm.) was incubated with 5 ml. of medium containing 5 μc. of 3,4-ditritiated L-proline (173 μc./μmole) or 1 μc. of DL-proline-1-^{14}C (3·1 μc. per μmole) (obtained from the New England Nuclear Corporation, Boston, Mass.). Incubation was carried out at 37° under 95 per cent oxygen–5 per cent carbon dioxide. The medium contained NaCl (0·022 *M*), KCl (0 003 *M*), $MgSO_4$ (0·0012 *M*), $CaCl_2$ (0 0013 *M*), KH_2PO_4 (0·0004 *M*), $NaHCO_3$ (0 025 *M*), and D-glucose (0 01 *M*). Tritium and carbon-14 were determined by liquid scintillation counting. The granulomas were obtained after six days of growth as described by Robertson and Schwartz (ref. 6); the deficient animals were deprived of ascorbic acid for two weeks. Proline and hydroxyproline were isolated from acid hydrolysates of the collagen by paper chromatography (solvent: *n*-butanol/acetic acid/water; 4:1:1), and determined, respectively, as described by Troll and Lindsley (ref. 12) and Neuman and Logan (ref. 13).

were about the same. The ratio of the total radioactivity of hydroxyproline to the total radioactivity of the water was 1·1 ± 0·2 (average of six experiments). It appears then that about half the proline tritium was lost during hydroxylation. This conclusion was substantiated by studies on granulomas from normal animals in which ^{14}C-proline and tritiated proline were used together; the ratio of the specific activities of ^{14}C-proline to ^{14}C-hydroxyproline was, within experimental error, half that of the corresponding ratio for the tritriated imino-acids. These findings are consistent with hydroxylation mechanisms involving either a 4-keto intermediate or 3,4-unsaturation, assuming random distribution of tritium at proline carbons 3 and 4 and absence of significant isotope effects. In several experiments, glutamic acid was isolated from collagen and found to contain negligible isotope. These observations agree with those of Green and Lowther[10], and indicate that metabolism of proline to products other than hydroxyproline is not appreciable in carrageenin granulomas.

Addition of ascorbic acid to granuloma minces obtained from scorbutic guinea pigs led to a marked increase in the formation of tritiated water (Table 2). Parallel studies showed that addition of ascorbic acid also increased incorporation of tritiated proline into collagen hydroxyproline; the increase in tritiated water was equal to that of tritiated hydroxyproline. In very recent studies (published while our work was in progress), Robertson and Hewitt[11] reported that addition of ascorbic acid to deficient tissues increased the specific activity of collagen ^{14}C-hydroxyproline.

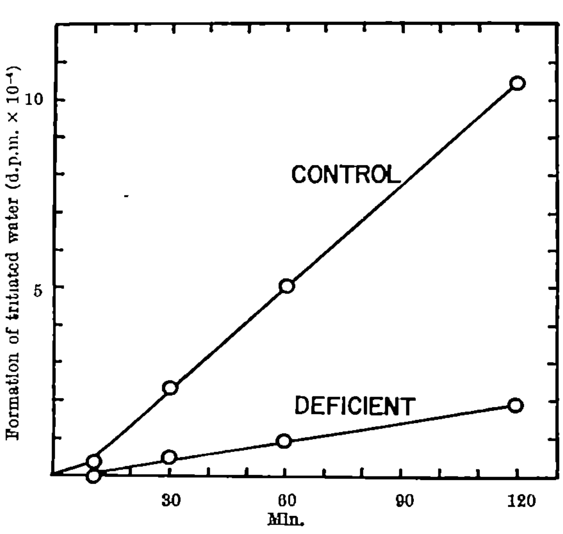

Fig. 1. Formation of tritiated water from 3,4-ditritiated L-proline by granulomas from ascorbic acid-deficient and control guinea pigs. (Conditions as in Table 1)

Table 2 STIMULATION OF THE OXIDATION OF PROLINE BY ASCORBIC ACID-DEFICIENT GRANULOMAS BY *in vitro* ADDITION OF ASCORBIC ACID

Exp. No.	Tissue	Ascorbate added (μgm./ml.)	Tritiated water (d.p.m.)
4	Deficient	None	19,700
	,,	800	45,500
5	,,	None	14,400
	,,	20	25,700
	,,	100	23,100
	Normal	None	27,200
	,,	20	30,600
	,,	100	26,500

* The experimental conditions were as given in Table 1 (with 3,4-ditritiated L-proline); after 120 min. the reaction mixtures were lyophilized and the water was trapped and counted

At least two schemes for conversion of proline to collagen imino-acids may be considered as follows:

$$\begin{tikzcd}
\text{Proline} \arrow[r, "1"] & {[(\text{pro})_x\text{-peptide}]^{\mathrm{I}}} \arrow[r, "2"] & {[(\text{pro})_{n-x}\text{-}(\text{hypro})_x\text{-peptide}]^{\mathrm{II}}} \arrow[d, "3"] \\
 & & \text{collagen}
\end{tikzcd}$$

Scheme *A*

$$\begin{tikzcd}
 & {[\text{pro}]^{\mathrm{I}}} \arrow[drr] & & \\
\text{Proline} \arrow[ur, "1"] \arrow[dr, "2"'] & & & \text{collagen} \\
 & {[\text{pro}]^{\mathrm{II}}} \arrow[r, "3"] & {[\text{hypro}]^{\mathrm{III}}} \arrow[ur] &
\end{tikzcd}$$

Scheme *B*

In *A*, proline is incorporated into collagen through a series of precursor peptides of types I and II; about half the proline residues are converted to hydroxyproline. In *B*, there are two types of proline-containing pools, of which one (I) contains proline destined to become collagen proline, and the other (II) proline that will become collagen hydroxyproline. The latter is oxidized to a hydroxyproline intermediate (III), not in equilibrium with free hydroxyproline and not yet in peptide linkage. (There may be more than one intermediate of types II and III.) According to scheme *A*, the ratio of the specific activity of collagen hydroxyproline to that of collagen proline should not exceed unity. However, Green and Lowther[10] observed ratios as high as 1·6, and we have also observed similar ratios. Robertson *et al.*[7] have stated other cogent arguments against mechanisms of type *A*.

The present findings appear to support a mechanism of type *B*. Accumulation in ascorbic acid deficiency of an intermediate (or intermediates) in the pathway from proline to collagen hydroxyproline would lead to much greater dilution of proline destined to become collagen hydroxyproline than proline which is to become collagen proline. A block at step 2 or 3 in scheme *B* would be accompanied by a decrease of tritiated water formation. However, a block at step 2, if it were between free proline and the first bound-proline intermediate, could only lead to accumulation of proline and would therefore dilute proline destined for both pathways. Accordingly, ascorbic acid appears to be required for the formation of an intermediate of type III, or possibly one of type II. (It is tempting to suggest, in line with present beliefs about protein synthesis, that I and III may be imino-acyl RNA compounds.) Reduced formation of tritiated water from tritiated proline in ascorbic acid deficiency might reflect general reduction in protein synthesis. However, such an explanation is unlikely because of the relatively high incorporation of proline into collagen proline as compared with hydroxyproline. We have also found similarly high incorporation of glycine and tyrosine into the collagen of deficient granulomas. Our results do not support a systemic ascorbic acid-mediated mechanism or the suggestions that ascorbic acid acts by stimulation of the maturation of fibroblasts or in the maintenance of collagen. The rapid increase of tritiated water formation on addition of ascorbic acid to deficient granuloma minces is consistent with the belief that ascorbic acid is involved in the hydroxylation step. According to the present proposal, hydroxylation of proline (and possibly of lysine) occurs prior to assembly of collagen peptides. Such reactions may therefore control the rate of collagen synthesis. Proof of this mechanism must necessarily depend on separation of the hydroxylation steps from protein synthesis, and on characterization of the intermediates involved. The use of tritiated proline provides a convenient tool with which to follow hydroxylation of proline, and for study of the mechanism of this reaction.

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ENZYMATIC CONVERSION OF D-GLUTAMIC ACID TO D-PYRROLIDONE CARBOXYLIC ACID BY MAMMALIAN TISSUES

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It is known that when DL-glutamate is administered to rats most of the D-component is excreted in the urine as D-pyrrolidone carboxylic acid[1,2]. Sarah Ratner suggested in 1944 that this conversion was probably enzymatic in nature[1], and recent work on rat tissue slices[2] is consistent with this view. In the course of work in this laboratory on the mechanism of the enzyme-catalysed synthesis of glutamine, evidence for the formation (in the presence of adenosine triphosphate and Mg^{++}) of activated enzyme-L-glutamate and enzyme-D-glutamate complexes was observed when ammonia was rigorously excluded[3-5]. Although the enzyme-L-glutamate complex reacts readily with small amounts of ammonia to yield L-glutamine, the reaction of the enzyme-D-glutamate complex with ammonia is very slow with low concentrations of ammonia. Mild heating of either complex gives pyrrolidone carboxylic acid. These observations suggested the possibility that the enzyme that catalyses the synthesis of L-glutamine might also be responsible (in the presence of low physiological concentrations of ammonia) for the formation of D-pyrrolidone carboxylic acid from D-glutamic acid.

To test this hypothesis, we incubated the purified glutamine-synthesizing enzyme from sheep brain[6] with D-glutamate under various conditions; although some D-pyrrolidone carboxylate formation occurred with large amounts of enzyme, this reaction did not occur at a significant rate at 37° with catalytic quantities of enzyme. It thus appeared probable that another enzyme activity was present in animal tissues that was able to catalyse the conversion of D-glutamate to D-pyrrolidone carboxylate. We therefore examined several mammalian tissues for such an activity and found it in the kidney and liver of the mouse, rat and man. In each case, the activity per gram of tissue was higher in the kidney than the liver.

Since homogenates of mouse kidney are somewhat more active than those of the other tissues examined, we purified the activity (about thirty- to fifty-fold) from this source. Approximately two-thirds of the activity was found in the supernatant solution after centrifugation of the homogenate at 100,000*g* for 2 hr., and most of it precipitated between 30 and 60 per cent of ammonium sulphate saturation at *p*H 7 and 0°. After separation of the protein from ammonium sulphate by passage through a column of 'Sephadex', further purification was achieved by adsorption of impurities on alumina $C\gamma$ gel.

The purified fraction catalysed the conversion of D-glutamate to D-pyrrolidone carboxylate in the presence of Mg^{++} or Mn^{++}. The reaction was conveniently followed with ^{14}C-D-glutamate by using paper chromatography; thus both disappearance of glutamate and formation of pyrrolidone carboxylate were observed (Fig. 1). Disappearance of D-glutamate has also been followed by use of the ninhydrin colour reaction. The formation of D-pyrrolidone carboxylate was shown conclusively by isolation of the product in a large-scale experiment; crystalline pyrrolidone carboxylate was obtained in analytically pure state. Hydrolysis of this material in 2 *N* hydrochloric acid for 3 hr. at 100° gave glutamate quantitatively. The glutamate thus obtained was not susceptible to the action of L-glutamic acid decarboxylase of *Clostridium welchii*.

Under the conditions used, no reaction occurred in the absence of enzyme or with heat-inactivated enzyme (Table 1). Little conversion occurred in the absence of added Mg^{++} or Mn^{++}. The *p*H optimum was about 8·3 with mouse kidney and liver preparations and Mg^{++}; at this value of *p*H, Mn^{++} was more effective than Mg^{++} when these metal ions (as magnesium chloride and manganese chloride) were compared at concentrations of 0·008–0·142 *M*. Addition of adenosine triphosphate (0·015 *M*) had either no effect or slightly inhibited the reaction. Addition of ammonia (0·005 *M*) with or without adenosine triphosphate did not affect the rate significantly, and the enzyme preparation did not catalyse the synthesis of either L- or D-glutamine. Incubation of the purified mouse kidney fraction (1 mgm.) with ribonuclease (50 μgm.) for 30 min. had no effect on activity, while all activity was destroyed by incubating the preparation with trypsin (50 μgm.). Treatment of the enzyme with charcoal did not affect the activity.

Table 1. ENZYMATIC CONVERSION OF D-GLUTAMATE TO D-PYRROLIDONE CARBOXYLATE

Reaction mixtures*	Pyrrolidone carboxylate formed (mμmoles)
Enzyme + D-glutamate + Mg^{++}	17 5
Enzyme + L-glutamate + Mg^{++}	0
Enzyme + D-glutamate + Mn^{++}	58·1
Enzyme + L-glutamate + Mn^{++}	0
Enzyme + D-glutamate	3 4
Enzyme† + D-glutamate + Mn^{++}	0
D-Glutamate + Mg^{++}	0
D-Glutamate + Mn^{++}	0

* Each experiment contained buffer and other components in the concentrations given in Fig, 1, except that 0 25 mgm. of enzyme preparation was used; incubated for 30 min. at 37°.

† Heated at 100° for 5 min.

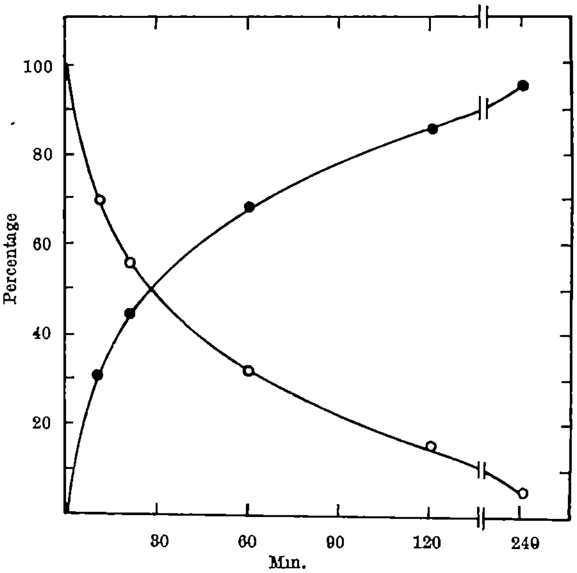

Fig. 1. Time course of the enzymatic conversion of D-glutamate to D-pyrrolidone carboxylate. The reaction mixtures consisted initially of purified enzyme from mouse kidney (0·5 mgm.), D-glutamate-1-^{14}C (0 07 μmole, 70,000 c.p.m.), $MgCl_2$ (4 μmoles), and 2-amino-2-(hydroxymethyl)-1,3-propanediol-HCl buffer (20 μmoles; *p*H 8·3); final volume, 0·12 ml.; 37°. The reaction was followed by paper chromatography (*n*-butanol/acetic acid/water; 4 . 1 : 1) of deproteinized reaction mixtures, and determination of the radioactivity found in the areas corresponding to glutamate (open circles) and pyrrolidone carboxylate (closed circles); ordinate represents per cent of total radioactivity found in each area

Under the conditions given in Fig. 1, the reaction went to approximately 95 per cent completion. The position of equilibrium is thus similar to that observed in studies on the non-enzymatic cyclization of glutamic acid[7]. That the enzymatic reaction is reversible was shown by incubating ^{14}C-D-pyrrolidone carboxylate in the system described in Fig. 1; under these conditions, about 5 per cent of the added substrate was converted to glutamate. In the presence of higher concentrations of D-glutamate (for example, 0·01 *M*) and Mn^{++}, the initial reaction-rate was approximately forty times greater than in the experiment described in Fig. 1.

The purified mouse kidney fraction did not catalyse the disappearance of L-glutamic acid, L-glutamine, D-glutamine, D-α-aminoadipic acid, D-homoglutamine, D-aspartic acid, or L-aspartic acid. On the other hand, when D-α-methylglutamic acid, DL-α-methylglutamic acid, DL-β-methylglutamic acid, and DL-α-aminotricarballylic acid were incubated with the enzyme, there was disappearance of amino-acid (never exceeding 50 per cent of the total with the racemic mixtures) as determined by the ninhydrin colour reaction. These findings constitute presumptive evidence that the enzyme acts on certain substituted D-glutamic acid derivatives.

The enzyme activity appears to be present in sufficient amount to account for the observed *in vivo* conversion of administered D-glutamate to D-pyrrolidone carboxylate. At the present time there is no evidence that D-glutamic acid or other D-amino-acids are of significance in mammalian metabolism. The enzyme activity that catalyses the conversion of D-glutamate to D-pyrrolidone carboxylate seems therefore to occupy the same uncomfortable metabolic status as that of D-amino-acid oxidase. It is of interest that both these enzymes are found in the kidney and liver, the former being more active in each case. Although it cannot be excluded that other as yet undiscovered more 'natural' substrates for these enzymes exist, it may be speculated that their functions are to destroy ingested D-amino-acids and those formed by the bacterial flora (and possibly by other reactions). It seems unlikely that they are artefacts of isolation since their catalytic actions appear to be reflected *in vivo* when D-amino-acids are administered. While many D-amino-acids are converted to their respective α-keto acid analogues (which may be re-aminated by L-specific systems), D-glutamic acid is removed from the organism by an intramolecular acylation reaction, analogous in some respects to the mechanisms for removal of benzoic and phenylacetic acids. The possibility that the enzyme described here acts on D-glutamate ingested in the diet or formed by the bacterial flora is consistent with the presence of D-glutamic acid in a number of lower forms. If such is the case, one might expect to find D-pyrrolidone carboxylic acid in the urine of mammals. It is of interest that human urine contains a considerable quantity of 'bound' glutamic acid[8], although a fraction of this can be accounted for as phenylacetylglutamine[9], some D-pyrrolidone carboxylate may be present. (We found that samples of human kidney and liver obtained at necropsy (8–14 hr. post-mortem) were 50–80 per cent as active as rat kidney and liver, respectively. Putnam *et al.*[10] found no ^{14}C-pyrrolidone carboxylic acid and only ^{14}C-glutamic acid in the urine after intravenous injection of ^{14}C-DL-glutamic acid to a patient with multiple myeloma; however, the patient had extensive renal damage and may also have had impaired liver function.)

Apart from the question of the physiological significance of the enzyme, the mechanism of this optically specific cyclization and the role of metal ions in the reaction pose interesting problems. Finally, the enzyme may, like D-amino-acid oxidase, have practical value in biochemical investigations.

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SALT-MARSH BACTERIA

Bacteria of a Developing Salt Marsh

IN a study of the bacterial flora of a salt marsh at Gibraltar Point, Lincolnshire, numbers of heterotrophic aerobes were estimated by colony counts on ZoBell's sea-water agar medium No. 2216[1]. Numbers increased with passage up the shore from uncolonized to colonized areas. The lowest counts were obtained from samples taken at the edge of the channel cut by the River Steeping through the bare mud flats, higher counts from the mature marsh and the highest from the *Salicornia* zone. This area also showed the greatest seasonal variation in numbers. Samples from areas dominated by *Spartina* often gave even lower counts than those taken from the bare mud flats at a point between the Steeping site and the first colonized zone. It is interesting to compare these results with the distribution of fungi reported for the same marsh by Pugh[2], who observed a comparable decrease in the *Spartina* zone but found the highest numbers of fungi in the mature marsh.

Colonies selected at random from the plates were isolated and purified by repeated streaking on ZoBell agar. 320 isolates were obtained over a period of four months, 40 from each of eight sampling sites, namely, the two uncolonized sites referred to above, which are flooded by most high tides; areas of the immature marsh dominated by *Salicornia* and *Spartina* and flooded only by spring tides; and four sites in the mature marsh dominated by *Halimione*, *Agropyron*, *Puccinellia* and *Limonium* and flooded only by exceptionally high spring tides. The isolates were preserved as desiccates until they were to be examined, when moist slopes on ZoBell agar were prepared. After 24–48 hr. growth these were examined microscopically, and used for inoculating media for physiological tests. The chief characters so far determined are given in Table 1, which shows for each test the percentage of isolates which gave a positive reaction.

Only 11 per cent of the isolates were Gram-positive, most of these being found in the mature marsh. Whereas Gram-positive rods, especially *Bacillus* spp. and mycelial forms, were almost confined to this area, cocci were more evenly distributed. Gram-negative rods, often pleomorphic, predominated in the marsh as a whole, and especially in the immature marsh. These results differ from those of Wood[3], who found the bacteria of coastal muds in Australia to include 23 per cent of Gram-positive rods, 28 per cent of cocci and only 49 per cent of Gram-negative rods. However, he pointed out that the numbers of *Bacillus* spp. and of cocci varied in different areas, also that seasonal changes and different techniques would affect the numbers recorded. Other workers, including ZoBell[4], have found up to 95 per cent of the bacteria of marine muds to be Gram-negative rods. Taylor[5] reported similar findings for fresh water muds. In drier soils, although Gram-negative forms predominate, Gram-positive forms seem to be rather more numerous[6] than in these water-logged muds, which agrees with the finding that the Gram-positive forms of the marsh were found mostly in the drier areas.

A third of the isolates were pigmented, whereas Wood found only 15 per cent of pigmented forms. Those from the mature marsh included a higher proportion of pink forms and some with the diffusible green fluorescent pigment usually associated with *Pseudomonas* spp., while the immature marsh yielded more isolates with yellow or orange pigments. 9·5 per cent of the isolates, all from uncolonized area and immature marsh, were colourless at first but later developed an orange-brown pigment in the centre of the colony.

Acid was formed from glucose under aerobic conditions by 42 per cent of the isolates, but only 7 per cent were active under anaerobic conditions. This and other biochemical activities, for example, the hydrolysis of starch and gelatin, were shown by a higher proportion of the mature marsh isolates, those from the immature marsh being for the most part rather inactive. Surprisingly, in view of the widespread blackening of the muds, only 22 per cent of the isolates produced hydrogen sulphide from peptone. Obligate anaerobes, including sulphate-reducers, must presumably be responsible for most of the sulphide production.

In many respects the bacterial flora described by Wood showed characters intermediate between the

Table 1. CHARACTERISTICS OF SALT MARSH BACTERIA

	Percentage of isolates showing character		
	Uncolonized areas and immature marsh (frequently flooded)	Mature marsh (more rarely flooded)	Whole marsh
Gram-positive reaction	6	16	11·0
(*a*) Rods	1	4	2·5
(*b*) Mycelial forms	0	8	4·0
(*c*) Cocci	5	8	4·0
Gram-negative rods	94	84	89·0
Pigmented forms (total)	29	35	32·0
(*a*) Pink	1	8	4·5
(*b*) Yellow or orange	27	20	23·5
(*c*) Fluorescent	1	7	4·0
Non-pigmented forms	71	65	68·0
Non-motile forms	67	72	70·0
Motile forms	33	28	30·0
(*a*) Polar flagellate	29	25	27·0
(*b*) Peritrichous	4	3	3·0
Spore-formers (*Bacillus* spp.)	0	2	1·0
Acid from glucose (Hugh and Leifson's test)			
Aerobic (oxidative)	40	46	43·0
Anaerobic (fermentative)	3	11	7·0
Starch hydrolysed	23	36	29·5
Gelatin hydrolysed (Frazier plate)	38	67	52·5
H_2S from peptone	17	27	22·0
NH_4Cl utilized as sole nitrogen source	2	16	9·0

Figures for each group of four sites given as the nearest whole numbers. 320 isolates in all were examined, 160 from each group of samples.

typical floras of soil and of marine mud. The flora at Gibraltar Point seems to resemble the latter more closely. While this may be due merely to the waterlogged nature of the muds, it is of special interest since there is evidence[7] that considerable amounts of sandy silt are swept in from the sea in this area. It is possible that a high proportion of the organic debris, especially in the lower part of the marsh, is of marine origin and may encourage the growth of marine bacteria.

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Effect of Salinity on the Growth of Certain Salt-marsh Bacteria

A COMPARISON of media made up with sea-water and with river-water (to be published) showed that the former was more satisfactory for the enumeration and isolation of bacteria from certain salt-marsh muds. Samples of saline mud from colonized and uncolonized areas at Gibraltar Point, Lincolnshire, gave rise to 6–90 times as many colonies on ZoBell's sea-water agar medium 2216 (ref. 1) as on a variant of the same medium made up with river-water. Comparable samples of river-mud gave about three times as many colonies on the river-water medium as on the sea-water medium.

The effect of salt concentration on the growth of bacteria isolated from the sea-water agar plates has been investigated. Forty isolations were made from mud samples from each of three areas, namely, the Halimionetum, the Salicornietum and an uncolonized area on the edge of the River Steeping as it crosses the bare mud-flats. Twenty isolations were also made from fresh-water mud from the River Steeping above the level of tidal influence. A few isolates from each group later failed to grow, for unknown reasons. The actual number of isolates from each group which were tested is shown in the tables.

The method used was similar to that used by Tyler, Bielling and Pratt[2]. The test media consisted of 0·1 per cent tryptone (Oxoid) in demineralized water, with the addition of mineral salts to give concentrations equivalent to those in sea-water (sodium chloride, 24·0 gm.; potassium chloride, 0·7 gm.; crystalline magnesium chloride, 5·3 gm.; crystalline magnesium sulphate, 7·0 gm. per litre), and similar tryptone solutions containing the same salts in half, twice, four times and six times the above concentrations. Tyler *et al.* varied the concentration of sodium chloride only; but it was decided in this experiment to vary the concentrations of all the mineral salts since the bacteria in the muds, which are exposed during low tides to the effects of evaporation and dilution by rain-water, would have been subjected to variations in concentration of all the salts.

The bacteria were grown on artificial sea-water—tryptone agar slopes with about 2 ml. of 2·4 per cent sodium chloride solution added. After two or three days incubation at 25° C. the bacterial suspension at the base of the slope was transferred to a sterile spectrophotometer tube and the optical density of the suspension was adjusted to 0·30 ± 0·05 with sterile 2·4 per cent sodium chloride solution. Conical flasks containing 30 ml. quantities of the test media were inoculated with 0·1 ml. of this adjusted suspension.

Growth was measured after two days incubation at 25° C., in terms of the optical density of the cultures at 500 mμ in a Unicam *SP* 1400 prism absorptiometer. The optical densities observed are recorded elsewhere[3]; but the results are summarized in Tables 1 and 2.

Table 1. PERCENTAGE OF ISOLATES CAPABLE OF GROWING AT ANY GIVEN DEGREE OF SALINITY

	Salicornietum mud	Halimionetum mud	River steeping uncolonized mud	Non-saline mud
A.S.W.* × 6	53	2	19	0
A.S.W. × 4	66	30	59	0
A.S.W. × 2	87	95	92	17
A.S.W.	100	97	97	55
A.S.W. × ½	89	92	97	78
Demineralized water	3	22	11	94
No. of isolates tested	38	40	36	18

*A.S.W., artificial sea-water.

Table 2. PERCENTAGE OF ISOLATES SHOWING MAXIMAL GROWTH AT ANY GIVEN DEGREE OR DEGREES OF SALINITY

	Salicornietum mud	Halimionetum mud	River steeping uncolonized mud	Non-saline mud
A.S.W.* × 6	0	0	0	0
A.S.W. × 4	18	0	5	0
A.S.W. × 2	58	17	33	0
A.S.W.	60	60	53	17
A.S.W. × ½	18	47	27	22
Demineralized water	0	0	0	89
No. of isolates tested	38	40	36	18

Note. Where an isolate showed equally good growth at two or more different concentrations of salts, it has been included in more than one set of figures.
* A.S.W., artificial sea-water.

A far larger proportion of the isolates from the Salicornietum, which was frequently flooded by the tide and where the chloride content has been found to reach high values (up to 57 gm. per kgm. of soil-water), grow albeit feebly, at a salinity six times that of artificial sea-water, that is, 53 per cent, compared with 2 and 19 per cent from the other sites. The organisms tested in this manner included pigmented and non-pigmented Gram-negative rods, and eighteen isolates which produced an orange-brown pigment in the centre of the colony after prolonged incubation. Fifteen of this latter type were among the organisms which grew at the highest salt concentrations. In the preceding communication it was noted that these organisms were confined to the more frequently flooded areas of the marsh. Most of the isolates tested grew poorly, if at all, in the medium with no added salts and showed maximum growth in the normal artificial sea-water medium. In contrast, the isolates from the fresh-water site grew best in the medium with no added salts and only half of them grew at all in the normal artificial sea-water medium.

Thus, within a saline area, variations in the salt tolerance of bacterial populations occurred, which may be related to the amount of tidal flooding to which they are subjected. This might be due to either a change in the species or to the selection of salt-tolerant variants of forms common to both environments. Furthermore, adequate comparisons of the numbers of bacteria in saline and non-saline parts of a river cannot be made using a single medium because of the wide range of behaviour of the bacteria in respect of salinity.

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THE GENE-FOR-GENE CONCEPT

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THE gene-for-gene hypothesis, propounded by Flor in 1942[1] to account for genetic interrelationships between flax (*Linum usitatissimum* L.) and flax rust (*Melampsora lini* Desm.) has in recent years been applied to a number of different parasitic systems[2,3]. According to Flor's interpretation, for each resistance gene in flax there is a specific and related gene for virulence in those rusts to which it is susceptible. There is, however, no apparent uniformity among pathologists as to the criteria by which gene-for-gene relationships are identified, or as to the tests by which such relationships can be unequivocally demonstrated. There are several reasons for this: owing to the specificity of the relationship, a gene in either member of the host : parasite system is identifiable only if the specific gene in the other member, with which it interacts, has been previously identified and is available for carrying out the identifications; there is also the fact that the genetic ratios that are obtained in segregating populations, either of the host or of the parasite, are in large part determined by the genotype of the tester stock or strain that is used, with the result that a manofactorial ratio, when it is obtained, need not indicate that the characteristic being studied is determined by a single genetic factor[3].

Along with the difficulties that may be encountered in carrying out genetic analyses for gene-for-gene relationships there is the fact that the gene-for-gene concept has not been clearly defined; and lacking a clear definition it is also difficult to assess the limits within which such relationships are likely to occur, or to recognize systems in which a search for gene-for-gene relationships is likely to be successful. There are systems, for example, in which the parasite is obligate during part of its life-cycle only, and those in which it is alternately obligate on two, and occasionally more, hosts; there are other systems, involving hyperparasitism, that are more complex than these. To meet with such a variety of situations, it is apparent that a clear definition of the gene-for-gene concept is needed. Our attempts to formulate a definition are recorded here.

In formulating our proposed definition we have found it necessary to take into account the processes by which gene-for-gene relationships arise and are maintained in biological systems. In natural populations selection pressure can bring about changes in gene frequencies at certain loci while at the same time existing frequencies at other loci remain unchanged. Given that populations do respond to changes in selection pressure, it follows that where the pressure is specific, and where the population under pressure makes an adjustive response, the response is also specific. The genes for production of penicillinase that are brought forward in penicillin-treated populations, and those for DDT-dehydrochlorinase in DDT-treated populations are cases in point: the action of these genes is specifically directed toward the agency which brought about the change. In the same way, the introduction of new genes for resistance into host populations, either by mutation or through the activities of plant breeders, usually brings about adjustive changes in the parasitic population and the effects of the new genes for virulence that are brought forward in these populations are specifically directed toward the resistance genes that have been introduced. An example of this sort is given by Turner[4], who has provided evidence that the pathogenic specificity of *Ophiobolus graminis* var. *avenae* toward oats is due to its capacity to produce avenacinase, an enzyme which destroys the biological activity of avenacin, which is a glucosidic inhibitor present in the oat plant. Turner, in ascribing pathogenic specificity to this mechanism, directs attention to the fact that a mutation in the parasite which inactivates the host inhibitor would be of great survival value. The survival of such a mutant gene in a parasitic population would, however, be maintained only so long as the genetic conditions in the host that determine its survival value remain unchanged. A gene for virulence in a parasitic population will become ineffective following the subsequent appearance of new resistance genes in the host. In summary: with the introduction of new resistance genes in a host population, specific and related genes are brought forward in the population of the parasite; these remain effective and are maintained in the parasitic population only so long as the related genes for resistance are present in the host.

Since the operation of selection pressure, either in maintaining or in bringing about changes in gene frequencies, is not limited to parasitic systems we have next to consider whether gene-for-gene relationships are likely to arise, through selective processes in other biological situations. A clear example is seen in these situations that involve mimicry, where the genes for colour and pattern which are to be of selective value to the mimic are predetermined by those genes for colour and pattern that have become established in the organism which is mimicked. We are convinced by this, and by other examples, that if the gene-for-gene concept is to be defined solely in terms of selective mechanisms then, owing to the ubiquity of these mechanisms, the concept would be applicable to most if not all biological situations in which organisms come into contact with, and exert selection pressure on, one another. We conclude that the definition must include some further qualification if the gene-for-gene concept is to be stated in such a way that the limits of its applicability are clearly defined.

To date, the gene-for-gene concept has been applied only to parasitic systems. Since parasitism is a form of symbiosis, we have extended our inquiry to include other forms of symbiosis, in order to determine whether the gene-for-gene concept applies to these also. We shall consider here mutualistic symbioses, in which it is considered that both members of the association profit by the presence of the other. In such systems it can be presumed that a mutation in one member that threatens the continued survival of the other, from which it profits, will not have been preserved. On the other hand, a mutation in one member that favours the other can be preserved only if at the same time it favours the organism in which it had occurred. A mutant gene is not likely to persist in an organism that derives no benefit from it. It is seen that genes accumulate in symbiotic systems because they are beneficial to the organism in which the mutations have occurred; the fact that the symbiotic partner also benefits is purely incidental. So far as the gene-for-gene concept is concerned, this illustrates that: (1) a mutation in one organism the sole action of which is to benefit another organism is not likely to persist; for those mutations that do persist, if they benefit another organism they do so accidentally; (2) since the new gene poses no threat to the organism that accidentally profits, it evokes no adjustive response and therefore there is no basis for specific gene-for-gene relationships.

Evidence for lack of genetic specificity between symbiotic partners had been provided by Pant and Fraenkel[5], who have shown that yeast symbionts taken from the gut of two beetle genera, *Stegobium* and *Lasioderma*, can be interchanged; the yeasts retain their morphological and physiological characteristics after being provided with new symbiotic partners. A similar case showing lack of specificity involves certain symbionts of the genus *Triatoma*; these may be transferred to sterile individuals of the genus *Rhodnius*, where they provide the latter with the necessary micronutrients for development[6]. In these cases, at least, there is no evidence of the high order of specificity, based on gene-for-gene relationships, that is so characteristic of parasitism. Quispel[7], in commenting on the relations that exist between symbionts, asserts that "Two organisms don't enter into symbiosis to give something to the partner, but in order to take as much advantage of the partner as possible". To this it can be added that it is only when the advantage taken by one works to the disadvantage of the other that an adjustive response is required, and a gene-for-gene relationship can arise; but when this occurs the symbiosis is no longer mutualistic but antagonistic or, to use the more common description, one organism has become parasitic on the other.

The consideration of symbiosis shows that gene-for-gene relationships arise only when the relationship between the symbiotic partners becomes antagonistic. Symbiosis frequently involves a high degree of intimacy in the association between partners; there can therefore be nothing in the intimacy of the association, *per se*, that necessarily leads to gene-for-gene relationships. The earlier example, mimicry, showed that the processes leading to gene-for-gene relationships can take place even when the relationship between the participants is relatively remote. But the intimacy of the relationship between host and parasite does provide a distinction that may be useful in coming to a precise definition. To take the example given earlier, it is possible to study the genetics of colour and pattern in one organism, and at the same time be quite ignorant of the fact that the pattern being studied is a mimic pattern. With parasitism, however, it is not possible to study resistance to infection independently of the parasite, or to study virulence of the parasite independently of the host. Both organisms must be present in the study. This is a necessary condition, since one and the same morphological expression, the reaction-type, provides the needed information, whether it is the genetics of the host or of the parasite that is being studied. If this is taken into account, a definition can be formulated that does not violate the gene-for-gene concept as it is presently held and at the same time places rather precise limits on its applicability. The concept thus defined would be limited to parasitism. Quispel[7] finds no distinct dividing line between mutualistic and antagonistic symbioses, and points to the fact that any given symbiotic pair can exhibit both kinds of relationship. Where this occurs it is our opinion that the antagonistic part of the relationship will have its basis in gene-for-gene specificities; in fact, we believe that where doubt exists the demonstration of such specificities is likely to be the surest diagnosis of an antagonistic symbiosis.

These considerations have brought us to the following definition of the gene-for-gene concept:

A gene-for-gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population, and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized.

The lack of clear definitions in biology can be expected to lead to confusion. An example relating to Flor's gene-for-gene concept has been given recently by Flangas and Dickson[8], who contend that some sort of structural homology at the sub-genic level is required of the units that interact in a gene-for-gene relationship. We believe, on the other hand, that functional specificities between related genes need neither imply nor require any structural specificities between the genes concerned. Speculations concerning the mechanisms by which related genes interact have therefore been considered irrelevant to the problem of formulating a definition of Flor's gene-for-gene concept.

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LETTERS TO THE EDITOR

ASTRONOMY

Expansion of the Universe

In a recent issue, Dr. Alastair Ward[1] suggests an interesting experiment to check whether or not the universe is expanding. By using the Mossbauer effect we can study photon interactions and attempt to produce an increase in wave-length λ that is proportional to distance r. This would then explain the Hubble law ($\delta\lambda/\lambda \sim r$) as derived from the red shift in the spectra of distant galaxies. But physicists should bear in mind the uncertainties in the astronomical data. For example, it is quite possible that the red shift, and hence the apparent velocity of recession, increases as distance squared.

Fig. 1 shows the most comprehensive collection of red shift values available at the present time for field galaxies[2]. The logarithm of the observed red shift is plotted against the observed bolometric magnitude M which has been corrected for absorption within the local galaxy. Most of the scatter among the points is due to intrinsic differences in absolute brightness and random motion of galaxies. There are many types of galaxies from *E0* to *Sc*, and each type has a characteristic magnitude. Deviations also occur in the brightness of galaxies within a single classification.

In a flat space-time with no intergalactic absorption the apparent magnitude M_0 of a galaxy at a distance r kiloparsecs is given by:

$$M_0 = M_{\text{abs.}} + 10 + 5 \log r \qquad (1)$$

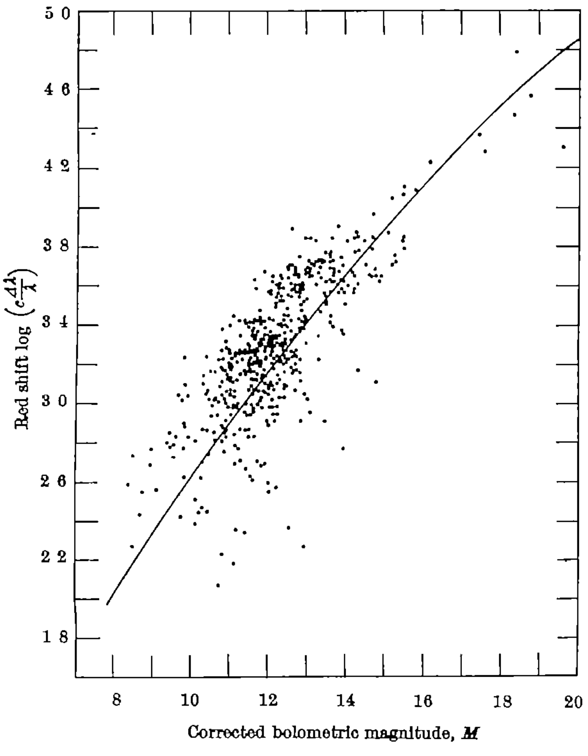

Fig. 1. Red shift for field galaxies

where $M_{\text{abs.}}$ is the absolute magnitude of the galaxy. As a first approximation we may follow Humason, Mayall and Sandage[2] and assume that $M_{\text{abs.}}$ is the same for each field galaxy. We would then expect to find the relation:

$$M = 5 \log \frac{c\delta\lambda}{\lambda} + \text{constant} \qquad (2)$$

If a least-mean-square solution of the form of (2) is fitted to the points in Fig. 1 then a gradient of 3·03 is obtained. If the analysis is restricted to the 430 galaxies with magnitudes brighter than + 14 (91 per cent of the sample) then the line of best fit is:

$$M = 2{\cdot}26 \log \frac{c\delta\lambda}{\lambda} + 4{\cdot}63 \qquad (3)$$

A gradient of 5 corresponds to a linear law while a gradient of 2·5 corresponds to a quadratic law. Thus the measurements for field galaxies are more consistent with a quadratic law, and in particular (3) gives:

$$\frac{\delta\lambda}{\lambda} \sim r^{2{\cdot}21} \qquad (4)$$

A linear law can only be obtained from these data by postulating that systematic biases, such as selection effects, exist, and by weighting the observations in some way so as to remove the suspected bias.

Intergalactic absorption may also be present, dimming the galaxies and displacing the points to the right. A galaxy of absolute magnitude $M_{\text{abs.}}$ will be dimmed by δM, where:

$$\delta M = \alpha \log_{10}^{-1} \{0{\cdot}2(M_0 - M_{\text{abs.}} - 10)\} \qquad (5)$$

and where α is the absorption coefficient in magnitudes per kiloparsec. In the Milky Way the interstellar density is 10^{-24} gm. cm.$^{-3}$ and the dust produces a coefficient of $\alpha = 1$. Between the galaxies[3] the density of material may be as high as 10^{-27} or 10^{-28} gm.cm.$^{-3}$ and values of $\alpha \sim 10^{-4}$ might be expected. The theoretical curve in Fig. 1 corresponds to a red shift which increases as r^2 and with a slight amount of absorption present ($\alpha = 6 \times 10^{-5}$ mag./kpsc.). The absolute magnitude[2,4] of a field galaxy was taken as $-$ 20·07. It can be seen that the theoretical curve is a tolerable fit to the data. Humason, Mayall and Sandage[2] have pointed out that more significance can be given to the points in the upper half of the diagram since these represent the larger galaxies and there is less likelihood of observational selection taking place. The theoretical curve forms a satisfactory envelope for these points when displaced to the left by 2·0 magnitudes to allow for the increased absolute magnitude of the brightest galaxies.

The curve may also be tested with the data from clusters of galaxies. Humason, Mayall and Sandage have given the red shift measurements for a number of clusters as shown in Fig. 2. The magnitude corresponds to the brightest cluster member and for the brightest member Humason, Mayall and Sandage[2]

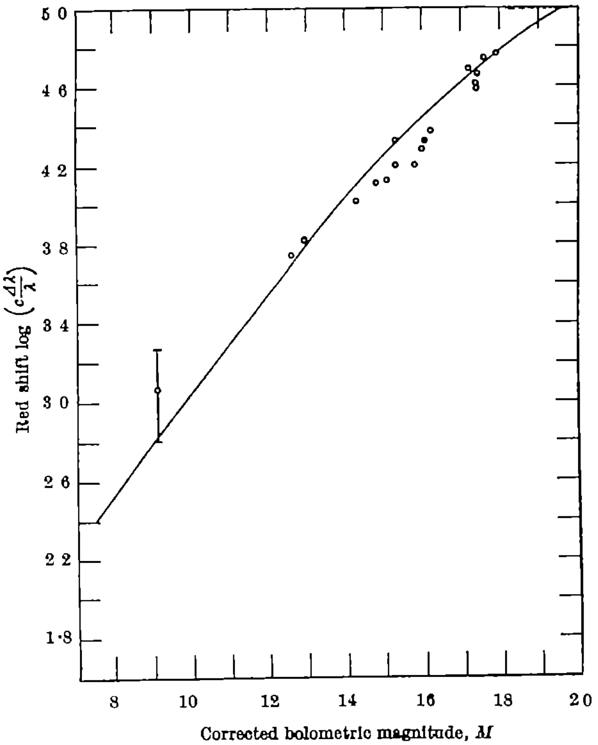

Fig. 2. Red shift for brightest members of clusters

and Sandage[4] give $M_{\text{abs.}} = -21{\cdot}64$. The curve was derived using the same values of density and absorption as for the field galaxies ($\alpha = 6{\cdot}0 \times 10^{-5}$ mag./kpsc., $\rho = 6{\cdot}6 \times 10^{-27}$ gm.cm.$^{-3}$). A bar has been placed on the point for the Virgo cluster to indicate the standard deviation of the red shift among the members of the cluster.

Thus it is premature to assume that the Hubble law is linear and that the apparent velocity of recession increases proportionally with distance. Indeed a quadratic law adds weight to the argument of a non-expanding universe and increases the need for interpreting the red shift as due to some cause other than the Doppler effect. If $\delta\lambda/\lambda \sim r^2$ in all directions of observation then a Doppler interpretation puts us at the origin of a parabolic velocity field and leads towards the unpalatable conclusion that the Milky Way is at the centre of the universe ! A quadratic law is best interpreted in terms of a static universe where the red shift is caused by some propagation effect. Mechanisms similar to the photon interaction proposed by Dr. Ward are interesting in this respect. Alternatively a quadratic law can be derived from the formulation of general relativity[5-7] using a static model as proposed by the author.

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PHYSICS

Hydromagnetic Heat Transfer

Consider the convective transfer of heat between an electrically conducting viscous fluid (of density ρ, shear viscosity μ, thermal conductivity $\varkappa$, specific heat at constant pressure C_p, and finite electrical conductivity σ) and a boundary surface at a different temperature and with characteristic length L, possibly associated with a complex geometry, in the presence of a steady externally applied magnetic field of magnitude B. A full mathematical analysis of general validity for this situation is still not available and it is useful to consider elementary dimensional arguments in this connexion. It will be seen that such arguments provide the possibility of determining the gross electrical conductivity of a continuous fluid in terms of a chosen standard fluid.

For forced convection, let the fluid be caused to move past the surface with steady speed v. The boundary temperature will be assumed to be greater than that of the main fluid by an amount $\Delta T (\neq 0)$; but the opposite situation could equally well apply. Difficulties over the detailed specification of ΔT will be avoided in the usual way by using the logarithmic mean temperature difference across the boundary layer region.

If a quantity of heat q passes from unit area of the boundary into the fluid per unit time then dimensional analysis leads readily to a functional relation between the relevant parameters of the flow. Defining the Nusselt number, Nu, Reynolds number, Re, and Prandtl number, Pr, by:

$$Nu = \frac{qL}{\varkappa\Delta T}, \; Re = \frac{\rho v L}{\mu}, \; Pr = \frac{\mu C_p}{\varkappa} \qquad (1)$$

and the dimensionless grouping $\xi = \frac{B^2\sigma L^2}{\varkappa\Delta T}$ (Gaussian units), then it follows that:

$$Nu = f_1(Re) f_2(Pr) f_3(\xi) + C \qquad (2)$$

where f_1, f_2, f_3 are three as yet undetermined functions. C is a constant associated with conductive heat transfer, and can be regarded as remaining independent of the magnetic field to very good approximation. Equation (2) then implies the condition $f_1(Re) \rightarrow 0$ as $v \rightarrow 0$, since in this limit $Nu = C$. Some information about the limiting form of f_3 for vanishing ξ is easily deduced when it is noted that $B^2\sigma L^2$ refers to the fluid-magnetic field coupling. When this coupling is zero (that is, $B = 0$) the heat transfer is purely hydrodynamic so that $f_3 (\xi) \rightarrow 1$ as $\xi \rightarrow 0$. Although it is unphysical in the present context it can be noted that if the coupling were ever complete (that is, $B^2\sigma L^2 \rightarrow \infty$) the fluid and field would be frozen together, the heat transfer then being by conduction relative to the field.

In the limit $\xi = 0$, empirical knowledge of equation (2) is already available[1]. Thus it is known that now many situations of practical interest are well described by the substitution $f_1(Re) f_2(Pr) = D(Re)^n (Pr)^m$, where (D, n, m) are flow parameters. For liduiq metals[2] $Pr \sim 10^{-2} - 10^{-3}$, $n = m$ and C in equation (2) is of the order 10; for turbulent conditions $D \sim 10^{-2}$ and $n \sim 3/4$. Making the reasonable assumption that the separation of the functions f_1 into independent hydrodynamic and magnetic contributions is valid, and introducing the Peclet number,

Pe, according to $Pe = Re \times Pr$, then equation (2) can be written generally:

$$\frac{Nu - C}{D(Pe)^n} = f_3(\xi) \qquad (3)$$

for hydromagnetic laminar or turbulent conditions.

Measurements of heat transfer for a known geometrical and magnetic arrangement allow the data $f_3(\xi)$ as function of ξ to be determined for a chosen fluid, which could conveniently be mercury. With the function f_3 known for one representative fluid this information is applicable to other fluids within the continuum approximation, and under comparable heat transfer conditions. If the geometry and magnetic field are known then ξ can be determined for some other fluid of interest by making heat transfer measurements and using the empirical data for f_3 in equation (3). By this method, equation (3) can be used to determine σ for one fluid in terms of a standard fluid; the method may have some merits in connexion with fluids that are difficult or dangerous to handle, since equation (3) can be applied to a closed loop system.

The same approach can be applied to natural convection where the gravitational acceleration g and the fluid density volume coefficient of expansion β are relevant while the fluid velocity is not. In this case Re is replaced by the Grashof number $Gr = \dfrac{\beta \Delta T g L^3}{\nu^2}$ and Pe by the Rayleigh number $Ra = Gr \times Pr$.

Finally, the fluid may be in steady rotation with angular speed Ω. In this case the right-hand side of equation (2) is multiplied by the additional function $f_4(Ta)$, where $Ta = \dfrac{\Omega L^2}{\nu}$ is the Taylor number. For free convection without a magnetic field f_4 has been found experimentally[3] in one instance to be reducible to a linear form if L is suitably defined, but almost no general data are at present available.

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GEOLOGY

Age of the Cuddapahs, India

IN discussing the age of the Cuddapahs, India, Dr. U. Aswathanarayana[1] includes lead isotope analyses of the Cuddapah galenas.

The extremely low lead-207/lead-204 ratios of these samples in comparison with their other ratios have attracted our attention since they are not readily explained by any of the now accepted lead isotope models with parameters that are in the range of those usually used. When we brought these unusual abundances to his attention, Dr. Aswathanarayana sent us his original spectrograms so that we could carry out our own measurements on them. We did this with results that were substantially different from those obtained in India, as can be seen here. It is to be emphasized that our values are not from our own measurements but are merely recomputations of some of the Indian spectra.

Table 1. ZANGAMRJUPALLE GALENA (WALTAIR MEASUREMENTS)

Calculated by	^{206}Pb/^{204}Pb	^{207}Pb/^{204}Pb	^{208}Pb/^{204}Pb
Aswathanarayana	15·86	14 49	34·70
Russell and Ostic	16·0	15 1	34 8

We have made considerable effort to try to determine the nature of the discrepancy between the two calculations, which results primarily from the different way in which base lines are drawn under the peaks at the two laboratories. It appears that the smaller resolution of the Waltair instrument exaggerates the effects of tailing of the peaks due to scattering of the ions in the analyser tube. It has been our practice to apply corrections for this tailing, which for our own spectrograms can change the results by as much as 0·5 per cent. To make this correction three numbers are required. These are the proportions of a peak observed at distances from its centre equivalent to one-half mass, one mass and two mass units respectively. These values vary approximately proportionally to the pressure in the analyser tube and may change with variations in the source conditions such as caused by the replacement of a filament, etc. Even with good resolution it is quite difficult to estimate the magnitude of these correction factors. However, we have tried to estimate representative values from lead tetramethyl spectra we have available from different laboratories. These estimates are given in Table 2 with sufficient other information about each spectrum to indicate the importance of adequate resolution in carrying out this type of measurement.

The way in which these terms enter into the calculations depends, of course, on the way in which base lines are drawn under the peaks. We draw the base line under the 249 mass by joining the minima on either side of it with a straight line. The base lines for the three major peaks are drawn by joining, with a straight line, the corresponding base lines measured well beyond the limits of the trimethyl group of the spectrum. This is similar to the procedure carried out at the University of Toronto. With this measuring procedure the ratio lead-207/lead-204 is that most critically affected by the tailing correction, the correction amounting to not less than the tailing at half mass plus twice the tailing at one mass distances. This figure is also given in Table 2 although we do not imply that any measurements are in error by the amount listed, since judiciously drawn base lines can allow for much of the tailing, particularly under the mass 249 peak.

We direct attention to this source of uncertainty in these measurements because, as Dr. Aswathanara-

Table 2. ORDER OF MAGNITUDE OF TAILING CORRECTIONS ESTIMATED FOR MASS SPECTROMETERS OF DIFFERENT RESOLUTION

Laboratory	Resolution	Collector slit width	Beam width at collector	Estimated tailing $m + \frac{1}{2}$	$m + 1$	$m + 2$	Correction of ^{207}Pb/^{204}Pb (per cent)
British Columbia	470	$1·7 \times 10^{-3} R$	$0·4 \times 10^{-3} R$	~ 0 002	~ 0·001	~ 0 0002	~ 0 4
Toronto	310	$2\ 3 \times 10^{-3} R$	$0\ 9 \times 10^{-3} R$	~ 0·01	~ 0·002	~ 0·0004	~ 1 4
Andhra	290	$2·0 \times 10^{-3} R$	$1\ 5 \times 10^{-3} R$	~ 0·02	~ 0·005	~ 0·0007	~ 3·0

R, Radius of curvature of the instrument.

yana points out, these samples may have an important bearing on understanding the geochronology of India, and the uncertainty in isotopic composition suggested by the present comparison is equivalent to a factor of two difference in age of the two samples as calculated in the paper cited. (We also contend that the calculation of the age of galenas by any technique requires some *a priori* evidence that the sample dated can be expected to satisfy the model used, but this is another matter.) Moreover, the analyses originally quoted suggest the primary development of lead isotopes in terrestrial systems having a uranium/lead ratio which we feel to be improbably low. We are also anxious to direct attention to the importance of pressure scattering as a source of uncertainty in lead isotope analyses since it does not seem to have been given sufficient attention by the laboratories carrying out such measurements.

We thank R. G. Ostic and E. R. Kanasewich for carrying out a number of calculations related to the present discussion and for their critical comments on this manuscript.

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Russell and Slawson have clearly brought out, presumably for the first time, the significance of the pressure-scattering correction which may change the lead-207/lead-204 ratio by about 3 per cent when low resolution mass-spectrometers are used for the isotopic analysis of lead. This correction, coupled with differences in the mode of measurement of peak heights, is responsible for the discrepancies in lead isotope ratios given by Russell and Slawson and those given by me[1]. It is possible that the correction may improve the accuracy of the lead isotope ratios, but this requires to be corroborated. The objective of this communication is to discuss the conclusions that flow from the revised model age of the Zangamrajupalle galena.

The corrected isotopic constitution of the lead of Zangamrajupalle (see preceding communication) (lat. 14° 45′ 22″ N.; long. 78° 51′ 33″ E.) galena, which occurs as veins in the Cumbum shales of the Upper Cuddapahs, and its model ages are given below in Table 1.

Table 1

^{206}Pb/^{204}Pb	^{207}Pb/^{204}Pb	^{208}Pb/^{204}Pb	Model ages (m.y.)		
16·0	15·1	34·8	1,400*	1,460†	1,470‡

* ^{206}Pb/^{204}Pb and ^{207}Pb/^{204}Pb age—Houtermann's formula, constants of Russell and Farquhar (ref. 2).

† ^{206}Pb/^{204}Pb and ^{207}Pb/^{204}Pb age—Houtermann's formula, constants of Moorbath (ref. 3) (read from the unpublished tables (No. 5) of R. P. C. Pockley).

‡ ^{208}Pb/^{204}Pb age—formula of Russell, Farquhar and Cumming (ref. 4).

The revised model age (1,400–1,470 m.y.) of the Zangamrajupalle galena has far-reaching implications. Thus the Cuddapahs may not be the time-equivalents of the Delhis, since the post-Delhi pegmatites were found to be 735 m.y. old[5]. This view has been advocated by Holmes[5]. The Cuddapahs may belong to the end phase of the Eastern Ghats cycle (1,600 m.y.) and the period immediately following[5,6]. As pointed out by Holmes[5], the two may be related in the same way as the Indo-Gangetic alluvium is related to the Himalayas.

It must be emphasized that the foregoing conclusions are tentative and require to be substantiated by further work, which is in progress.

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Occurrence of Graywacke in the Lower Siwaliks, Simla Hills

SIKKA *et al.*[1] have reported the occurrence of graywacke in the Nahan Series (Lower Siwaliks) near Kalka, Simla Hills, Panjab. The rock has been described as "a grey to greenish micaceous 'sandstone' type" in the hand specimen which in thin sections is seen to consist of "angular grains of quartz (approximately 50 per cent) embedded in a matrix composed of fragments of slate, schist, quartzite, chert, felspar, chlorite and authigenic sericite". The authors appear to be of the opinion that the presence of this rock type in the Siwaliks casts doubts on their fresh-water origin since "it is generally accepted that the graywackes are characteristic of the geotectonic belts".

It is well known that the two distinctive features of a graywacke are its texture and composition. The authors made no precise mention of the texture of the rock although, according to Pettijohn[2], "it alone distinguishes graywacke from all other sandstones". The description of the composition of the rock is also confusing since rock fragments and felspar grains have been regarded as the constituents of the matrix. It is clear that rock fragments and felspar grains do not constitute the 'matrix' but form an integral part of the framework of the graywackes. Since the grain/matrix ratio is important in defining these rocks, it is essential that the amount of 'true' matrix is indicated separately. If this distinction is not clearly brought out, the graywacke proper cannot be distinguished from the sub-graywackes which have a different origin and geologic significance. From the description given by Sikka *et al.*[1], it is not at all clear whether the rock is a graywacke or a sub-graywacke.

Krynine[3] had noted the occurrence of graywacke in the Siwaliks of north-western India and considered them non-marine. It is interesting to note, however, that the same author redefined the term in 1945[3] and created two types of graywackes—low-rank and high-rank. The low-rank graywacke of Krynine so far as composition is concerned is equivalent to the sub-graywacke as defined by Dapples *et al.*[4] and Pettijohn[5].

It is more than likely, therefore, that much of the so-called graywacke of the Siwaliks may, in fact, turn out to be sub-graywacke (the low-rank graywacke of Krynine) if a proper petrographic study is made. If this is the case, the notion that the Siwaliks are entirely of fresh-water origin may still be true because the sub-graywackes are neither necessarily marine nor are they characteristic of the geotectonic belts.

The presence of cross-bedding and ripple-marks, the moderate sorting of the Siwalik sandstones and their profuse richness in plant remains (even in the Nahans themselves) and in the mammalian fauna of the Upper Siwaliks suggest their deposition under shallow water conditions. Indeed their nature and mode of formation are identical with those of the sub-graywackes and they may well have formed on flood plains or in deltas in the coastal plains. The presence of red shales and numerous bands of conglomerates also supports the existing view that they were deposited under subaerial conditions. Moreover, in the vast thickness of the Lower Siwaliks not a trace of marine fossils has been found, nor any other evidence suggestive of geosynclinal conditions. The molluscan fauna wherever found suggests fresh-water conditions.

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A Pedunculate Cirripede from the Upper Silurian of Oesel, Esthonia

The discovery of a pedunculate cirripede in Silurian rocks carries the history of that order a long way back; for until now the earliest record of a pedunculate is from the Rhætic, and that of the goose-barnacles (Lepadidae) is from the Pliocene. Some fifty of these fossils were discovered when etching out the chitinous skins of *Eurypterus fischeri* Eichw. from the Upper Silurian limestones of the Island of Oesel in Esthonia. Some were still attached to the legs of the *Eurypterus*, clustering on them as do the young *Lepas* on bits of sponge, cuttle-bone, etc.

I name this fossil *Cyprilepas holmi* gen. et sp. nov. (Fig. 1). As the figure shows, there is a capitulum of two valves united on one side and resembling the shell of the Recent ostracod *Cypris* and of the *Cypris*-larva of all cirripedes; and a cylindrical peduncle with one or more annular constrictions and, in a few cases, with a terminal attachment-disk. Their total length ranges from 2·2 mm. to 4·8 mm. The chitin is virtually structureless and devoid of growth-lines; but in two cases it displays a small patch of hexagonal markings quite similar to those left on the chitinous covering of the Recent goose-barnacle *Lepas* when its calcareous plates have been dissolved. This may be evidence of the existence of a pair of calcareous plates before the fossil was etched from the rock; but there is nothing to suggest that there were five plates, as in *Lepas*. The chitin of the stalk appears, in some examples, to be covered with minute papillæ. This feature and the terminal disk can both be matched in very young post-larval *Lepas*.

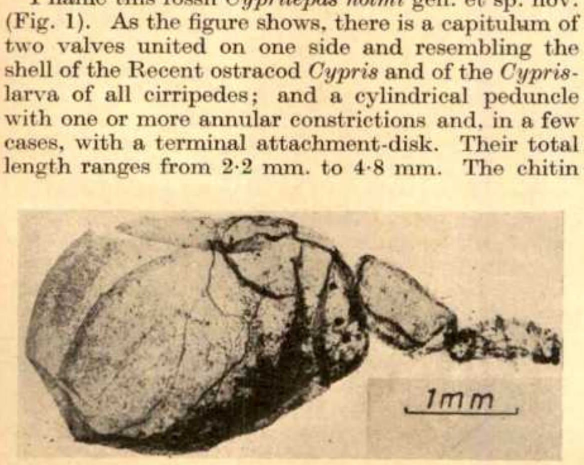

Fig. 1. *Cyprilepas holmi* gen. et sp. nov.; the holotype, *B.U.*743

Duplicates are available for distribution to interested individuals and museums.

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GEOCHEMISTRY

Nitrate and Iodate in Antarctic Salt Deposits

Antarctic salts have been described by Ferrar[1], Ball and Nichols[2] and several other authors, but nitrates and iodates have not previously been reported. Although Jensen's[3] analyses showed small amounts of nitrogen, presumably in ammoniacal form, he specifically mentions the absence of nitrate. He considered this deficiency due to nitrifying bacteria being unable to survive in the cold climate, and believed it to be an important factor in preventing plant life.

Salt pans, efflorescent crusts on rock faces, and salt layers within the moraine were sampled by the 1959–60 Victoria University Antarctic Expedition during its study of the 'ice free' Victoria Valley region[4] (situated opposite Ross Island in the Victoria Land mountains). We report here the presence of considerable amounts of nitrate and iodate in two samples obtained 30 miles west of McMurdo Sound.

The salts contained some acid-insoluble mineral matter, but consisted largely of calcium sulphate and carbonate, and sodium sulphate, nitrate and chloride either as single salts or as mixtures in various combinations.

Of the eighteen samples examined, all showed nitrate in quantities ranging from a trace to as much as 90 per cent sodium nitrate in one sample. The analysis of this latter sample is given in Table 1. Nitrate, first identified by optical methods, was later determined by the phenoldisulphonic acid method, with the estimation being made spectrophotometrically.

Table 1. Analyses of Salts containing High Iodate and Nitrate Respectively

Victoria University of Wellington sample No.	Location	Description	Composition (per cent)	
10567	¼ mile south-west of Lake Vashka	Surface sample from salt-filled depression	$CaSO_4.2H_2O$ $CaCO_3$ $NaIO_3$ $NaNO_3$	85 14 0·13 trace
10569	3 miles south of Lake Vashka on Range west of Balham Valley (ref. 4)	Encrustation on Beacon sandstone cliff	$NaNO_3$ $CaSO_4.2H_2O$	90 10

The important 'caliche' deposits of Chile have not yet been adequately explained despite the many theories advanced[5]; the existence of nitrate in the Antarctic is even harder to explain. Iodate is associated with the nitrate in the Chile deposits but is also found with other salts in Victoria Valley.

Iodate to the extent of 0·13 per cent sodium iodate was found in one sample (Table 1) which consisted mainly of calcium sulphate, with small amounts of calcium carbonate and traces of sodium chloride and nitrate. Another specimen composed largely of sodium sulphate contained 0·02 per cent sodium iodate. Waters from the nearby Lake Vashka also contain iodate.

The iodate was determined by addition of potassium iodide to an acid extract of the salt, followed by titration with standard phenylarsenoxide, using starch as the indicator. Iodate was established as the oxidizing agent by reduction with sulphite, followed by nitrous acid oxidation, when extraction with chloroform gave the characteristic coloration for elementary iodine.

An explanation of the existence of iodine in an oxidized form can be made on thermodynamical grounds, since at neutral *p*H values the atmospheric oxygen-iodine potential is such that some oxidation may occur, and subsequent hydrolysis will give iodate as the stable form[6], as is found in sea water[7-9].

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Uranium in Recent Japanese Sediments

Considering the amount of work that has been done concerning the uranium content in recent marine or lacustrine sediments, especially during the past decade, the available data are rather less than for uranium of rocks or mineral assemblages[1-3].

Very little is known about the uranium content of sediments from various environments in Japan. The primary purpose of an investigation that we recently made was to determine the uranium in such sediments, but with regard to non-contaminated areas, and thus to obtain a figure for the ground-level of uranium for natural environments in Japan.

Samples of sediments, of which two were marine, three from inlets, four sections of a core obtained from a brackish lake, one from an oilfield and one from a gasfield, were examined. The locations of the samples are shown in Fig. 1. The microquantities of uranium present in the samples were determined colorimetrically by complexing with dibenzoylmethane[4,5].

Uranium was isolated from the leached solution of 1 or 2 gm. of sediment with a liquid–liquid extraction of ethyl acetate, followed by treatment with anion exchange resin ('Amberlite *IRA*-400', 80–100 mesh) which was used in a column of 8 mm. diam. and 5 cm. long. The uranium was afterwards eluted with 1 *M* perchloric acid, and the effluent evaporated to dryness.

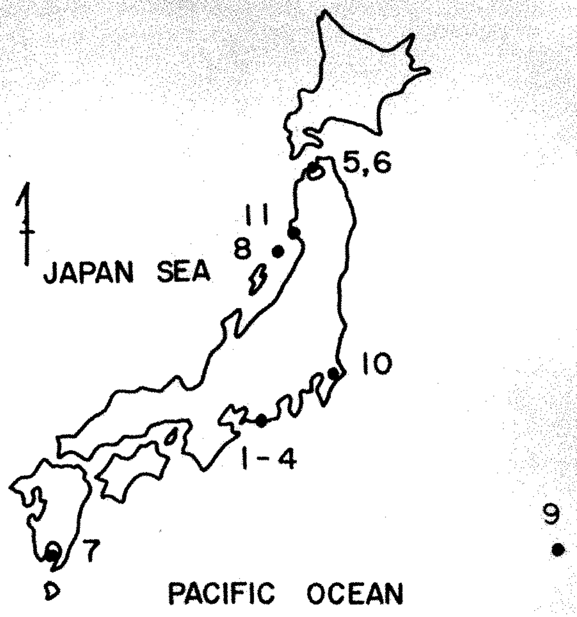

Fig. 1. Location of sediments sampled

The small residue of uranium resulting from the chemical separation was dissolved in nitric acid and analysed by measuring the maximum absorbance at 420 mμ of the complex formed with dibenzoylmethane.

The results are summarized in Table 1. It can be seen that the sediments from Lake Hamana (samples No. 1–4) and Kagoshima Bay (sample No. 7) show higher values of uranium than the others. This discrepancy is probably due to the fact that the bottom layers of Lake Hamana and of the Kagoshima Bay present reducing conditions, so that tetravalent uranium is quickly adsorbed or precipitated as a hydrolysate, and accumulated in the natural environment.

Table 1. Uranium in Recent Japanese Sediments

No.	Sample remarks	Water depth (m.)	Uranium (μgm./gm.)
1	Lake Hamana St. 4, No. 3	6	$14{\cdot}2 \pm 0{\cdot}3$
2	No. 4		$17{\cdot}9 \pm 0{\cdot}4$
3	No. 5		$8{\cdot}3 \pm 0{\cdot}2$
4	No. 6		$18{\cdot}1 \pm 0{\cdot}4$
5	Mutsu Bay *E*-16	50	$2{\cdot}6 \pm 0{\cdot}0_5$
6	*E*-25	50	$2{\cdot}1 \pm 0{\cdot}0_4$
7	Kagoshima Bay St. 10	200	$8{\cdot}7 \pm 0{\cdot}2$
8	Japan Sea (off Sakata)	200	$3{\cdot}5 \pm 0{\cdot}0_7$
9	Pacific red clay	6,215	$1{\cdot}3 \pm 0{\cdot}0_3$
10	Tohgane gasfield	—	$1{\cdot}7 \pm 0{\cdot}0_3$
11	Funakawa oilfield	—	$3{\cdot}7 \pm 0{\cdot}0_7$

However, fairly constant values of uranium were obtained in samples from elsewhere in Japan. Kulp *et al.*[6] summarized the uranium values of various types of sediment as follows: shelf, hemipelagic and pelagic, containing 3·0, 2·5 and 1·0–1·8 μgm. of uranium per gram of sediment respectively.

In comparison with the uranium content of average igneous rocks (3·5 μgm./gm.)[1], the uranium contents of recent Japanese sediments are slightly less, but are of the same order of magnitude as those in the classification given by Kulp *et al.*

An approximate estimation of the uranium values of recent Japanese sediments is given as an average of

2·5 p.p.m. (excluding those sediments with an extraordinary content of uranium).

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BIOPHYSICS

Mechanism of the Reaction of Polynucleotides and Hg^{II}

The reversible reaction of deoxyribonucleic acid (DNA) and Hg^{II} has been known for some nine years[1] during which it has been studied by a wide variety of techniques[1-4]. All the studies concur in demonstrating that the binding is quite strong but can nevertheless be completely reversed by a variety of common chemical reagents which complex Hg^{II}. After reversal the DNA is both structurally and functionally intact despite the large changes in physical properties which accompany and characterize the reaction. Initially thought to be a reaction which occurred primarily with phosphoryl groups[1] it was later convincingly suggested by Thomas[2] that the reaction occurred primarily at the bases. A few years later this observation was confirmed and extended to a greater variety of polydeoxyribonucleotides[3,5] and most recently to the polyribonucleotide of tobacco mosaic virus[6].

The mechanism of the reaction has remained a puzzle, however, in spite of many suggestions. The purpose of this communication, therefore, is to outline briefly a step-function mechanism which can incorporate the known facts and is capable of predicting many as yet unknown experimentally.

In their recent work Yamane and Davidson[5] showed that H$^+$ is released when Hg^{II} reacts with DNA. They examined the hydrogen ion release as a function of added increments of Hg^{II} and were able to obtain plots of $\Delta H^+/\Delta Hg^{++}$ as a function of r for several kinds of DNA as well as *A–T* polymer, a synthetic polydeoxyribonucleotide. The quantity ΔH^+ is the average release of hydrogen ion when a small quantity ΔHg^{++} is added to a solution of a polynucleotide^{II}. The quantity r is the number of moles of Hg^{II} added per mole of nucleotide.

The mechanism assumes that all or nearly all the Hg^{II} added up to $r = 0{\cdot}5$ reacts to form 2 : 1 base—Hg^{II} complexes; that the complexes bridge the double strands of the DNA helix; that Hg^{II}—(thymidine)$_2$ complexes are much more readily formed than any of the other possible 2 : 1 compounds; that a chain shift induced by Hg^{II} occurs such that complementary bases opposite each other in the original unperturbed structure are displaced by an Hg^{II} bridge to the nearest (opposite) neighbour and that as a first approximation the bases in the natural DNA's are distributed as random along the chain. Knowing the formulæ of all the 1 : 1 nucleoside—Hg^{II} complexes[6] it is not hard to make reasonable formulæ for all the 2 : 1 nucleoside complexes (the 2 : 1 adenosine—Hg^{II} complex has been reported[6]) and with these and the assumptions given above to construct step functions to fit the curves of Yamane and Davidson. Fig. 1 shows an example of the fit of such a function to the data for *E. coli* DNA. The curves for calf-thymus and *M. lysodeikticus* DNA's and *A–T* polymer can be similarly constructed. The positions and extents of the plateaux and abrupt drops which are obvious in the curves of Yamane and Davidson find a natural and general explanation in the mechanism. In addition, the mechanism lends itself readily to the more elaborate treatments of 'binding' available[7]. It is already sufficiently successful quantitatively to permit the prediction that the Hg^{II} reaction of polynucleotides can be used in fundamental studies of nucleotide sequence and in determination of the base composition of polynucleotides. These and other ramifications will be discussed at length elsewhere.

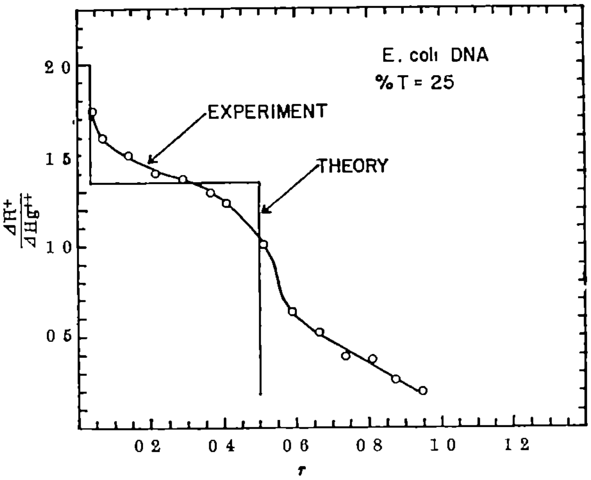

Fig. 1. The fit of a step-function theory to the data of Yamane and Davidson

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CHEMISTRY

A Transparent Semiconducting Indicator Electrode

During the course of an investigation of the photosensitivity of silver halide precipitates, the need for a transparent, conducting electrode sensitive to halogens became evident. A new practical solution to the problem has been found by the use of thin

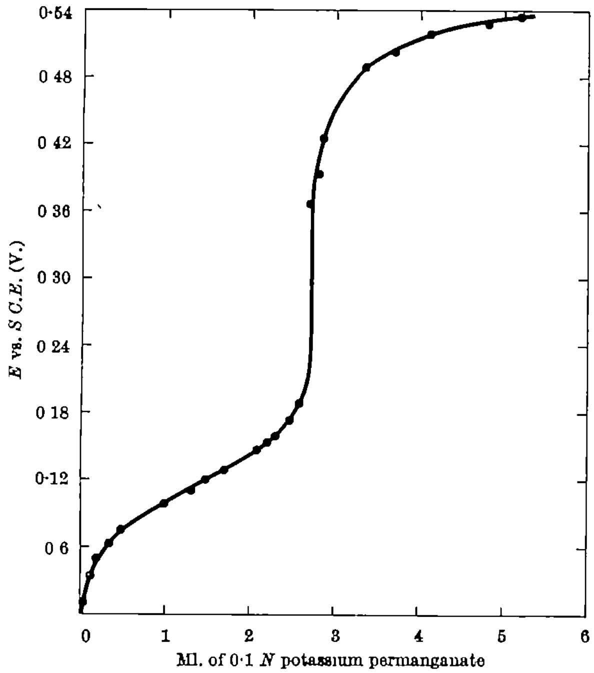

Fig. 1. Titration of 10 ml. of 0 0269 *N* ferrous sulphate with 0 1 *N* potassium permanganate

layers of stannic oxide coated on glass (*NESA* *) as an indicator electrode.

The electrode is prepared from a piece of *NESA* glass 4 cm. × 2·5 cm. in area and 3 mm. thick; the semiconducting layer on one surface is 0·05–0·5μ thick and has a specific resistivity of about $2{\cdot}5 \times 10^{-4}$ ohm-cm. Since stoichiometric tin oxide ordinarily has a far higher specific resistivity, the layer probably contains added impurities; for example, layers of stannic oxide containing antimony oxide, deposited on glass, are known to exhibit low specific resistivity[1]. Silver paste is applied over the semiconductor layer at the upper end to facilitate good electrical contact, by means of an 'alligator' clip, with the external circuit. The lower half of the electrode is partially immersed in the solution to be titrated; the reference electrode is either platinum sheet connected to the titration solution by a salt bridge or a fibre-tipped saturated-calomel electrode. Changes in potential are measured by a Keithley 200*B* vacuum-tube voltmeter.

The electrode is highly sensitive to halogen solutions added to 0·001 *M* potassium halides, acquiring a positive potential with respect to the reference electrode. At bromine concentrations from $2{\cdot}5 \times 10^{-6}$ to 8×10^{-5} *M*, the potential at the stannic oxide electrode was found to vary linearly with the square root of the bromine concentration. Small, but distinct, break points in plots over a larger concentration-range suggest the formation of charged polynuclear halide species, or of hydrolysis products which contribute to the potential. The latter is a distinct possibility since hypobromite ion was found to yield a potential at the electrode. At a given concentration of halogen, the potential decreased in the order: $Cl_2 > Br_2 > I_2$. The order is similar to that for the oxidation potentials of halogens referred to the normal hydrogen electrode.

The electrode was highly sensitive to the following ions: MnO_4^-, Ce^{++++}, $Cr_2O_7^{=}$, $S_2O_3^{=}$, Ti^{+++}, Tl^+ and NO_2^- ions. These observations suggested the feasibility of the stannic oxide as an indicator electrode for potentiometric investigation of oxidation-reduction reactions.

Fig. 1 shows a titration of ferrous ion with standard permanganate ion in 1 *N* sulphuric acid solution. On addition of the titrant, electrode equilibrium was rapidly established. It can be seen that the rate of change of potential at the equivalence point is large, enabling a ready determination of the end-point. To illustrate further the utility of stannic oxide as an indicator electrode, iodine was titrated by standard thiosulphate ion. Rapid changes of potential also occurred at or near the equivalence point for the titration. However, on the addition of excess thiosulphate ion, the electrode responded sluggishly and came slowly to equilibrium.

The results seem to show that stannic oxide can be utilized as an indicator electrode and that the properties of transparency in the visible region, reproducibility and durability suggest a variety of uses. For example, in certain circumstances, such as oxidation-reduction in concentrated chloride solutions, stannic oxide might be superior to platinum as the indicator electrode, for the formation of $PtCl_4^{=}$ tends to make the latter inaccurate[2].

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Hydrogen–Deuterium Exchange on Iso-structural Sesquioxides of the First Long Period

Investigations of the relationship between the electronic structure and the catalytic activity of transition metal oxides have tended to centre around either the electron (or positive hole) concentration[1] or the population of atomic *d* orbitals[2]; but there have been no attempts so far to follow up in this field the newer studies on electrical and magnetic properties which reflect the extent of *d* orbital overlap in the oxides of the first long period[3,4]. Moreover, although it is now widely recognized that the energy-levels of transition metal ions in solids are strongly influenced by the symmetry of the crystal field, there are no correlative studies of catalytic activity in which this important parameter has been standardized. It is for these reasons that we have undertaken a short study of the H_2–D_2 exchange on sintered specimens of titanium, vanadium and chromium sesquioxides. The ions Ti^{3+}, V^{3+} and Cr^{3+} follow in the sequence d^1, d^2 and d^3, and in the almost cubic field of the corundum lattice (which is common to all three oxides) one would expect the progressive filling of the degenerate $d\varepsilon$ set to ensure a gradual decrease in the amount of orbital overlap along this sequence.

Ti_2O_3, V_2O_3 and Cr_2O_3 are immune to bulk reduction by hydrogen, so the H_2–D_2 exchange can be examined at quite high temperatures and the use of well-crystallized specimens becomes practicable. Ti_2O_3 was prepared by reduction of TiO_2 in a $TiCl_4/H_2$ stream at 750° C.; V_2O_3 was prepared by reduction of V_2O_5 in hydrogen at 800° C.; Cr_2O_3 was obtained by ignition of $(NH_4)_2Cr_2O_7$ at 800° C. A circulating

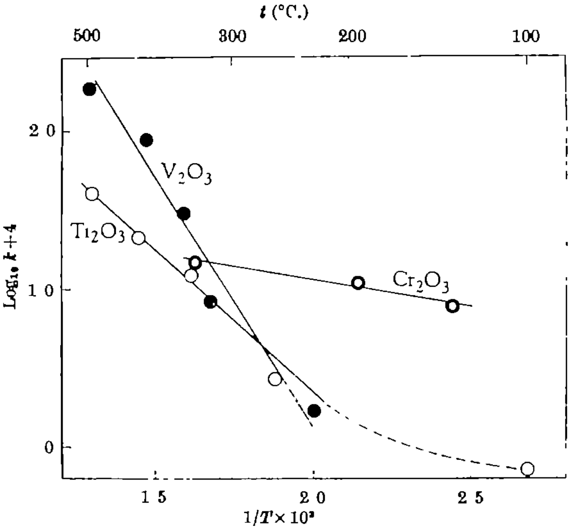

Fig. 1. The activity of Ti_2O_3, V_2O_3 and Cr_2O_3 in H_2–D_2 exchange

system[1] was used for the exchange experiments. The course of the reaction was followed by withdrawing samples from time to time and analysing them in a mass spectrometer. The kinetics can be expressed in terms of a first-order rate constant k, referred to unit surface area. Thus:

$$k = \frac{1}{At}\ln\{[HD]_\infty/([HD]_\infty - [HD]_t)\}$$

where A is the area of catalyst used. Arrhenius plots showing the values of k(m.$^{-2}$min.$^{-1}$) at various temperatures between 100° and 500° C. are given in Fig. 1.

It is immediately apparent that the simple correlation of an activity increase in the sequence d^1, d^2, d^3 claimed for this reaction by Dowden, Mackenzie and Trapnell[2] is not borne out by the results presented here. $V_2O_3(d^2)$ is the most active oxide at high temperatures and the least active at low temperatures. Below 300°, however, the well-known activity of chromia for this reaction is clearly demonstrated. H_2–D_2 exchange has normally been studied on 'unglowed' chromia gel, which is active even at −196°; but the crystalline oxide evidently retains the characteristic property which determines catalytic activity at low temperatures. It suggests that Cr^{3+} stabilized in the octahedral holes of the oxide lattice is genuinely responsible. The greatest interest is attached to the high activity of Ti_2O_3 and V_2O_3 at high temperatures, as reflected by their much larger activation energies. Now the corundum structure is basically a hexagonal close-packed lattice of oxide ions with the cations regularly occupying two-thirds of the octahedral holes. There is, however, some distortion. The result is that each cation has one near neighbour and three other cation neighbours somewhat farther away. A pair-wise interaction due to the overlap of the occupied d orbitals of the near neighbours is then possible in these transition metal sesquioxides[5], and, other things being equal, this overlap will be greatest for Ti^{3+} with its smaller nuclear charge. The resultant coupling of the spins leads to an unusual antiferro magnetism in Ti_2O_3 and V_2O_3, but Cr_2O_3 has much more conventional magnetic behaviour, suggesting that this pairing is either absent or very weak. It would appear that the onset of catalytic activity in Ti_2O_3 and V_2O_3 does not occur until these spins are uncoupled. This begins to occur with V_2O_3 at about −100°, but is not complete until 300° (ref. 6). With Ti_2O_3 the unpairing begins at about 100°, but is much less susceptible to a rise in temperature[7]. The catalytic activity is seen to follow this general pattern, suggesting that the critical step is indeed the formation of a covalent d bond between the metal and adsorbed hydrogen, as Dowden, Mackenzie and Trapnell have surmized[2]. The relationship with conductivity is also interesting. Once the temperature is high enough for the magnetic exchange interaction to be removed, the semiconductivity of Ti_2O_3 and V_2O_3 disappears and the band structure approximates to that of a metal[3,4]. The d-metals are generally good catalysts for hydrogen chemisorption and for the H_2–D_2 reaction, and the relatively high activity of Ti_2O_3 and V_2O_3 which we find above 300° now extends the analogy.

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Reduction of Osmic Acid by Ultrasonic Waves

It is assumed that water decomposes under the influence of the ultrasonic waves into free radicals according to the equation:

$$H_2O = H + OH$$

The radicals are responsible for some secondary reactions which depend on the medium treated.

In aqueous solutions some substances react with hydrogen atoms, others with OH radicals. On the other hand, in an ultrasonic field reduction or oxidation occurs according to the properties and structure of the reacting ions and particles.

Beuthe[1] has observed that a dilute solution of potassium permanganate becomes decolorized when, under ultrasonic treatment, and mercuric chloride, to which ammonium oxalate has been added, is reduced to mercurous chloride. Moreover, it has been shown that some other substances, inorganic and organic, are reduced in an ultrasonic field[2–6].

In our experiments an ultrasonic generator (quartz) of 500-kHz. frequency and 5-W./cm.^{2} power, and a cylindrical glass vessel with a very thin bottom the diameter of which (3·5 cm.) was equal to the diameter of the vibrating quartz plate, were used.

The quartz plate and the reaction vessel were cooled down by flowing oil through. Solutions of osmic acid, in concentrations of 1–2 per cent, were at first saturated with hydrogen and in the course of the ultrasonic treatment about 50 hydrogen bubbles per min. were passed through them. After subjection to ultrasonic treatment the solution was poured into a long test-tube. In every case 20 ml. of osmic acid solution were treated with the ultrasonic waves.

Effect of Osmium Tetroxide on the Efficiency of the Reaction. Osmium tetroxide solutions (0·1–1·9 per cent) were treated with ultrasonic waves for 3 min. The interdependence of the concentration and the

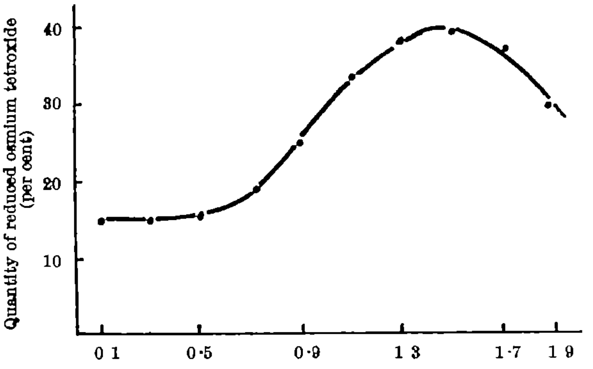

Fig. 1

amount of reduced osmium tetroxide is shown in Fig. 1. A decrease in the efficiency of the osmium tetroxide reduction reaction in solutions of concentration higher than 1·5 per cent is explained by the fact that a certain maximum concentration of colloidal osmium tetroxide particles disperses a part of ultrasonic energy.

After treatment, the solutions of low concentration (0·1–0·5 per cent) were of an ashen colour with a bluish shade while the solutions of higher concentration (above 0·5 per cent) were dark blue.

Osmic acid in the ultrasonic field is reduced under the influence of hydrogen to colloidal OsO_2 according to the equations:

$$H_2 = H + H$$

$$OsO_4 + 4H = OsO_2 + 2H_2O$$

The degree of the colloidal division of OsO_2 is so great that only after a few days do OsO_2 particles show a tendency to settle in the solutions of the highest concentration. But when 0·5–1 ml. of the saturated solution of ammonium sulphate is added, then a sudden coagulation of OsO_2 particles takes place. A visual observation of the velocity of the sedimentation of OsO_2 particles leads to the conclusion that most of them belong to a monodispersal system, as the absolute number of OsO_2 particles settles down with the same velocity. The solutions of colloidal OsO_2 obtained are able to decompose hydrogen peroxide.

The solutions of osmic acid acidified with 2 *N* hydrochloric acid give less colloidal OsO_2. Under these conditions H radicals at *p*H less than 3 change into H_2^+ ions according to the equation:

$$H + H^+ = H_2^+$$

with strongly oxidizing properties.

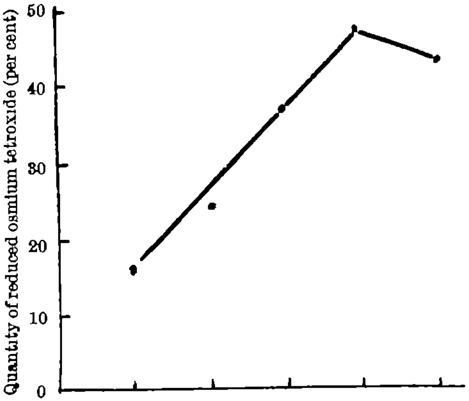

Fig. 2

Effects of Duration of Ultrasonic Treatment on the Quantity of Reduced Osmium Tetroxide. 20 ml. of 0·7 per cent osmium tetroxide solution were treated for 2, 4, 6, 8 and 10 min. The interdependence of the quantity of reduced tetroxide and duration of the treatment are shown in Fig. 2.

After the treatment for 1 min. no change occurred. Probably after that duration of treatment the concentration of H radicals is too low to start the reduction of osmium tetroxide. During the course of further treatment the solutions turned blue because colloidal OsO_2 was formed, the particles of which showed no tendency to sediment even after some days. Colloidal OsO_2 precipitated by ammonium sulphate and then air-dried turned black. Precipitated OsO_2 can decompose hydrogen peroxide.

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Centrifugal Fractionation of Wool Wax

It has recently been reported[1,2] that complete or almost complete fractionation of wool wax into oxidized and unoxidized fractions occurs during centrifugal recovery from the scouring liquor. We have already[3] found evidence of a rapid fall in the high peroxide value of the wax emulsified in the hot scouring liquor, a slow fall continuing even in the cold, and unless centrifugal separation takes place during or immediately after scouring, all the wax in the liquor is almost peroxide-free and centrifuging does not give fractions of very different peroxide value. The peroxides presumably break down to the usual ultimate oxidation products such as ketones and fatty acids, and these fatty acids tend to remain in the alkaline liquor as soap, thus contributing to the fractionation of oxidized products during centrifuging. This is supported by the much higher acid value which Anderson and Wood[1] found in their 'unrecovered' compared with their 'recovered' wax.

That fractionation occurs, involving not only fatty acids, has long been accepted in the British lanolin industry. Thus, pharmaceutical-quality wool fat (anhydrous lanolin) refined from centrifuged wool grease, compared with wool fat refined to a similar acidity and general standard from the 'unrecovered' grease (which, in Britain, is often recovered by 'acid-cracking'), differs in odour, colour, melting-point, free cholesterol content, etc., and individual users of pharmaceutical wool fat often show a preference for one particular type, not necessarily that of centrifugal origin.

It seems unlikely that centrifugal fractionation of oxidized substances is as complete as has been claimed. Anderson and Wood[1] found centrifuged wool grease to have approximately four times the acid value and hydroxyl value of new wax solvent-extracted from the base of the fleece, and their ultra-violet absorption spectra of the two waxes showed considerable differences. Also new wax has been reported[4-6] as substantially colourless, neutral, and of saponification value as low as 73, whereas most centrifuged wool grease is very different in all these respects (for example, the saponification value is usually > 90).

It appears probable that more than direct oxidation is responsible for the darkening in colour and other changes which are undergone by wool wax on the fleece and which are involved in the centrifugal fractionation. In contrast to the lightening in colour which occurs during oxidative bleaching or surface autoxidation of most types of wool wax, we have found that when peroxides in wool fat are rapidly broken down by heating or treatment with sulphur dioxide, potassium iodide, etc., the colour darkens and the acid value rises, the breakdown being accelerated by presence of metals (iron, copper, etc.) or by an acid pH such as prevails in oxidized wool fat[7]. The causes of the change may possibly be summarized as: autoxidation, catalysed by metallic impurities present[5,6], the resultant peroxides undergoing natural or accelerated decay under the influence of chemical or biological reduction (possibly catalase-like[8]) and/or metallic catalysts, with simultaneous formation of dark-coloured metallo-organic compounds and absorption of substances (including nitrogen, phosphorus and perhaps sulphur[4,10]) from the suint, excreta and, possibly, degraded wool.

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BIOCHEMISTRY

Incorporation of Carbon-14 from Labelled Sodium Bicarbonate into the Retina of the Rat

Since respiration and glucose metabolism in retina are more rapid if bicarbonate is added to the incubation medium[1,2], it was of interest to investigate the metabolic pathways by which carbon dioxide can be assimilated during such incubation. In the experiments reported here the bicarbonate was labelled with carbon-14 and the radioactive products formed during incubation were separated and located by chromatography followed by autoradiography.

One or two rat retinæ were placed in 200 μl. phosphate Ringer solution containing 1 μmole glucose and 1 μmole sodium bicarbonate; the solution was gassed with oxygen and incubated for 10 min. at 37°. 50 μl. aqueous carbon-14 labelled sodium bicarbonate (containing 0·4 μmole/10 μc.) was then added and incubation was continued for 5–30 min. and stopped by addition of 1 ml. absolute ethanol. The metabolites soluble in aqueous ethanol were separated and located by chromatography and autoradiography by a method similar to that described by Moses[3].

Ten active compounds were detected by this treatment, but in most of them radioactivity was very slight. The more active compounds were identified by co-chromatography of the extract with suitable markers. The identification of fumaric, malic, aspartic and glutamic acids and of glutamine was confirmed by elution of these compounds on to fresh chromatograms, which were run with authentic markers, in two different solvent systems. The remaining compounds had low activity and were not identified; one may have been lactic acid. An attempt was made to isolate keto acids from an *m*-phosphoric acid extract of the incubation mixture as the 2 : 4-dinitrophenylhydrazones[4]. The hydrazone fraction showed an activity of 100 counts/min./retina compared with 2,000 counts/min./retina for the whole extract, but it was not possible to identify individual keto acids. The activity both of the whole extract and of the hydrazone fraction was similar for extracts incubated for 5 or for 30 min. The count for the whole extract corresponded to the incorporation of approximately 0·1 per cent of the added bicarbonate. This is lower than that recorded by Katz and Chaikoff[5] for rat liver and the pattern of incorporation is also different from that reported for other mammalian tissues[5,6]. A higher rate of incorporation might have been expected from Crane and Ball's[7] results, but these workers added pyruvate as well as glucose to the incubation medium. In our experiments carbon-14 was distributed among a number of intermediary metabolites; much shorter incubation periods would have been required to prevent further reaction of the first products of fixation, but the small amount of carbon-14 assimilated would probably have precluded their identification, and experiments of this kind are unlikely to throw light on the effect of bicarbonate in glucose metabolism in retina.

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Micro-fractionation on Paper of Acidic Polysaccharides and Related Substances

In 1955, Scott[1,2] described a method for the preparative separation of polysaccharides containing different amounts or types of anionic residues, based on the formation of insoluble complexes with cetyl pyridinium chloride (CPC) or similar long-chain cations. The resulting complexes can be selectively dissociated by varying concentrations of electrolytes (sodium sulphate, sodium chloride, magnesium chloride)[3,4].

We have now extended this principle for the separation of polyuronides, sulphated polysaccharides and related substances, on paper strips in the quantitative range 5–70 μgm., using upward elution from the corresponding CPC complexes in a magnesium chloride gradient (0–3·5 *M*).

The elution apparatus consists of a glass cylinder (7·5 × 42 cm.) fitted for magnetic stirring and containing 10 ml. of water. Into this, a saturated solution (20° C.) of magnesium chloride is introduced by an electrically driven syringe at a rate of 1·8 ml./hr. The polysaccharide mixtures are prepared for separation in the following way. The end (4–5 cm.) of a strip (5 × 46 cm.) of Whatman No. 4 paper (with a line ('origin') drawn 3 cm. from its lower edge) is dipped in 10 per cent (w/v) solution of CPC in 95 per cent ethanol for 10 min. The paper is dried and a solution (in 50 μl.) of polysaccharides (5–70 μgm. of each constituent) is applied as a spot on the 'origin'. Up to three samples can be examined simultaneously, arranged as in conventional chromatography. The paper is dried gently, and excess CPC is then removed by washing in 95 per cent ethanol or other suitable solvent. The paper is again dried, and it is then equilibrated with water vapour by being suspended in a moist chamber for 12 hr.

The resulting paper is rapidly transferred to the elution apparatus, where it is suspended with the lower edge just immersed in the eluting medium, which is gently stirred magnetically. The apparatus is closed and the introduction of magnesium chloride is commenced at once. In the course of 5 hr., the solvent rises to a height of about 30 cm. The electrolyte gradient is approximately linear up the paper.

The eluted paper is washed (5 min.) in 95 per cent ethanol and the separated components may be detected by a variety of methods, for example, Alcian Blue[5], a general reagent for mucoproteins, hyaluronic acid, etc., mucicarmine[6], for mucopolysaccharides, etc., Azur *A*[7] for metachromatic substances such as chondroitin sulphate and for some sialic acid-containing substances, and periodic acid–Schiff reagent[8] for components containing oxidizable carbohydrates. The method has also been used successfully with mucopolysaccharides labelled with carbon-14 or sulphur-35 and for their dinitrophenyl derivatives.

The procedure gives clear and reproducible separations of substances such as: heparin, chondroitin sulphates, colonic mucoproteins, dextran sulphate, hyaluronic acid.

In a typical separation, lasting 4 hr. 40 min., in which the solvent front was at 32·0 cm. (measured from the origin), heparin migrated 4·2 cm., chondroitin sulphate 8·4 cm., hyaluronic acid 12·9 cm. The procedure appears to offer considerable promise for the separation and comparison of these types of mucosubstances.

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Effect of Adenine Nucleotides on Phagocytizing Guinea Pig Leucocytes

PHAGOCYTOSIS has been used predominantly as an indicator for estimating the effect of chemical agents on the reticuloendothelial system[1]. As an outcome of investigations on the mechanism of action of biologically interesting compounds on phagocytosis, it has now been found that adenosine triphosphate (ATP) is a powerful inhibitor of this phenomenon without affecting respiration, glucose utilization and lactic acid production of the leucocytes. Similar effect was observed, though less pronounced, with adenosine diphosphate (ADP), adenosine monophosphate (AMP) and diphosphopyridine nucleotide (DPN).

The effect of ATP at various concentrations on phagocytosis by guinea pig exudate mononuclear cells of the heat-treated *Mycobacteria X* was investigated (Fig. 1). Leucocytes were collected by saline washing the peritoneal cavity of bled animals 5 days after intraperitoneal injection of 10 ml. 12 per cent sterile solution of sodium caseinate. The centrifuged (10 min. at 220*g*) leucocytes were resuspended for the phagocytosis experiments in *p*H 7·4 bicarbonate buffered Krebs-Ringer solution containing 5 per cent heat-inactivated rabbit serum and 0·15 per cent glucose. The total yield was 50–150 × 10^6 leucocytes (80–85 per cent monocytes) per animal. Three times saline-washed and heat-treated (1 hr. at 60° C. in saline) *Mycobacteria X* (ref. 2) from 3–4-week-old cultures on Dubos-'Tween'-albumin medium were used as a 0·100 optical density (625 mμ) saline stock suspension. Phagocytosis was measured by a modification of the method described by Suter[3] using 0·2 ml. of the *Mycobacterium X* stock suspension per 0·8 × 10^6 leucocyte. After incubation for 1 hr., in presence or absence of the compound to be tested, the coverslips were washed in saline twice to eliminate the extraneous bacteria, fixed in methanol, dried, stained with cold carbolfuchsin[4] for 8 min., washed with water, decolorized, counterstained for 1 min. with 0·3 per cent aqueous methylene blue, dried and mounted. Counting was effected until a total of approximately 100 bacteria-containing leucocytes were enumerated. The phagocytic index (*P.I.*) was calculated by using the formula:

$$P.I. = \frac{\text{no. of leucocytes containing bacteria}}{\text{total No. of leucocytes counted}} \times 100$$

Each point on the graph represents the mean values of five experiments. Statistical analysis, by calculating the probabilities on the basis of the null hypothesis for true difference between the control and test values,

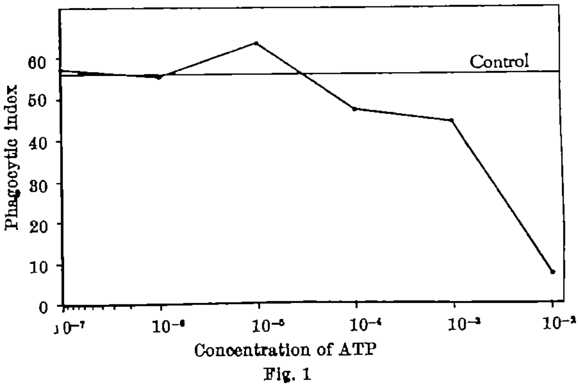

Fig. 1

Table 1. EFFECT OF ATP ON RESPIRATION, GLUCOSE UTILISATION AND LACTIC ACID PRODUCTION OF GUINEA PIG MONOCYTES*

	Results per 10^7 cells/hr.					
	Oxygen (μl)		Glucose (μM)		Lactic acid (μM)	
	a	*b*	*a*	*b*	*a*	*b*
Control	8 60	10 21	0·465	0 460	1 821	1 892
ATP $5 \times 10^{-3} M$	10 00	9·84	0·461	0 475	2 130	2 178
Control	7·60	9 15	0 656	0 663	1 631	1 518
ATP $1 \times 10^{-5} M$	7 79	8 55	0 665	0 668	1 524	1·808

a, Resting cells. *b*, phagocytizing cells.

* Uptake of oxygen was examined in a Warburg respirometer. The main compartment contained $35–40 \times 10^6$ cells in a total volume of 2 5 ml. phosphate buffered Krebs-Ringer solution, containing 5 per cent heat-inactivated rabbit serum and 100 mgm per cent glucose. The side arm contained either (*a*) 0 5 ml. of Krebs-Ringer solution, or (*b*) the compound in the medium alone, or (*c*) *Mycobacterium X* (same count as for phagocytosis experiments) in the medium alone, or (*d*) *Mycobacterium X* and the compound tested together in 0 5 ml. of the medium. The centre well contained 0 2 ml. of 10 per cent potassium hydroxide. After equilibration for 20 min., the side-arm was tipped 30 min. after the start of the readings. Measurements of uptake of oxygen were continued for a total period of 210 min. The reaction was stopped by the addition of $10^{-4} M$ sodium azide and the contents of the flasks were centrifuged. The supernatant fluid was used for the glucose (ref. 14) and lactic acid (ref. 15) determinations

gave $P < 0{\cdot}001$ for all concentrations above $1 \times 10^{-6} M$, $0{\cdot}05 < P < 0{\cdot}1$ for $1 \times 10^{-6} M$ and $0{\cdot}1 < P < 0{\cdot}2$ for $1 \times 10^{-7} M$.

Fig. 1 shows that, depending on the concentration, ATP slightly enhances or considerably inhibits phagocytosis by guinea pig leucocytes. In contrast to this effect on phagocytosis, ATP does not affect noticeably the respiration, glucose utilization and lactic acid production of resting and phagocytizing mononuclear cells (Table 1). At $1 \times 10^{-2} M$ AMP inhibited phagocytosis, but had no effect at lower concentrations. With ADP the cells came off the coverslips at concentrations of $1 \times 10^{-3} M$, $5 \times 10^{-3} M$ and $1 \times 10^{-2} M$. The drastic effect of ATP on phagocytosis, which involves cytoplasmic deformation, is not unexpected since cytoplasmic streaming, which is the basis of cell motility, has been considered to be akin to muscle contraction and relaxation processes[5–7]. That AMP also has some inhibitory action at the concentration of $1 \times 10^{-2} M$, where ATP exerts considerable inhibition, suggests, however, that the effect of ATP may not be solely related to its utilization as source of energy. This is also consistent with the absence of action of ATP on the glycolysis of leucocytes. Thus, simpler mechanisms, such as multipoint attachment to functional groups of the cellular surface structure (that is, cross-linking by secondary valence forces (cf. ref. 8)), should be considered for the observed inhibition of phagocytosis.

Also DPN has a marked inhibitory effect on phagocytosis at both $5 \times 10^{-4} M$ and $5 \times 10^{-3} M$ (22 and 45 per cent decrease of the phagocytic index relative to the control), while DPNH is an inhibitor only at $5 \times 10^{-3} M$ (38 per cent decrease). On the respiration and on the glycolysis of mononuclear cells, however, addition of DPN appeared to have some stimulatory effect. Only small difference was found in the uptake of oxygen by resting and phagocytizing leucocytes. There was no difference in glucose utilization and no change in lactic acid production[9].

In contrast to the findings of Sbarra and Karnovsky[10], and Cohn and Morse[11] on the effect of metabolic inhibitors on polymorphonuclear cells, iodoacetate, fluoroacetate and sodium fluoride at $1 \times 10^{-4} M$, and sodium azide and *p*-chloromercuribenzoate at $5 \times 10^{-5} M$ were either without effect on phagocytosis in our assay system, or produced slight stimulation. However, 2,4-dinitrophenol was markedly inhibitory at $5 \times 10^{-4} M$. Cysteine, glutathione and *p*-mercaptobenzoate at $5 \times 10^{-3} M$ were without effect, which is in general agreement with the observations of Lisowski[12] with various sulphydryl compounds. The sulphydryl inhibitor dicarbonyl compounds, diacetyl, acetylacetone and acetonylacetone (all at $5 \times 10^{-3} M$), were inhibitory to phagocytosis to various degrees. However, acetone and also ethanol had a considerable stimulatory effect both on the percentage of active leucocytes and on the number of particles taken up by the phagocytes.

The observations[13] that bacterial lipopolysaccharides stimulate the phagocytic activity of guinea pig polymorphonuclear cells could not be observed in our system. There was no effect with concentrations of 0·1–50 μgm./ml. Pre-incubation of the cells with this material had some inhibitory effect on phagocytosis.

Because it is conceivable that components of the serum may interact with some of the compounds tested, the use of media containing no serum was attempted. Considerable drop in the phagocytic index was observed when the experiment was carried out in bicarbonate buffered Krebs-Ringer solution in the absence of serum. Rabbit leucocytes were found to be much more sensitive (*P.I.* = 2·5) to the absence of serum than were guinea pig leucocytes (*P.I.* = 23·0), while control experiments conducted in presence of sera of various species and at various levels gave *P.I.* values between 55·0 and 69·0 for the leucocytes of both species.

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Determination of Volatile Anæsthetics in Rhesus Monkey Tissue

METHODS for the facile quantitation by infra-red spectrophotometry of such anæsthetics as diethyl ether or chloroform and its congeners in animal tissues have been developed within our organization[1–3]. Extensive results which we obtained from anæsthetized dogs are being reported[3]. The results of experiments on higher primates are somewhat different and some of their peculiarities are of more general interest.

The healthy, large animals used were secured in a supine position and anæsthetized without premedi-

Table 1. Rhesus Monkeys anæsthetized without Premedication for 2·5 hr., killed with Pentobarbitone and dissected immediately

Levels of anæsthetic expressed as parts per million parts by weight. Tissue samples weighed about 1 gm.

Anæsthetic	Ether	Chloroform	Methoxyflurane	Halothane	
Weight (gm.) Sex	8·9 M[1]	6·2 M	7·7 M	10·8 M	8·1 F
Area analysed					
Arterial blood[2]	550	112	125	146	126
2 hours	540	108	122	133	118
2·5 hours	560	116	123	160	133
Exhaled air[2]	6·5%	1·72%	0·31%	[3]	1·46%
2 hours	7·3	1·40	0·28		1·43
2·5 hours	5·8	2·05	0·33		1·50
Brain					
Frontal Cortex	618	310	269	78	71
Temporo-Parietal Cortex	733	351	[3]	98	83
Cerebellum	541	454	268	76	70
Medulla	663	482	267	124	107
Thyroid	558	359	184	196	260
Thymus	2,500	1,918	2,590	378	521
Right Ventricle	800	197	290	151	115
Lung	[3]	99	192	< 50	29
Skeletal Muscle	572	144	84	38	43
Liver	556	303	363	56	39
Pancreas	570	336	245	141	133
Spleen	531	242	159	39	30
Adrenal	1,231	1,652	1,682	[3]	340
Kidney	672	203	238	152	40
Testes	697	192	297	93	< 10[4]
Omental Fat	3,280	1,660	925	146	31[5]
Bile	[3]	318	129	< 50	23
Urine	[3]	86	28	< 50	< 10

[1] Atropine to control secretions.
[2] Samples taken at 2 and 2·5 hr. of anæsthesia when anæsthesia status was considered very stable.
[3] Samples lost, not taken or analytically unusable.
[4] Ovary.
[5] Very obese.

cation by inhalation of diethyl ether, chloroform, methoxyflurane ($CH_3OCF_2CCl_2H$) and halothane ($CF_3CBrClH$) vapours, administered by conventional clinical equipment for 2·5 hr. The ordinary details are described elsewhere[3] and followed modern human clinical practice on the whole[4].

Expired air samples were collected within 5 min. at 2 and 2·5 hr. of anæsthesia through a one-way valve leading into 2-litre gas-tight saran bags. The concentration of anæsthetic in the bag was determined directly in a 10-cm. gas cell in a Perkin-Elmer 221 infra-red spectrometer. Blood samples were taken from the femoral artery while the air samples were being collected. After 2·5 hr. of anæsthesia, the animals were killed abruptly by means of a large intravenous dose of sodium pentobarbitone. The tissues were immediately dissected out and prepared for analysis. Blood and tissue samples of diethyl ether, chloroform and methoxy flurane were extracted by carbon disulphide and the content of anæsthetic in the extract quantitated by infra-red spectrometry[1]. In the case of halothane, tissue samples were analysed by extracting into *n*-hexane with quantitation of the bromine contained in halothane by means of X-ray fluorescence spectrometry as explained elsewhere[2]. The overall analytic error is about ± 10 per cent with either method of analysis and all sources of error would lead toward lower than actual values.

The data obtained are presented in Table 1. The arterial blood-levels of all the drugs are several-fold lower than required in the dog for a comparable anæsthesia. Such data as exist for man[4,5] are most comparable with the monkey. Brain-levels, generally, of ether, chloroform and methoxyflurane were found to be higher than arterial blood-levels, while those of halothane are distinctly lower. This difference was not seen in the dog. No obvious differences in anæsthetic concentration among different brain areas were detected, although there is a trend toward higher concentrations in the cerebellum and medulla.

The very high concentration of all these anæsthetics found in the whole adrenal and in the thymus defies immediate explanation. It cannot be a simple solution, for in the cases of the fluorinated anæsthetics it exceeds the level found in omental fat. The low level of halothane in the ovary of the one female examined must be accepted with reservation, although we have no reason to doubt its correctness. The relative ease with which determinations of many anæsthetics can now be made, despite the complex apparatus required for quantitation, intimates that this, and many other questions, will soon be answered.

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Anaerobic Formation of *n*-Hept-1-ene from *n*-Heptane by Resting Cells of *Pseudomonas aeruginosa*

Two of us (E. A. and J. C. S.) have previously shown[1,2] that *Pseudomonas aeruginosa* metabolizes anaerobically *n*-heptane through an adaptative dehydrogenase coupled with pyocyanin and/or diphosphopyridine nucleotide (DPN). The formation of the corresponding 1-olefine as the product of the reaction was suggested on the basis of certain indirect evidence, but still had to be analytically demonstrated. The

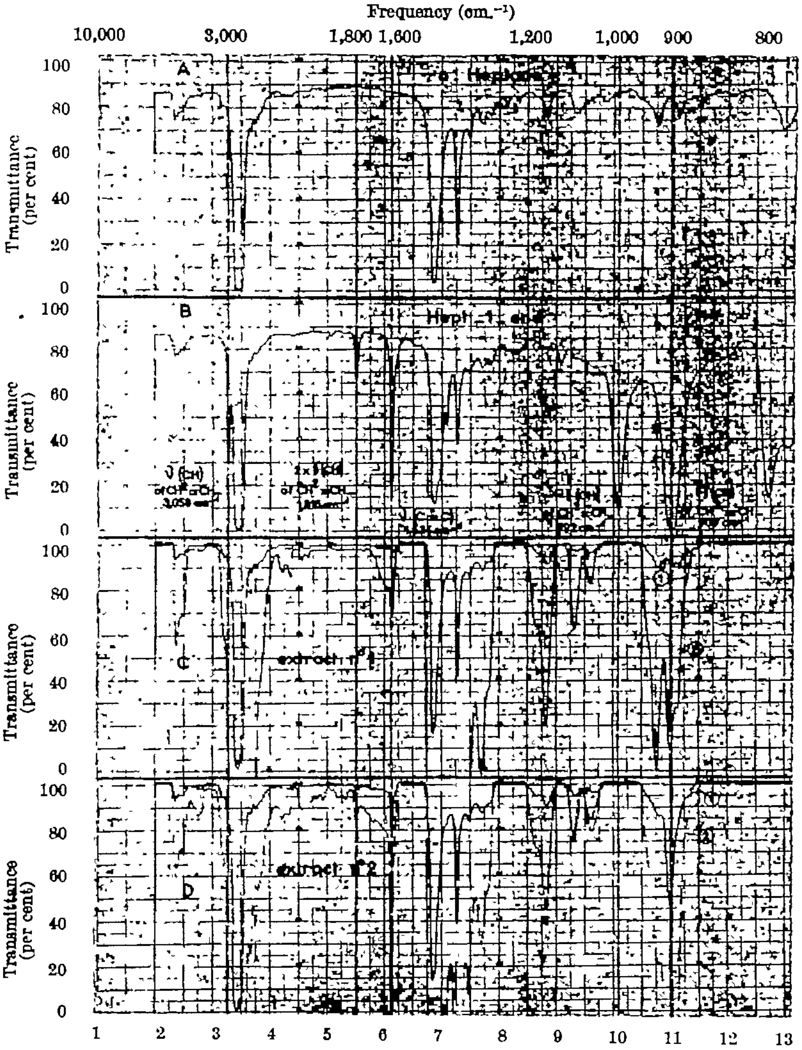

Fig. 1. *A* and *B*, Infra-red spectra of pure *n*-heptane and *n*-hept-1-ene in a 0·025-mm. cell; *C* (extract No. 1), carbon tetrachloride extract of an experimental system initially containing 70 mgm. *n*-heptane; 1·0 mm. cell; curve 1, without scale expansion, curve 2, with 10-times scale expansion; *D* (extract No. 2), spectra of a carbon tetrachloride batch utilized for successive extraction of nine experimental systems, each containing initially about 40 mgm. *n*-heptane; 1·0 mm. cell, curve 1, without scale expansion; curve 2, five-times scale expansion

results reported here provide an infra-red spectrophotometric confirmation of this pathway.

In order to minimize the possible interference of intra-cellular material with the spectrophotometric characterization of the olefine, the experiments were carried out with intact cells of *P. aeruginosa* (strain *S* 20) cultivated on *n*-heptane as already described[2]. The reactions were performed in Thunberg's tubes, under the following conditions: bacterial cells; 10–20 mgm. dry-weight; purified pyocyanin[4], 5 μmoles; *n*-heptane, 40–70 mgm.; total volume completed to 25 ml. with 0·05 *M* phosphate buffer (*p*H 7·0). Prior to the introduction of the substrate, the systems containing all the other constituents were frozen at − 15° C., this precaution excluding any possibility of an aerobic oxidation of the alkane by the bacteria during the interval of time between the preparation of the systems and the evacuation of the tubes.

In these experimental systems, the substrate (*n*-heptane) is in considerable excess, and complete reduction of the external electron acceptor (pyocyanin) theoretically corresponds to the formation of 490 μgm. (5 μmoles) of heptene.

After the evacuation of air, the systems were allowed to equilibrate at + 23° C. and the reaction pursued for about 30 min., until complete discoloration of pyocyanin. The tubes were then cooled again to 0° C., opened, and the systems were extracted with 4 ml. of cold carbon tetrachloride. The solvent was thoroughly mixed, separated by centrifugation at 0° C. and its spectrum recorded with a Perkin–Elmer model 21 infra-red spectrophotometer in a 1 mm. cell. The absorption of the solvent (carbon tetrachloride) was compensated for by using a variable thickness cell and adjusting for the absorption band of carbon tetrachloride at 9·4μ (1,064 cm.⁻¹).

The following products have been utilized for the dehydrogenation assays and the recording of reference spectra: (1) *n*-heptane A.P.I. grade, 99·8 per cent pure (commercial product from Phillips Co.); (2) *n*-hept-1-ene (purity 99·80 ± 0·10 per cent), *trans*-*n*-hept-2-ene (purity 99·85 ± 0·05 per cent), and *trans*-*n*-hept-3-ene (purity 99·80 ± 0·15 per cent), kindly provided by the British Petroleum Research Laboratories (Sunbury-on-Thames); (3) pure 1-*n*-heptanol, heptanal, heptanoic acid and methylheptanoate kindly provided by Organico Research Laboratories (Marseilles).

Preliminary assays have shown that the initial quantity of *n*-heptane introduced in the bacterial systems must not exceed 70 mgm., in order to avoid the masking of the olefine spectrum by the excess alkane. The minimum concentration of *n*-hept-1-ene detectable in carbon tetrachloride with a 20-times ordinates scale expansion is of about 70 μgm./ml. Under these conditions, only the characteristic bands of absorption described by Sheppard and Simpson[5] at 909 cm.⁻¹ [γ(CH)] and 1,634 cm.⁻¹ [ν(C = C)] are faintly visible. With the same scale expansion, the peak at 3,058 cm.⁻¹ [ν(CH)] becomes apparent for a concentration of 700 μgm./ml. With a concentration ten times higher, the two last peaks at 1,815 cm.⁻¹ [2 × γ(CH)] and 992 cm.⁻¹ [γ(CH)] become faintly visible without scale expansion and are quite marked with a 5-times scale expansion.

It has been verified that repeated extraction of control systems containing the bacteria, pyocyanin and buffer in the absence of *n*-heptane does not introduce into the carbon tetrachloride any contaminating material which could interfere with the spectrophotometric detection of the olefine.

The original spectra reproduced in Fig. 1*C* (extract No. 1) correspond to a single carbon tetrachloride extraction of an experimental system initially containing 70 mgm. of *n*-heptane. The characteristic bands of hept-1-ene at 909 cm.$^{-1}$ and 1,634 cm.$^{-1}$ are clearly visible without scale expansion (curve 1) and much more marked with a 10-times scale expansion (curve 2). On curve 2, the absorption band at 3,058 cm.$^{-1}$ forms a distinct shoulder on the adjacent band of *n*-heptane. The spectra in Fig. 1*D* have been recorded on a sample of carbon tetrachloride which had been used for the successive extraction of 9 experimental systems, each containing initially about 40 mgm. *n*-heptane. On these spectra, the three aforementioned bands are clearly visible and also the band at 1,815 cm.$^{-1}$. The absence of the fifth characteristic band (992 cm.$^{-1}$) is due to the fact that the very strong absorption of carbon tetrachloride in this range cannot be exactly compensated for and was actually overcompensated.

The chemical structure of the olefine formed in the reaction is demonstrated by the absence of the single characteristic absorption band[3] of *trans*-hept-2-ene and *trans*-hept-3-ene. The absorption bands of the corresponding *cis*-heptenes[3] are not interfering with the bands of the other *n*-heptenes and are too weak to be apparent in the carbon tetrachloride mixtures. The complete absence of the characteristic bands of 1-*n*-heptanol, *n*-heptanal, heptanoic acid and methylheptanoate should be also noted.

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Increased Sensitivity of Ethylenediamine Analysis for Norepinephrine

ATTEMPTS have been made to increase the sensitivity of analysis for catecholamines using the ethylenediamine condensation method described by Weil-Malherbe and Bone[1]. The objective was to be able to determine catecholamine-levels of 0·05 μgm. and below. Examination of the intensity of fluorescence of norepinephrine after various periods of heating demonstrated, in agreement with others[2,3], that 1–2 hr. heating at 50° C. was optimum. It was also possible to obtain the same intensity of fluorescence by permitting the reaction to proceed at room temperature for about 7 hr. or at 5° C. overnight. At 50° C. the intensity of fluorescence was found to be maximum in 20 min. for epinephrine and in 40–50 min. for dopamine. When using the Klett fluorimeter, the fluorescence for epinephrine was stable while that for norepinephrine and dopamine was not.

As reported previously[2], intense ultra-violet light caused a rapid decrease in intensity of fluorescence. In the Klett fluorimeter, the intensity of the ultraviolet light was great enough to cause a significant drop in fluorescence during the time it took to null the meter. Exposure to the incident beam for more than 5 sec. could be avoided by measuring the deflexion of the galvanometer beam rather than using the galvanometer as a null point meter. During this time the decrease in fluorescence was minimal. With the Aminco-Bowman spectrophotofluorometer, the fluorescence from norepinephrine and dopamine decreased more slowly, and, for practical purposes, could be considered stable. When the proper activation and fluorescent wave-lengths were used, the intensity of the fluorescence from norepinephrine was somewhat greater than from epinephrine. This improved sensitivity of the method still would not permit the analysis of 0·05 μgm. or less of norepinephrine. Examination of the extractability of the fluorescent product of norepinephrine with various volumes of isobutanol yielded the results given in Table 1. It was observed that a 12-fold concentration of the fluorescent product is possible by decreasing the volume of isobutanol from 3·0 to 0·25 ml. By using micro-cuvettes and the following slit arrangement: (1/16, 1/32, 1/8, none, none, none and 1/16 in. openings for slits No. 1, 2, 3, 4, 5, 6 and 7 respectively), one is able to detect as little as 0·0075 μgm. of norepinephrine.

Table 1. FLUORESCENCE OBTAINED WITH 0 2 μGM. OF NOREPINEPHRINE FOLLOWING EXTRACTIONS OF 5 ML. OF 0·2 *N* ACETIC ACID WITH VARIOUS VOLUMES OF ISOBUTANOL

Sample No.	Isobutanol Vol. (ml.)	Fluorescent units
1	3 00	0 012
2	1 50	0 024
3	0·75	0 045
4	0 50	0 075
5	0 25	0 162

This simple modification of the procedure has been used successfully for the determination of catecholamine-levels in isolated rabbit arterial strips.

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Phytic Acid as a Phosphorus Reservoir in the Developing Potato Tuber

PHYTIC acid is present in the mature potato tuber, as a mixture of dicalcium monomagnesium salts of inositol hexa- and penta-phosphoric acids[1,2]. The present communication provides information on the effect of maturity and post-harvest storage temperature on the phytic acid content of the potato tuber. Table 1 shows that the phytic acid phosphorus content of freshly harvested tubers increases with increasing maturity, but does not change greatly on subsequent storage at either 25° or 0°. Table 1 also shows that the percentage of total phosphorus present as phytic acid increases with increasing maturity, especially during later stages of development. Thus two-thirds of the increase in phosphorus content per 100 gm. of tuber observed between 80 and 102 days after planting appears as phytic acid phosphorus, whereas at earlier stages of development the incoming phosphorus is preferentially incorporated into starch[3]. Between two-thirds and four-fifths of the total organically bound acid-soluble phosphorus is present in the tuber as phytic acid phosphorus[2]. Although the phytic acid contributes a substantial portion of the total phosphorus of the tuber, its concentration in tubers amounts to less than 0·1 per cent of the fresh weight of the tuber.

Table 1. PHYTIC ACID CONTENT OF POTATO TUBERS

Post-harvest conditions	Days after planting 60	80	102	60	80	102
	var. White Rose			var. Kennebec		
	Mgm. phytic acid phosphorus per 100 gm. of tuber*					
Freshly harvested	—	9·0	14·9	—	10·3	16·0
6 wks at 25°	6·5	8·4	14·4	8·9	11·3	16·8
6 wks. at 0°	6·2	8·8	13·3	8·5	10·5	16·7
	Phytic acid phosphorus as per cent of total phosphorus					
Freshly harvested	—	16·9	24·2	—	16·6	22·3
6 wks. at 25°	13·8	15·9	23·5	15·3	18·2	23·3
6 wks. at 0°	12·7	16·6	21·7	14·6	16·9	23·2

* Phytic acid phosphorus was estimated as the sum of the phosphorus content of (*a*) the acid insoluble precipitate from treatment of a trichloroacetic acid extract of potatoes and (*b*) that fraction of acid soluble phosphorus that is retained on a 'Dowex-1-chloride' column after chromatography with eluting agents of Khym and Cohn (ref. 5). The phytic acid fraction can be quantitatively removed from the column by elution with 1 *M* hydrochloric acid. Proof of the identity of at least 99 per cent of the phosphorus of fractions (*a*) and (*b*) with phytic acid phosphorus has been published elsewhere (ref. 6).

These results suggest that phytic acid, together with starch, may function as a reservoir for accumulating phosphorus in the maturing potato tuber. By preventing the accumulation of relatively high concentrations of inorganic phosphate and thereby limiting the metabolic activity of the tuber[4], phytic acid might serve to prepare the tuber for post-harvest dormancy. In this sense phytic acid phosphorus, in contrast with starch phosphorus, can be considered as ballast material rather than as a ready source of phosphorus.

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2-Hydroxyphenylacetic Acid, a New Constituent of Higher Plants

IN connexion with our work on the biogenesis of plant stilbenes the formation of hydrangeic acid in *Hydrangea macrophylla* was studied[1]. To evaluate possible intermediates we attempted to identify all phenolic and acidic constituents of the species *Hydrangea macrophylla* by a new technique which uses fractional sublimation in a horizontal tube prior to paper chromatography. This makes possible the clear identification of those constituents which are present in very small amounts.

This work was extended to 19 species of 9 genera of the family of Saxifragaceae[2]. Most of the compounds found are well known as plant constituents, for example, caffeic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, gentisic and ferulic acids. The only compound found which has so far not been detected in higher plants was 2-hydroxyphenylacetic acid. The only natural occurrence of this C_6–C_2 compound so far reported seems to be in the mother liquor of *Penicillium* cultures[3]. This compound could be isolated from various species of the genus *Astilbe*. In *Astilbe chinensis* it is the major acid constituent and amounts up to 0·05 per cent dry weight of the leaves whereas in roots only 0·005 per cent was found.

After extraction by ethanol and evaporation of the ethanol the free acids were obtained by treatment of the residue with ether. The glucosides remained in the aqueous phase and their aglucones were extracted after acid cleavage. Sublimation of the crude acids (140°, 0·01 torr) yielded a mixture consisting mainly of 2-hydroxyphenylacetic, vanillic and 4-hydroxybenzoic acid. These compounds were separated by thin-layer chromatography ('Kieselgel *G*,' Merck, diethylether: di-*n*-butylether, 100 : 1). 2-Hydroxyphenylacetic acid was eluted from the gel. After a second sublimation white crystals of m.p. 148° were obtained. Identity with an authentic sample of this compound was proved by mixed melting point, infra-red and ultra-violet spectra (absorption maxima 275 mμ ($\varepsilon = 2{,}090$) and 227 mμ ($\varepsilon = 2{,}250$), minimum at 243 mμ).

Since 2-hydroxyphenylacetic acid seems to be present only in the genus *Astilbe* and could not be found in any other genus of the family of Saxifragaceae (*Hydrangea*, *Philadelphus*, *Rodgersia*, *Ribes*, *Escallonia*, *Bergenia*, *Deutzia* and *Heuchera*) which have been investigated so far, the occurrence of this compound seems to be of some taxonomic value.

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Organic Acids of the Rhizome of *Iris pseudacorus* L.

SHIKIMIC, quinic, succinic, malic, citric, fumaric and lactic acids have been separated on 'Dowex 1' ion-exchanger from combined aqueous and alcohol extracts of *Iris pseudacorus*, using the method of Hulme and Wooltorton[1]. These acids have been identified from their elution positions, their R_F values in various solvents and by chemical tests where appropriate.

The major acid was identified as shikimic acid: (*a*) by its R_F values of 0·19 in 1-pentanol/5 *N* formic acid (1 : 1; v/v) and 0·54 in phenol/90 per cent (w/v) formic acid/water (300 : 4 : 100; w/v/v)[2]; (*b*) by co-chromatography with an authentic sample of shikimic acid (Light and Co.); (*c*) by its colour reactions with potassium permanganate[2] and with sodium metaperiodate/piperazine/sodium nitroprusside[1]; (*d*) by the similarity of its infra-red spectrum with that figured by Davis *et al.*[3] (Fig. 1).

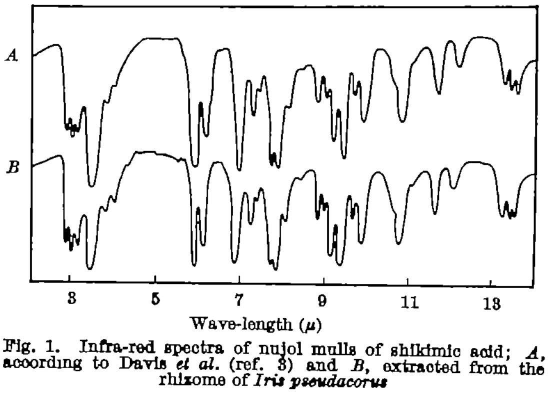

Fig. 1. Infra-red spectra of nujol mulls of shikimic acid; *A*, according to Davis *et al.* (ref. 3) and *B*, extracted from the rhizome of *Iris pseudacorus*

Table 1. Seasonal Variation in Acid Content of Iris Rhizome
(Expressed in mgm 100/gm fresh wt.)

Date	Shikimic	Quinic	Malic
Feb. 17	739	99	163
March 8	878	167	225
April 1	610	85	137
June 10	545	93	67

Table 1 shows the changes in amounts of the three principal acids, as extracted from *Iris* rhizome on four occasions during the first six months of 1960.

These results show that there is a build-up of all three acids during the late winter months, and a pronounced drop following leaf emergence. Quinic-U-^{14}C has been shown to give rise to radioactive shikimate in several higher plants[4], and the presence therefore of quinic acid in *Iris* rhizome in concentrations related to those of shikimic is of considerable interest. The importance of shikimic acid in further biosynthesis is under investigation.

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Alkaline Phosphatase of *Escherichia coli* and the Inactivation of Tryptophanase

In continuation of the studies on pyridoxamine phosphate oxidase and pyridoxal phosphatase activities by *Escherichia coli*[1,2] and their significance in the control of amino-acid metabolism, we have now prepared both acid and alkaline phosphatases from extracts of the strains of *E. coli* which we are using in Leeds and examined their action on the tryptophanase system. We had previously shown that such extracts had a high acid phosphatase and low alkaline phosphatase activity, but that a mammalian acid phosphatase did not dephosphorylate pyridoxal phosphate whereas an alkaline phosphatase did[3].

The acid phosphatase from *E. coli* is without activity on pyridoxal and pyridoxamine phosphates whereas the alkaline phosphatase hydrolyses the aldehyde phosphate but not the amine phosphate. This appears to contrast with the mammalian enzyme[1,3,4].

In the extracts of *E. coli* which we are using as a source of tryptophanase, 8 mgm. of extract protein dephosphorylates approximately 20 μgm. pyridoxal phosphate in 80 min. under conditions applied in the study of the kinetics of the tryptophanase system[5]. We have consequently investigated the role of this alkaline phosphatase in the inactivation of the enzyme by ageing.

The extract (tryptophanase) has been incubated at 37° for varying periods of time in a solution comprising a 0·05 *M tris* buffer *p*H 7·8, magnesium sulphate (0·2 × 10^{-2} *M*), potassium chloride (0·05 *M*), pyridoxal phosphate or water, both with and without the alkaline phosphatase preparation. The total volume of these systems has been 2·5 ml. After the desired time, 0·5 ml. of 1·96 × 10^{-2} *M* DL tryptophan has been added and the activity of the tryptophanase assessed by the amount of indole produced during a further incubation for 5 min.

Table 1

System tested	Indole produced (μgm.) after incubation of the system for			
	0 min.	60 min.	135 min.	200 min.
Tryptophanase	11	9	2·5	2·5
Tryptophanase and phosphatase	11	3·5	1·0	1·0
Tryptophanase and Pal.P 20 μgm	52	52	50	52
Tryptophanase and Pal.P and phosphatase	45	13	2·5	2·5

Table 1 shows the results taken from a number of similar experiments in which variations in the concentration of reactants have been used.

The concentration of Mg^{++} is less than the optimum required for the alkaline phosphatase studies of Turner[3], but corresponds to that used in studies on the enzymatic conversion of pyridoxamine phosphate to pyridoxal phosphate[5,6].

The results indicated: (1) a rapid loss in activity of the tryptophanase extract due to added phosphatase; (2) that added pyridoxal phosphate which stabilizes the tryptophanase for a time against the small amount of phosphatase present in the extract is also rapidly dephosphorylated by added phosphatase. It would appear from further experiments that the combined pyridoxal phosphate in tryptophanase is protected somewhat against the phosphatase, but further kinetic studies are proceeding.

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PHYSIOLOGY

Post-Operative Intra-Abdominal Adhesions

The ætiology of the persistent fibrous adhesions which may develop after an abdominal operation presents an important and as yet incompletely solved problem. The accepted view is that, in most cases, these adhesions represent the fibrous tissue laid down in the healing of raw peritoneal surfaces which may result from surgical trauma or from severe infection. The observations presented here are the results of experiments on adult rats.

Division of the mesentery of the small intestine, sufficient to impair its blood supply, resulted in adhesions which were confined to the region of the devascularized segment; the bulk of adhesion formation was roughly proportional to the length of the ischæmic bowel. Even in the presence of diffuse peritonitis, fibrous (as distinct from fibrinous) adhesions were localized to the ischæmic bowel. These adhesions were essentially nutritive; thus a sheet of fine polythene film placed around the devascularized segment to prevent the adhesions from reaching the gut wall was invariably followed by gangrene.

When the omentum with its blood supply intact was sutured as a pedicled graft around such segments of devascularized intestine, the graft would rapidly become attached to the underlying ischæmic intestine. It could then be shown by injection studies, using micro-radiography and micro-dissection methods, that these adhesions were vascular in nature and provided a graft of blood vessels to the ischæmic bowel.

In contrast, detached (and therefore ischæmic) omental grafts wrapped around similar devascularized segments of intestine had no such protective effect; in fact the extra bulk of ischæmic tissue merely provoked a tremendous adhesive reaction to adjacent healthy viscera.

Large defects produced by excision of the parietal peritoneum were found to have healed smoothly in 7 days; in contrast, meticulous repair of such raw areas precipitated adhesion formation to them presumably because of the inevitable local ischæmia which resulted from the dragging together of peritoneal flaps under tension.

Moreover, any other procedure which produced dead tissue on the parietal wall—crush injuries, tightly applied ligatures or free peritoneal grafts to parietal defects—produced adhesions to intra-abdominal viscera. Injection studies showed that these adhesions conveyed blood vessels which bridged across from adjacent healthy tissues into the devascularized area.

These experiments suggest that it is ischæmic tissue which stimulates the formation of persistent adhesions which are, at least in their early stage, essentially in the nature of a vascular graft to the devitalized zone.

The exact mechanism of the stimulus is unknown. It is not merely a foreign-body reaction and is not produced by suture material loosely stitched into the parietal peritoneum. It may prove to be a specific chemical stimulus to capillary growth produced by anoxic cells.

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Intracellular Localization of Catecholamines in Tissues of the Rat

PREVIOUS work in this laboratory has shown that radioactive catecholamines are removed from the circulation and retained at or near sympathetic nerve endings[1]. This fact has made it possible to study in detail the intracellular localization of these compounds. Epinephrine was found to be stored in relatively heavy granules in the adrenal medulla[2], and in similar granules in chromaffin cells of cyclostome hearts[3]. Recent work has suggested that norepinephrine is also retained in particulate material, as yet unidentified, in splenic nerves[4,5], brain[6], and puppy hearts[7]. We wish to report that norepinephrine is predominantly localized in small particles which sediment with the microsomes.

Sprague–Dawley male rats weighing 140–160 gm. were given 50 μc. of tritiated-norepinephrine (20 mc./mgm.) intravenously, and were killed 30 min. later by a blow on the head. Tissues were removed immediately and washed in ice-cold sucrose. Homogenization in 5 vol. of isotonic sucrose was performed with a loose-fitting glass pestle for 2 min. (heart) or 0·5 min. (other tissues) in a salted ice bath.

Subcellular fractions were separated by a single centrifugation in a continuous sucrose density gradient prepared as follows. A small vertical tube containing isotonic sucrose was tightly fitted over a 5-ml. flask so as to communicate through a fine needle. The lower flask was initially filled with 60 per cent sucrose and was mixed by a magnetic stirrer. A side-arm with a fine tip at the bottom of the flask permitted slow outflow into an ultracentrifuge tube. The dilute solution dripped slowly into the flask, progressively diluting its contents and producing a gradient in the outflow varying from 60 to 10 per cent sucrose. Tissue homogenates were carefully layered on the surface of the sucrose gradients, and the tubes were centrifuged in the Spinco ultracentrifuge using an *SW*39 rotor at 35,000 r.p.m. for 30 min.

Fig. 1 shows the appearance of a sucrose gradient with heart homogenate, after centrifugation. Five distinct layers were found: a nuclear and cellular sediment on the bottom, a hazy band of muscle debris, a thin red hæmoglobin layer, a yellowish mitochondrial region, and a faintly cloudy microsomal band below the clear supernatant fluid. Approximately 70 drops were collected with a needle through the bottom of each tube, and these were analysed for tritiated and endogenous catecholamines and succinic acid dehydrogenase activity. Total radioactivity was assayed in a liquid scintillation counter; about 90 per cent of the total radioactivity was found to be tritiated-norepinephrine by specific assay[8]. Endogenous catecholamines were assayed fluorometrically, either directly or after alumina column purification[9]. Results were found to be the same by the two techniques. Succinic acid dehydrogenase activity was assayed by a modified tetrazolium dye reduction method[10].

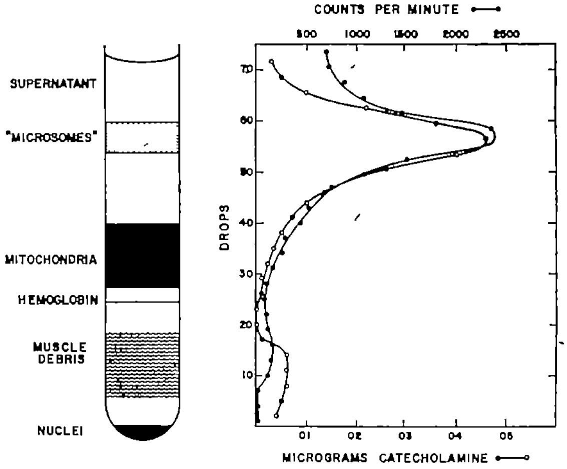

Fig. 1. Intracellular localization of tritiated norepinephrine and catecholamines in the rat heart

Fig. 1 shows the intracellular distribution of endogenous and tritiated-norepinephrine in the heart. The predominant peak of these catecholamines coincided with the 'microsomal' band. Small amounts were found in the nuclear pellet (1,092 c.p.m.) and muscle fragments. Negligible quantities were present in the mitochondrial fraction, where virtually all the succinic acid dehydrogenase activity was found. When the 'microsomal' particles from the heart were sedimented by centrifugation and then resuspended by gentle homogenization in isotonic sucrose, they retained 80 per cent of their tritiated norepinephrine. Particles suspended in water, however, lost more than 80 per cent of their ^{3}H-norepinephrine. In similar experiments, administered tritiated-epinephrine was also found in the 'microsomal' layer of the heart whereas tritiated-DOPA, tritiated-dopamine, and tritiated-normetanephrine appeared wholly in the supernatant fluid.

In the salivary glands both radioactive and endogenous epinephrine and norepinephrine were also found in the lightest particles. In the adrenal medulla most of the endogenous catecholamines and all tritiated-epinephrine and tritiated-dopamine were in heavy granules near the bottom of the tube. In contrast, tritiated-norepinephrine in the adrenal glands was predominantly found in the 'microsomal' band. Thus epinephrine and norepinephrine appear to be predominantly present in separate adrenal particles, but in the same heart particles. Tritiated-DOPA was found only in the adrenal supernatant. In the brain, radioactivity after the administration of tritiated-DOPA was found in an intermediate layer above the mitochondria, corresponding in sedimentation characteristics to the "pinched off nerve endings" studied by Whittaker[11]. Endogenous 5-hydroxytryptamine, assayed fluorometrically[12], was predominantly found in this layer as was tritiated-norepinephrine taken up by brain slices *in vitro*.

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Potentiation of Narcotic Action by the Brain treated with an Electric Shock

Differences in the biological action on animals and man of homogenates from normal brains and from brains which had undergone electric shock have been shown. They take the form of pharmacological activity on sleep[1] and against phenol intoxication[2] as well as of a protective effect against neurotropic viruses[3-5] and the behaviour in clinical trials[6].

In the present work the effect of homogenates from brain subjected to an electric shock on barbiturate-induced sleep was examined in an attempt to estimate the active compounds present in them.

Hogs weighing about 100 kgm. received 15 electric shocks on alternate days and were killed one day after the last treatment. Brains were removed under sterile conditions and stored at − 20° C. until needed. Before each experiment a 10 per cent homogenate in physiological saline was prepared. Controls were made with normal brains obtained and treated under similar conditions.

Experiments were performed on rabbits of average weight 1·5 kgm. 'Evipan' (N methylcycloesenylmethylmalonylurea Bayer) dissolved in physiological saline was administered through the marginal vein of the ear as rapidly as possible. The threshold dose for immediate sleep was 15 mgm./kgm.

The period of sleep with 'Evipan' alone was first established for each animal. The effect of the administration of brain homogenates on narcosis was afterwards assayed by injecting the homogenates (2·5 ml./kgm.) into the gluteal muscles, 30 min. prior to 'Evipan'. Each animal was treated, in separate experiments, with brain subjected to electric shock and normal brain, one half the group receiving brain subjected to electric shock, the others normal brain. Three days elapsed between each treatment.

Two doses of 'Evipan' were used: a threshold dose for sleep of 15 mgm./kgm. and a larger dose of 25 mgm./kgm.

Results are given in Table 1.

Though a small increase in period of sleep is observed after administration of normal brain, little statistical significance can be given to it. On the other hand, the increase due to brain subjected to electric shock is more pronounced and significant.

Interference of effects due only to the administration of normal brain substance can be excluded, in evaluating the results, by directly comparing the results in columns III and I. It appears that the effect of substances produced in brain following electric shock treatment is particularly significant with doses of 'Evipan' larger than the limiting dose.

The presence in brain subjected to electric shock of substances acting on sleep was foreshadowed by Longhi[1], who, by instilling such brain homogenates, into the third ventricle of dogs, induced sleep immediately while he did not with homogenates from normal brain.

The substances active on 'Evipan'-induced sleep shown by the work reported here are likely to be related to those mentioned above. Little can be inferred, however, about their nature.

It can only be said that they act in very small doses: in fact 2·5 ml. of a 10 per cent homogenate correspond to only 65 mgm. dry brain substance[7,8] administered per kgm. of animal treated. Moreover, the absorption, after intramuscular injection, is slow and presumably

Table 1. Sleeping Period with and without Administration of Brain subjected to Electric Shock

			I 'Evipan' alone	II 'Evipan' + normal brain	III 'Evipan' + ES brain	Differences		
						II–I	III–1	III–II
			$n=10$	$n=15$	$n=16$	$n=9$	$n=10$	$n=15$
	Significance	P	<0·001	0·002	<0·001	0·15	0·025	0·15
		t	6·094	3·994	4·482	1·625	2·691	1·468
Dose of 'Evipan'	15 mgm./kgm.	Sleeping time	5·1±0·8	7·5±1·9	11·0±2·5	5·6±3·4	9·7±3·6	3·1±2·0
			$n=14$	$n=14$	$n=14$	$n=14$	$n=14$	$n=12$
	25 mgm./kgm.	in min. and min./10	16·6±1·0	17·4±2·1	20·4±1·9	1·1±2·4	3·8±1·9	4·1±1·9
		t	15·84	8·447	10·61	0·4708	1·973	2·083
	Significance	P	<0·001	<0·001	<0·001	>0·5	0·075	0·05

10 per cent homogenates of brain of electric shock treated hogs (ES brain), and (controls) homogenates from untreated hogs, were injected intramuscularly to rabbits (2·5 ml./kgm. body-weight) 30 min. prior to intravenous administration of 'Evipan'.

not total. They seem also rather stable: brains used after a 10 months storage at − 20° C. were found still to possess the properties described.

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Effect on the Thyroid Gland of Protracted Administration of Posterior Hypothalamic Extracts

As a follow-up of our investigations of the neurosecretory function of diencephalon[1] we have examined the effect on the histophysiology and respiratory function of the thyroid glands of rats of protracted administration of extracts of different regions of the hypothalamus and of the cortex.

Table 1. EFFECT OF EXTRACTS OF POSTERIOR HYPOTHALAMUS ON THE THYROID GLAND OF WHITE FEMALE RATS

	Treatment	Thyroid gland mgm./100 gm.	*P*	Acinar cell height*	*P*	K^{131}I intake (24 hr.)†	*P*
(I)	Rat cortex (10)	9·70 ± 0·43	< 0·01	5·04 ± 0·17	>0·05	—	—
	post. hypoth. (10)	13·41 ± 1·09		5·95 ± 0·54			
(II)	Cattle cortex (11)	11·37 ± 0·26	= 0·01	4·85 ± 0·14	< 0·01	—	—
	post. hypoth. (4)	18·10 ± 2·48		6·65 ± 0·55			
(III)	Rabbit cortex (8)	10·61 ± 0·50	< 0·01	—	—	31·51 ± 2·42	= 0·02
	post. hypoth. (7)	14·07 ± 0·71				22·66 ± 2·50	

(I) 0·1 per cent extract; daily dose, 1·0 ml.; length of treatment, 14 days.
(II) Filtrate of 25 per cent homog.; daily dose, 1·0 ml.; length of treatment, 14–26 days.
(III) Extract containing 1/6 of posterior hypothalamus for one day. Length of treatment, 14 days.
* In units of ocularmicrometer 100 cell/thyroid gland.
† Activity of 10 mgm. tissue of thyroid gland in per cent of injected activity.

Daily administration for at least a fortnight of a physiological saline extract obtained from the posterior hypothalamus of rats, cattle and rabbits increased, statistically significantly, the weight of the thyroid gland of white adult female rats, kept on a standard mixed diet. This effect was not obtained by administration of saline extracts of the cortex. Quantitative examination of the height of thyroid acinar cells has shown an increase which was in the second experiment significant and in the first nearly so. The administration of an extract obtained from the anterior or medial part of the hypothalamus did not produce such a reaction. The increase of weight of the thyroid gland is not accompanied by an increased ability to concentrate iodine, and even the intake of radioactive iodine in 24 hr. is, referred to the unit of weight, lower than in the group treated with cortical extract (Table 1). The administration of posterior hypothalamic extracts, after previous treatment with thyrotropic hormone ('Ambinon'), impeded the decrease in weight of the thyroid gland to the normal size. The decrease in the height of acinar epithelium was moderately influenced by this treatment. Changes in the thyroid gland induced by previous treatment with 4-methyl-2-thiouracil (a 0·15 per cent diet for 15 days) were not influenced significantly by administration of a suspension of the posterior hypothalamus.

Administration of prepared suspensions of the brains of old rats (0·8 per cent), continued for a fortnight, did not cause any difference in the function of the thyroid gland. Treatment of infantile rats with a suspension of posterior hypothalami of rats (a daily dosage of an animal containing one-third of the posterior hypothalamus) for six days resulted in narrower acini which contained less colloid and had a higher epithelial lining as compared with the group treated with a cortical suspension. The increase in height of the acinar cells and the decrease of the acinar diameter is equally significant ($P<0\cdot01$, $P<0\cdot05$). In this experiment there was no difference in weight.

A systematic investigation of supposed diencephalic neurohumoral mediators affecting the thyroid gland has been initiated by other authors[2–5]. Ottaviani and Azzali[5], having injected a lipoid fraction of diencephalic extracts of cattle into dogs and hamsters for a variable period, noted in the case of low doses a histological picture which signified an increased secretory activity of the thyroid gland, and in the case of high doses a colloid storage. Shibusawa *et al.*[6] isolated from the raw extract of the urine an active thyroid-stimulating hormone, thought to be of anterior hypothalamic origin; and Schreiber *et al.*[7] demonstrated a hypothalamic factor, increasing the acid phosphatase activity of the thyreotrophic cells of the anterior pituitary.

Our own results indicate a hypothalamic factor appearing to be concentrated in the posterior hypothalamus, affecting the function of the thyroid even of infantile animals, which, however is considerably decreased or has even disappeared in old rats. From these results it may be supposed that this hypothalamic factor is distinguished from the TRF demonstrated by Shibusawa *et al.* not only by its localization but also by the fact that it decreases the intake of iodine simultaneously with increasing the weight of the thyroid gland. We do not think it likely that an effect which would primarily aim at the cortex of the adrenal gland would be based on adrenocortical-thyroid antagonism, because we have observed some adrenocortical stimulation only after administration of more concentrated anterior hypothalamic extracts without changes in the thyroid gland. At present we venture to suppose an inhibitory effect which seems to be similar to that of goitrogenic agents.

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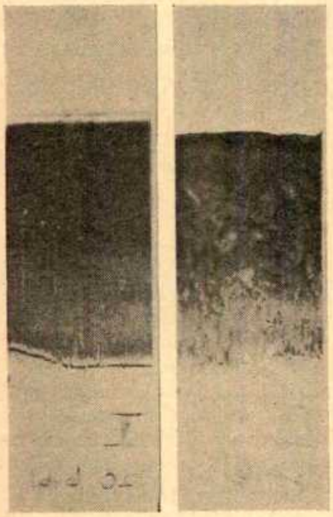
Bovine Bracken Poisoning

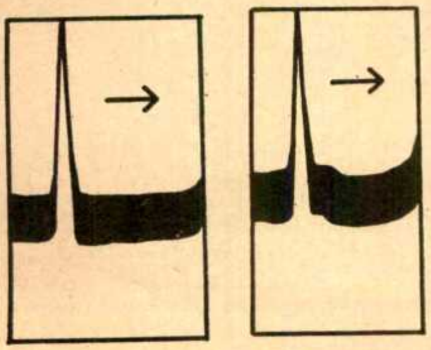
WHILE preparing a blood smear for the differential leucocyte count, it is found that the blood of cattle in the terminal stages of bracken poisoning does not spread evenly in the manner of a normal sample, but presents a blotched or streaky appearance[1] (Fig. 1). Bracken plasma contains increased amounts of fibrinogen[2], but streaking is not produced if commercial bovine fibrinogen is added to normal blood, to give equivalent concentrations[3]. Nevertheless, we have established that the streaking is connected with the fibrinogen, and have reason to suppose that a small, altered fraction is the cause. Ultracentrifuge readings suggest that since this fraction runs more rapidly, it has a larger molecular weight than the main body of fibrinogen which runs normally (Fig. 2).

Many references in the literature[4-6] indicate that heparinoid substances of large molecular weight with sulphated groups and strong negative charge have an affinity for fibrinogen, forming reversible polymers. (Compare the behaviour of polysaccharides containing sulphuric acid esters with dye molecules and the resulting metachromasia[7].) These abnormal fibrinogens have a tendency to be cold-insoluble and are often connected with a higher erythrocyte sedimentation-rate. They increase in amount in certain disorders notably of a rheumatic or febrile nature[5,8]. These considerations and the fact that the blood of irradiated rabbits contains increased amounts of heparin[9] led us to follow the blood heparin-levels in a calf with experimentally-induced bracken poisoning, the results obtained being positive with a terminal increase (Fig. 3). The release of heparin in abnormal amounts into the circulation suggests an involvement or destruction of body mast cells. Since these cells also store histamine, it is reasonable to suppose that this toxic amine may also be present in increased



Normal Bracken

Fig. 1



Normal Bracken

Fig. 2

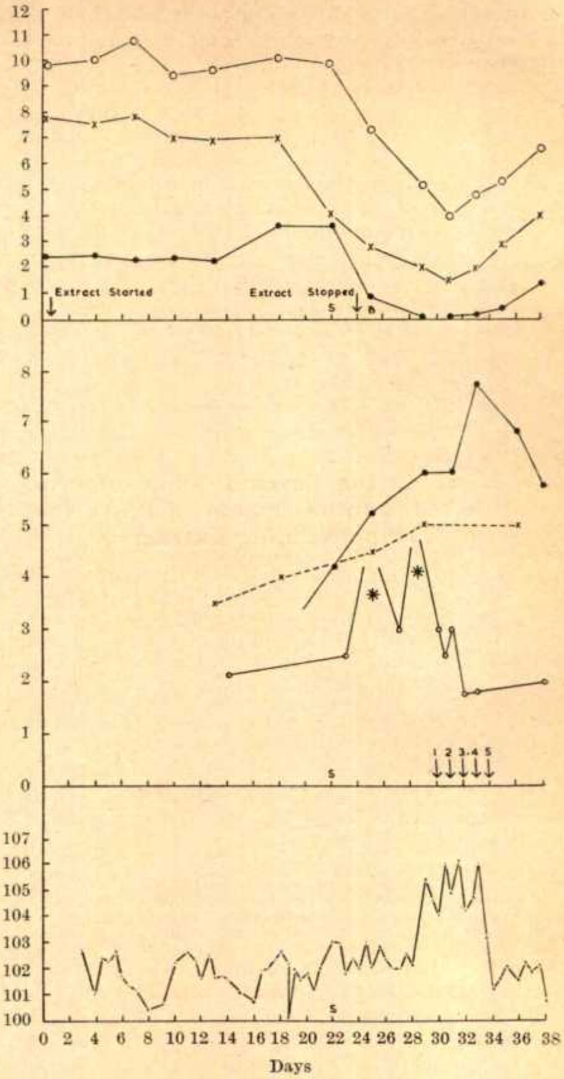


Fig. 3. Calf receiving alkaline ether-soluble fraction of bracken frond by drench

Top, ○, Leucocytes × 10^3/mm.^{3}; ×, platelets × 10^5/mm.^{3}; ●, neutrophils (percentage of differential count); *B*, 2·5 per cent basophils in differential count.

Middle, ●, Fibrinogen (optical density at 540 mμ × 10^{-1}); ×, erythrocyte sedimentation-rate (mm. per 24 hr.); ○, blood heparin-level (γ's protamine sulphate × 10, required to clot blood); *, incoagulable with 35 γ-protamine sulphate. Injections: (1) 250 mgm. toluidine blue in 250-ml. saline (intravenous); (2) 150 mgm. 'Anthisan', 500 mgm. batyl alcohol dispersed in physiological saline by 'Tween' 20 and 80, 18 mgm. 'Predsolan' (intramuscular); (3) 250 mgm. toluidine blue in 250 ml. as before, 150 mgm. 'Anthisan' (intramuscular); (4) 100 mgm. 'Anthisan', 700 mgm. batyl alcohol (intravenous); 20 mgm. 'Predsolan' (intramuscular), 4 ml. 'Distavone' (intramuscular); (5) 4 ml. 'Distavone' only (intramuscular).

Bottom, ·——·, Temperatures (° F.) morning and evening; *S*, blood smears begin to show irregular streaking quantities in a biologically active form[10]. Our present experiments are directed to test this theory. An increase in free heparin and histamine would help to explain the prolonged clotting-time and increased capillary fragility known to accompany bracken poisoning.

In spite of lack of definite knowledge about histamine-levels, we tried the following combined therapy: (1) Toluidine blue, intravenous (anti-heparin); (2) 'Anthisan', intramuscular (anti-histamine); (3) 'Predsolan', intramuscular (hydrocortisone treatment); (4) batyl alcohol, intravenous (bone marrow stimulant); (5) 'Distavone', intramuscular (precautionary antibiotic indicated by leucopenia). (The dosages and time-intervals were purely experimental and both these and the use of cortisone treatment and 'Tween' will obviously be open to revision.)

The recovery of the five-month-old calf, together with clinical records and details of the therapy, are given in Fig. 3. Two field cases of four-month-old calves have also responded to this treatment. It should be pointed out that recovery of calves of this age from acute bracken poisoning is rare.

Since bracken poisoning is a radiomimetic condition, we suggest that these findings may be relevant to irradiation sickness. It would be interesting to know if a combined therapy of this nature would give similar hopeful results. One of us (I. A. E.) thanks the Agricultural Research Council for support and R. M. H. acknowledges a Ministry of Agriculture, Fisheries and Food postgraduate studentship. We thank Mr. J. T. Bowen, Department of Biochemistry, University of Leeds, for carrying out the ultracentrifuge runs.

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HISTOLOGY

Histology of Omental Reactivity

Comparative histological examinations of the greater omentum have shown that the characteristic 'milk spots' of the human omentum are an equally constant and striking feature of this membrane in the pig but appear to be absent in dog and cat. These structures have been examined in whole spread preparations of human and pig material stained with Weigert's hæmatoxylin, Mallory and toluidine blue for cytological detail and by the Falgog method[1] for the vascular pattern.

In both species the spots consist of a thin reticular capsule and stroma, densely packed with cells and traversed by two or more blood-vessels which form a glomus-like network within the cellular mass.

In man, the cells of the milk spots are predominantly mature lymphocytes. In the pig, lymphocytes are replaced by large, pale-staining multi-nucleolated cells intermingled with plasma cells in a pattern essentially similar to the germinal centre of the lymphoid nodule. The very numerous mast cells distributed along the omental blood-vessels showed much the same abundance in man and in the pig.

Two preliminary types of experiment have been carried out in the pig: one, the induction of shock by traction on the omentum, and the other, the production of hæmorrhage in the peritoneal cavity by puncture of a gastro-epiploic vein. Traction shock produced a drop in the total apparent mast cell population. This was accompanied by an extrusion of the large, basophilic granules from approximately 40 per cent of the remaining mast cells. Intraperitoneal hæmorrhage produced a similar drop in the total apparent mast cell population and diffusion of the metachromatic intergranular substance from approximately 70 per cent of the remaining mast cells into the surrounding tissue.

Roberts[2] stated that antigen injected into the peritoneal cavity is taken up by the milk spots of the omentum and the draining lymph nodes. More recently, Walker and Rogers[3] have described the role of the rabbit omentum in antibody synthesis.

The experimental findings outlined here appear to throw some further light on the known reactivity of the omentum to trauma.

Further investigations are proceeding along two lines, namely, the significance of the glomus-like formations already mentioned and the histochemistry of the reactions which have been described.

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Staining Characteristics of Chromaffin Granules in Human Skin

Reports of the presence of chromaffin cells in human skin[1-3] are of importance, because this work could, if confirmed, open a new concept in the control of the peripheral circulation[4]. Changes have been found to occur in these cells in the cat's tail and nictitating membrane after reserpine and denervation which could indicate their association with the sympathetic system[5]. Burn and Rand[6] have further suggested that these cells might be the site of storage of noradrenalin in the sympathetic post-ganglionic mechanism.

Adams-Ray and Nordenstam[1,2] and Burch and Phillips[3] have emphasized the superiority of the Sevki stain (a modified Giemsa stain) for demonstrating red chromaffin granules in chromated skin. However, using similar techniques, Mercantini[7] found it impossible to differentiate mast cell granules staining purple or bluish-red from other granules staining clear-red or bluish-red presumed to be chromaffin granules. To support their claims that a red colour indicates chromaffinity Adams-Ray and Nordenstam[1,2] have referred to Pearse[8], but have, in addition, reported the same reaction in adrenal medulla treated identically. However, Pearse[9] states that the Sevki method applied to chromated tissue produces not a red colour but a green colour in the presence of adrenalin or noradrenalin. Also, Coupland[10] found that the Giemsa stain after formal dichromate fixation produced a green colour in adrenal medullary cells.

We have confirmed that the brown chromaffin substance in chromated adrenal medulla (Orth's solution) turns green when stained with the Sevki technique of Burch and Phillips[3]. This green colour in mouse adrenal was greatly reduced by previously treating the animal with reserpine.

We are unable to suggest why the previous investigators[1-3] did not find a green colour in adrenal medulla using similar techniques.

We have proceeded to examine fresh chromated human skin for the presence of chromaffin-positive granules which might turn green on staining with Sevki's method. The only cells producing a brown colour in the dermis were dermal chromatophores and red blood cells. The natural colour of the pigment in chromatophores was indistinguishable from that produced by a true chromaffin reaction whether or not the skin was chromated. These chromatophores frequently had bipolar cytoplasmic projections containing granules of variable size. On staining with the Sevki method the pigment in chromatophores frequently developed a green tinge whereas the brown chromaffin-positive red blood cells remained unchanged.

Many elongated cells with fine red granules, described by others to be chromaffin cells, were seen in the Sevki-stained sections. However, there was no suggestion of a green colour in these cells, which indicated that the granules were unlikely to contain adrenalin or noradrenalin.

Since mast cell granules stain in an identical manner and both these and 'chromaffin cell' granules are metachromatic with toluidine blue, we suggest that, despite differences in morphology, the 'chromaffin cells' in human skin are mast cells.

From these observations we conclude that if the post-ganglionic transmission theory of Burn and Rand[11] applies to human skin, the peripheral stores of noradrenalin are unlikely to exist in the 'chromaffin cells' described by Adams-Ray and Nordenstam.

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HISTOCHEMISTRY

Immunohistochemical Localization of Drug-binding Protein in Tissues of the Electric Eel

A PROTEIN having a high affinity for *d*-tubocurarine has been isolated from ammonium sulphate fractions of extracts of electric organ of electric eel[1,2]. Although *d*-tubocurarine combines with a variety of macromolecules[3-5], the reaction with the electric organ protein appeared to be somewhat unique, especially since the resulting complex is very stable to high ionic strength[1]. The protein also combines in a more or less specific way with a large number of neurotropic compounds, the relative affinities in general paralleling the effectiveness of these compounds in their pharmacological action upon the single isolated electroplax[1,2,5,6] and squid giant axon[7,8]. Although it was proposed[1,2] that the protein might function *in vivo* as a 'receptor' for acetylcholine and other neurotropic compounds, more recent evidence has led to a suggestion of an entirely different role[9,10], namely, that the protein might be the drug-binding component of conducting membranes only and thus is not involved in the physiological effects of acetylcholine and other depolarizing agents. The distribution of this material in the electroplax is thus of primary importance in determining whether or not the conclusions regarding its proposed biological significance are justified.

The high solubility of the protein in aqueous solution, the fact that it does not gel on heating, and its antigenic properties (as described here), indicate that it most likely is not a component of connective tissue fibres. The protein contains a significant amount of phospholipid, primarily in the form of lecithin, and this suggests the possibility of a membranous origin. One method for rather precise localization of the protein is by means of the fluorescent antibody technique, the results of which are reported in this communication.

Rabbit antiserum was prepared to an alum precipitate of the purified protein and used as an immunohistochemical reagent. Pooled antisera were first subjected to sodium sulphate fractionation to obtain a crude globulin fraction. This was tested against the original protein antigen by the Ouchterlony gel diffusion method which revealed the presence of two bands, one of far greater intensity than the other. This finding correlates with results of free electrophoresis and ultracentrifugation, both of which showed the presence of a 10 per cent impurity in the preparation[11]. The crude globulin was then conjugated with a fluorescein derivative according to the method of Coons and Kaplan[12]. After appropriate absorption and dilution procedures, the fluorescein-labelled antibody was used as a (specific) stain for the detection of the original antigen (receptor protein) in frozen sections of various electric eel tissues, including single isolated cells (electroplax) from the main organ. After staining, sites at which the protein is present were rendered visible as areas of bright green fluorescence under the fluorescence microscope. Specificity of staining was established by conventional means including the demonstration of: (1) inhibition of tissue staining by pre-treatment with unlabelled antibody; (2) failure of tissue staining by a fluorescein-globulin preparation with unrelated or no antibody activity.

When sections of single eel electroplax were examined by this method, the receptor protein was found to be present in, or in close proximity to, both

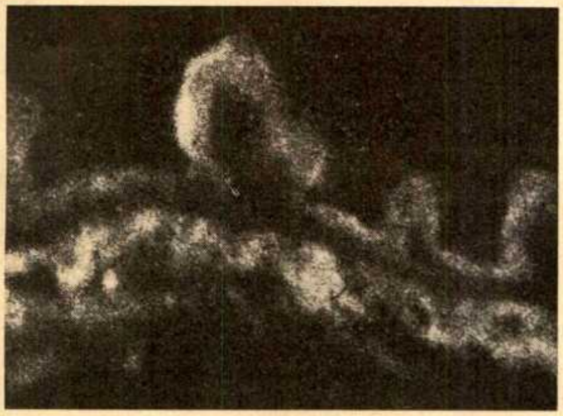

Fig. 1. Localization of drug-binding protein in the electroplax of electric eel. Areas where the protein is present are white. Specific staining may be seen at both the innervated (bottom) and non-innervated membrane (top), as well as in the loose connective tissue on the innervated side

the innervated and non-innervated membranes (Fig. 1). No protein could be demonstrated in the cell interior. In these sections, there appeared to be some specific fluorescence in the region of the nerve supply to the electroplax. Interestingly, prior application of either *d*-tubocurarine or protamine, both of which precipitate the protein *in vitro*, failed to reduce the intensity of tissue staining, suggesting that the drug combining sites and antigenic sites of the protein are distinct. This finding was confirmed in test-tube experiments, that is, neither *d*-tubocurarine nor protamine reduced the amount of receptor-antibody precipitate.

In other work using the fluorescent antibody technique[13], receptor protein was found to be absent in eel serum and in eel red blood cells. In eel muscle, it was found in the form of a fine trabecular network which surrounds and enmeshes muscle fibres. Muscle sarcolemma and muscle cell interior do not contain the protein. There was no observable difference in the pattern of staining in muscle which had previously been denervated. Heart muscle stained in a manner similar to that described for skeletal muscle. The protein could not be demonstrated in nerve cell bodies or in heavily myelinated axons. It was found, however, in the form of a membrane surrounding vagus nerve fibres.

Skeletal muscle and heart taken from other species did not stain with the fluorescein-tagged antibody, thus confirming the specificity of the reaction with electric tissue component.

These results point to a sharply localized and apparently characteristic distribution of this drug-binding protein in excitable tissues; together with the somewhat unique *in vitro* properties of this material, they strengthen the suggestion for an *in vivo* role in neurophysiological processes. However, the weak binding of acetylcholine and other potent depolarizing compounds, for example, decamethonium, carbamylcholine, etc., together with the fact that the protein is in or near both the innervated and non-innervated membranes of the electroplax, constitute difficulties in assigning to this protein the function of physiological acetylcholine receptor substance of the end-plate region. The present results would appear to strengthen the newer hypothesis[9,10] that the protein may serve as a model for the component of conducting membranes of electroplax and squid axon with which drugs in general combine when they exert their actions on these systems. On this basis, the weak actions of acetylcholine, decamethonium, carbamylcholine, prostigmine and eserine, on specially treated nerves, for example, cobra venom[8], or cationic detergent[14], are predicted, as is the much greater effectiveness of *d*-tubocurarine, chlorisondamine and protamine under similar circumstances.

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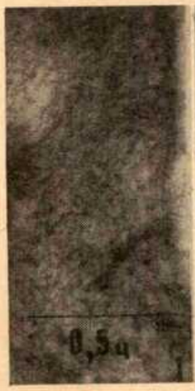
PATHOLOGY

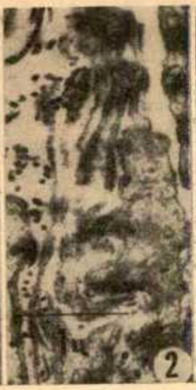
Fine Structure of Tumour Stroma and Blood Vessel Stroma in Human Supratentorial Meningioma

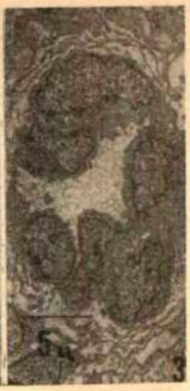
Meningiomas generally provoke a higher degree of vascular proliferation than do other benign tumour cells. Vascularization in the different meningioma groups is in some respects similar to, in others different from, the vessel systems in the respective tissue of origin.

The material used consisted of samples of supratentorial meningioma and tissue of origin, that is, dura and arachnoidea, from 14 patients and samples of normal cerebral tissue containing blood vessels from 5 patients with other brain diseases. The specimens were fixed in osmic acid, stained with phosphotungstic acid or uranylacetate and embedded in methacrylate and araldite. Ultra-thin sections were cut on a Porter–Blum microtome and examined with a Philips *EM* 100 *B* electron microscope. The material was also analysed with a light microscope, using different connective tissue stains.

Electron microscopic examination of the vessels in endotheliomatous meningiomas showed an abundance of structures generally formed of polysaccharides. Basement membranes could be seen in nearly every border area between endothelial cells, connective tissue processes and perivascular cells. Basement membranes were seen even within the connective tissue processes (Fig. 2) and in the cytoplasm of endothelial cells (cf. ref. 1). In the larger vessels the







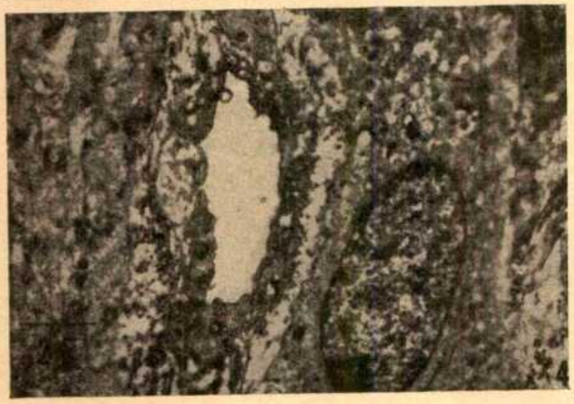


Fig. 1. Collagenous microfibrils in endothelium of vessel wall in a sphenoidal, fibromatous meningioma. (× 40,500)

Fig. 2. Basement membranes in connective tissue processes of vessel wall in a parasagittal, endotheliomatous meningioma. (× 15,200)

Fig. 3. Proliferation of endothelium of a capillary in an angiomatous meningioma. (× 1,800)

Fig. 4. Arteriole in an endotheliomatous meningioma of tuberculum sellæ. The number of collagen fibrils per area unit of ground substance is nearly the same in the vessel stroma (centre) as in the tumour stroma (right). (× 4,800)

amount of ground substance in the walls was more important in relation to the frequency of collagenous and reticular fibrils in the substance than in the vessel walls of fibromatous and angiomatous meningiomas. The periodicity of the collagen was about 640 Å. The breadth of the basement membranes was generally 500–1200 Å., but thicknesses of up to 2000 Å. could be observed. It has been emphasized[2] that meningiomas in their degenerative state, and especially as cysts, are rich in polysaccharides, and that the process of regression starts from the basement membranes.

Fibromatous meningiomas closely associated with the dura generally showed a sparser vascular network than the endotheliomatous meningiomas. The endothelial cells and the connective tissue layers in the vessel walls of fibromatous meningiomas contained bunches of microfibrils (Fig. 1). It is possible that these microfibrils mark a stage in the homogenizing collagenosis of vessels, typical of these meningiomas. The elastin frequently was situated peripheral to this layer, and collagenosis probably had begun from the inside of the vessel (cf. ref. 3). The elastic fibres had a very 'looping' course, but they were of normal fine structure. In the light microscopic examination this 'looping' elastin had a 'granular' appearance in cross-section. In the electron microscopic examination the number of collagen fibrils per area unit of ground substance was much greater in the tumour stroma than in the stroma of vessel walls. This relation was the same in the tissue of origin, that is, in the dura. In the endotheliomatous meningiomas the number of collagen fibrils per area unit of ground substance was nearly the same in the vessel walls, in the tumour stroma and in the tissue of origin, that is, the arachnoidea, of the same cases (Fig. 4). The periodicity of all collagen fibrils was the same in fibromatous and endotheliomatous meningiomas.

In the angiomatous meningiomas the blood vessels showed a high degree of endothelial proliferation (Fig. 3). Total obliteration of the vessels has been stressed as a contributing factor in the calcification process which is typical of this tumour[3]. Vascular dilatation was frequently observed. Dilated vessels have been seen even in the normal subdural appendicular vessel layer[2]. The number of fibrils per area unit of ground substance in the angiomatous meningiomas and in the subdural appendicular vessel layer in the same cases was greater in the vessel stroma than in other tissue stroma. The periodicity of collagen was the same as in other types of meningiomas.

The present findings seem to confirm the mesenchymal origin of all meningioma types as well as to clarify some reasons for the type of regressive alterations in meningiomas.

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Urethan Induction of Thymic Lymphoma in *C57bl* Mice

URETHAN (ethyl carbamate) has been reported to augment the induction of lymphoid leukæmias by X-ray, œstrogen or methyl cholanthrene[1]. Since urethan alone did not augment the incidence of tumours in these low-leukæmia strains, it was suggested that it be classified as a 'co-leukæmogen'. The drug has not been found to affect the incidence of leukæmia in the high-leukæmia *AKR* and *C58* strains[2], and it has caused only a slight shortening of the latent period in them. However, in Swiss albino mice injected at birth[3,4], as well as in adults given toxic amounts of the drug in the drinking water in two divided 10-day intervals[5], there has been a moderate augmentation of leukæmia incidence and shortening of the latent period. Untreated mice of this strain have a fairly low incidence of leukæmia that is influenced by the sex. Berenblum and Trainin[6] found that urethan augmented leukæmogenesis by X-ray in the low-leukæmia *C57bl* strain only if it was given during or after the X-ray treatment. They have presented evidence that the tissues of *C57bl* mice immediately after irradiation contain a factor which, together with urethan, augments the incidence of leukæmia in this (low-leukæmia) strain[7]. This factor was not found in the tissues of non-irradiated mice but it was still present in the tissues 30 days after irradiation. They suggest that urethan acts as a 'promoter' in a two-stage mechanism that implies the necessity of an 'initiating' factor.

The radiation-induced leukæmia of *C57bl* mice has been shown to arise in the thymus[6]. This communication reports the induction of thymic lymphoma in 12 out of 13 *C57bl* mice surviving repeated toxic dosages of urethan alone. The stock of mice was obtained from the Texas Inbred Mouse Company, Houston, Texas. The incidence of thymic lymphoma in untreated mice in this colony is 3 per cent at 15 months of age (Dmochowski, L., personal communication).

Urethan was injected subcutaneously into mice less than 24 hr. old at a dose of 1 mgm./gm. body-weight. Seven further injections at the same dose were given at weekly intervals. The young were separated from their mothers for the period of anæsthesia following injection. Despite this precaution, a high mortality was observed, due in part to maternal cannibalism. Thirteen animals survived the injection series, although one dying at 10 weeks of age was too autolysed for histological examination, leaving a total of 12 determinate mice. Tumours were found in all twelve of these at autopsy (Table 1) between 12 and 27 weeks of age. The mean age (17 weeks) at which tumours were discovered was considerably less than the mean latent period (24 weeks) reported for the radiation-induced lymphoma in this strain[9]. We have observed no lymphoid tumours in 30 untreated animals in this laboratory followed to about one year of age.

Table 1. INCIDENCE OF THYMIC LYMPHOMA IN URETHAN-TREATED *C57bl* MICE

				Tumour-bearing mice	
litter No.	No. of mice	Died during treatment	Net No. of mice	No.	Mean age (weeks)
1	7	3	4	4	16
2*	7	3	3	3	20
3	4	3	1	1	12
4	6	2	4	4	15
Total	24	11	12	12	(av.)17

* One animal in this litter died at 10 weeks without histological examination.

The tumours were indistinguishable histologically from the thymic lymphoma which is induced by irradiation in this strain[8]. However, the pattern of dissemination (Table 2) was not identical to that reported for the radiation-induced thymic lymphoma. Dissemination was found in 7 of the 12 animals (58 per cent). Sections of thymus, lymph nodes, lung, liver, spleen, kidney, testis and small intestine were examined routinely. A noteworthy absence of identifiable dissemination to the spleen may be a result of the early deaths.

Table 2. DISTRIBUTION OF LYMPHOMA IN TUMOUR-BEARING MICE

		Local spread	Dissemination			
	Thymus	Mediastinum	Liver	Lung	Kidney	Lymph nodes
No. examined	12	12	12	12	8	9
No. with tumour	12	9	6	5	3	2
Per cent with tumour	100	75	50	42	37·5	22

We have also injected *C57bl* mice intraperitoneally with the same dose of urethan beginning between 4 and 5 weeks of age and continuing weekly for a total of 4 treatments. Of 19 mice receiving this treatment, and followed to one year of age, only one developed lymphoma at 10 months.

It is apparent that urethan administered to newborn *C57bl* mice will induce thymic lymphomas with a frequency approached only by that following divided doses of whole body X-irradiation. It remains to be shown whether urethan acts directly on the thymus as the initiator of these tumours or whether its effect, like that of X-rays[10], is indirectly mediated. It may be significant that urethan has been shown to affect mitosis and maturation of bone marrow cells[11] and that it abolishes the protection of bone marrow shielding against radiation-induced thymic lymphoma[1].

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Lipid Content in Hyperdiploid and Hypertetraploid Cells isolated from the Hyperdiploid Ehrlich Ascites Tumour

THE hyperdiploid Ehrlich ascites tumour, described by Hauschka *et al.*[1], contains about 5 per cent hypertetraploid cells. A varying amount of non-tumour cells (leucocytes) always occurs. By counter-streaming centrifugation a fraction of hyperdiploid cells could be obtained free from hypertetraploid cells which were concentrated into another fraction[2]. Inoculated into another mouse this fraction developed into a tumour with a higher proportion of hypertetraploid cells than the original one. Repeated fractionation followed by inoculation of this fraction increased the mean number of chromosomes per cell to about twice the original one, the mean number of chromosomes being estimated as the amount of deoxyribonucleic acid phosphorus (DNAP) per cell.

The hypertetraploid cells with a volume twice that of the hyperdiploid ones could be expected to have the highest relative sedimentation-rate of all the cells of the tumour. Nevertheless at counter-streaming centrifugation they behave as if they were smaller or specifically denser than the hypderdiploid cells. When investigating the reason for this unexpected distribution it was observed that small structures, previously referred to as 'vacuoles'[3] and occurring on the surface as well as in the interior of the tumour cells, are more abundant in large than in small cells[2]. These vacuoles are spherical, are strongly refractive, stain intensely black when treated with sudan *B* according to Baker, and Chiffele and Putt[4], and are thus made up of lipids. Accordingly their specific density is low as appears from the fact that, when liberated from the surface of the cells by handling of the suspensions, they accumulate on the top of the supernatant on prolonged centrifugation (cf. also ref. 5).

Fairly pure suspensions of hyperdiploid and hypertetraploid cells were prepared as previously described[2]. DNAP was analysed by Schneider's method[8]. DNAP/cell varied slightly around $1\cdot00 \times 10^{-6}$ μgm. for the hyperdiploid and about $2\cdot00 \times 10^{-6}$ μgm. for the hypertetraploid suspensions. Total lipids were extracted with alcohol ether mixture by Bloor's method[6], and analysed for different fractions of lipids the amounts of which are referred to 1·0 μgm. DNAP (Table 1). As the volumes of the two kinds of cells are proportionate to their contents of DNAP, the figures obtained may be looked on as amounts per unit cell volume. It appears that the amount of 'total lipid fatty acids and cholesterol' is more than twice as great in the hypertetraploid cells as in the hyperdiploid ones. About half the difference is due to cholesterol, the other half being due to fatty acids. Although the amount of cholesterol is about four times higher in the hypertetraploid than in the hyperdiploid cells, the relationship between free and esterified cholesterol is the same. The amount of phospholipids is, however, only slightly higher in the hypertetraploid cells than in the hyperdiploid ones.

Table 1. LIPID FRACTIONS IN HYPERDIPLOID (6 SAMPLES) AND HYPERTETRAPLOID CELLS (7 SAMPLES) IN μGM. PER μGM. DNAP

	Total lipid fatty acids and cholesterol*	Total lipid fatty acids†	Phospholipids*	Free cholesterol‡	Total cholesterol‡
Hyperdiploid cells	22·0	12·0	9·3	1·7	3·7
Hypertetraploid cells	47·9	21·9	11·3	8·4	16·3

* Analysed according to Bloor (ref. 6).
† Analysed according to Stoddard and Drury (ref. 6).
‡ Analysed according to Schoenheimer and Sperry (ref. 7).

Analyses of lipid fractions in tetraploid and octoploid ascites tumour cells of other origin are being made.

The reported results suggest a lower specific density of the hypertetraploid than of the hyperdiploid cells. It is furthermore obvious that doubling of the chromosome number induces a remarkable change in the lipid metabolism.

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IMMUNOLOGY

Use of Ferritin-Conjugated Antibody to identify Nephrotoxic Sera in Renal Tissue by Electron Microscopy

EXPERIMENTAL acute glomerulonephritis is readily produced in rats by the injection of rabbit antibody into rat kidney[1]. It has been found by radioautography that labelled nephrotoxic antibody was present in glomeruli[2]. Investigations with the fluorescent antibody technique afterwards showed the deposition of nephrotoxic globulin in what appeared to be basement membranes[3,4]; but lack of resolution made precise localization difficult.

The conjugation of ferritin with globulin[5] has now provided a method for detecting the sites of antigen–antibody reaction within cells by means of electron microscopy[6]. The purpose of this communication is to report the use of this technique for the precise localization of nephrotoxic globulin.

Following injection of rabbit antibody against rat kidney, the rats were lightly anæsthetized and the kidneys removed. Very thin pieces of cortex were excised and fixed for 1 hr. at 4° C. in 5 per cent formalin phosphate buffered at *p*H 7·2. In view of the injury to fine structure caused by freezing, it was decided to facilitate the penetration of ferritin by cutting the tissue into very small fragments under a dissecting microscope at 4° C. These fragments were then immersed for 20 min. at room temperature in ferritin-conjugated antibody to rabbit globulin. (The antiserum was produced in the duck and conjugated with ferritin as previously described[5,7]. Before use it was absorbed with acetone-extracted, dried rat and mouse liver powders successively.) After a thorough washing in Tyrode's solution the tissue was fixed in osmium tetroxide, dehydrated in graded dilutions of ethyl alcohol and embedded in methacrylate. Sections 0·25μ thick were cut on a Porter–Blum microtome, and the glomeruli identified in a phase-contrast

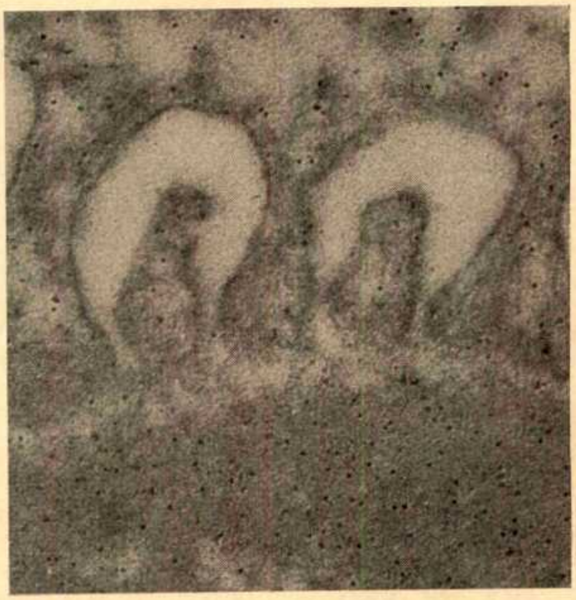

Fig. 1. Glomerular capillary wall of a rat kidney 10 days after intravenous injection of 0·8-ml. rabbit anti-rat kidney serum. The tissue was immersed in ferritin-conjugated antibody against rabbit globulin. Ferritin granules in the basement membrane and in the epithelial cytoplasm indicate the presence of the nephrotoxic rabbit globulin. (× 77,000)

microscope. The block was then trimmed so that the glomeruli could be thin-sectioned for electron microscopy.

Fig. 1 illustrates part of a glomerular capillary wall from a nephritic kidney treated with ferritin-conjugated duck antibody to rabbit globulin. The basement membrane occupies the lower third of the field, the cytoplasm of an epithelial cell with characteristic 'foot-processes' the upper two-thirds. Numerous dense ferritin granules are present within the basement membrane as well as the epithelial cytoplasm. Ferritin was also encountered in the cytoplasm of the endothelial cells (not shown in Fig. 1). There was no ferritin in the nuclei.

The specificity of the reaction of ferritin-conjugated antibody with rabbit globulin was determined by the following experiments. Pretreatment of the tissue with unconjugated duck anti-rabbit-globulin blocked subsequent tagging with ferritin-conjugated duck anti-rabbit-globulin. Purified ferritin alone and ferritin-conjugated antibody against influenza virus failed to localize in the glomerular capillary walls. Finally, specific ferritin-conjugated antibody to rabbit globulin did not tag normal rat kidney.

These results reveal that in nephritic rat kidneys the injected antibody is localized not only in the glomerular basement membrane but also in the cytoplasm of the epithelial and endothelial cells. The technique which has thus been developed should have application to many problems concerned with diseases originating from antigen–antibody reactions in tissues.

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RADIOBIOLOGY

Changes in Metabolic Activities of Hepatoma Cells following Increasing X-Ray Doses

IT has been reported that ærobic and anærobic glycolysis in the Yoshida ascites sarcoma are markedly impaired following *in vitro* irradiation with a dose of 20 kr. of X-rays. In these conditions uptake of oxygen is slightly decreased[1]. A lowered incorporation of alanine labelled with carbon-14 into protein of the ascites-sarcoma cells was also noted under anærobic conditions[2]. More recently the *in vitro* irradiation of Ehrlich ascites tumour cells with different doses of X-rays (20–300 kr.), was found to stimulate both oxygen consumption and ærobic glycolysis whereas anærobic glycolysis decreased as the X-ray dose increased up to about 200 kr. (ref. 3).

Preliminary experiments in this laboratory showed that *in vitro* X-irradiation with a dose of 20 kr. did not affect ærobic glycolysis and oxygen consumption but led to a marked inhibition of anærobic glycolysis in the Yoshida ascites hepatoma cells. The incorporation of labelled glycine into protein was impaired under anærobic but not under ærobic conditions. Further investigations dealt with the effect of increasing doses of *in vitro* X-irradiation on respiration, glycolysis and incorporation of labelled leucine into protein of ascites-hepatoma cells.

The experiments were carried out as follows: well-packed ascites cells, freed from blood elements, were resuspended 1 : 15 (v/v) in ice-cold medium. In glycolysis experiments, the Ringer phosphate-bicarbonate used for resuspending the cells contained sodium chloride 108 m.mole; potassium chloride 4·7 m.mole; calcium chloride 1·3 m.mole; magnesium sulphate 1·2 m.mole; sodium-phosphate buffer, *p*H 7·4, 5 m.mole; sodium bicarbonate 26·6 m.mole. When uptake of oxygen was studied, Ringer phosphate buffered solution contained: sodium chloride 139 m.mole; potassium chloride 5·5 m.mole; calcium chloride 1·0 m.mole; magnesium sulphate

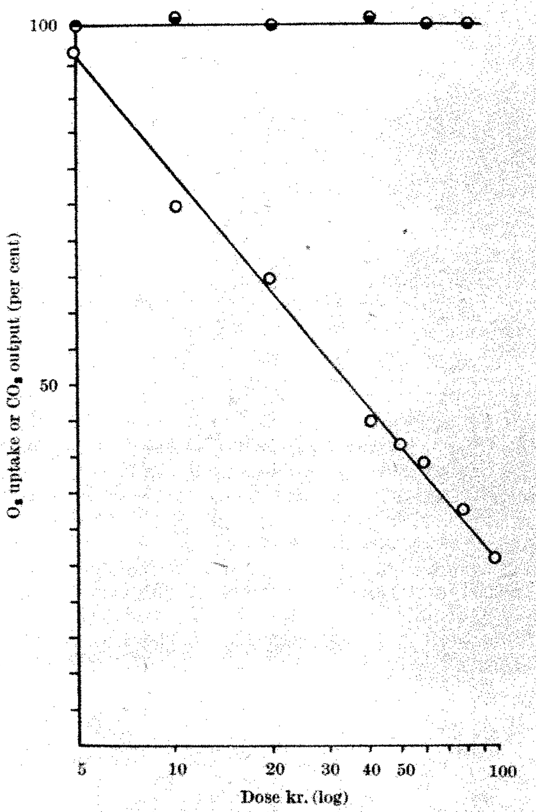

Fig. 1. Effect of increasing doses of X-rays on uptake of oxygen and anærobic glycolysis of the Yoshida ascites-hepatoma cells. ○, Anærobic glycolysis. Each flask contained 2·65 ml. of the cell suspension in the main chamber; 0·15 ml. of 0·3 *M* glucose and 0·2 ml. of a solution containing 20 μc. 5·25 μmoles of D–L leucine per ml., in the side-arm. Gas phase was N_2—CO_2 (95 : 5). 100 = 585 μl. CO_2. ◒, Oxygen uptake. Each flask contained 2·80 ml. of the cell suspension in the main chamber; 0·2 ml. of the above amino-acid solution in the side-arm; 0·2 ml. of 20 per cent potassium hydroxide in the centre well. Gas phase as air. 100 = 205 μl. O_2. The contents of the side-arm were tipped after a 10-min. equilibration

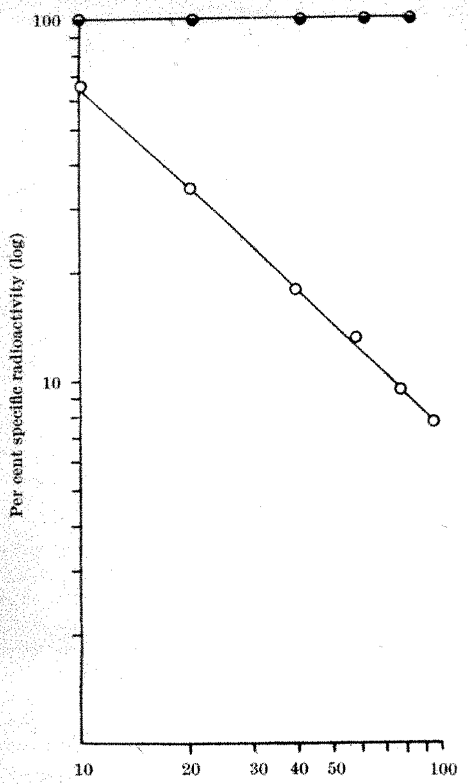

Fig. 2. Effect of increasing doses on incorporation of 1-^{14}C-leucine into protein of the hepatoma cells. ○, Anaerobic incorporation 100 = 380 c.p.m./mgm. protein; ●, aerobic incorporation 100 = 655 c.p.m./mgm. protein

1·4 m.mole; potassium dihydrogen phosphate 1·4 m.mole; sodium-phosphate buffer, *p*H 7·4, 10·6 m.mole.

The cell suspension was placed into a cylindrical 'Perspex' container and irradiated at room temperature with filtered X-rays delivered by a therapeutic beryllium-window X-ray tube operated at 80 kVp. and 5 m.amp., using a 0·1-mm. aluminium filter so that the half-value layer was 0·11 mm. aluminium (equivalent 2·2 mm. water) and the dose-rate at the target distance used (7·5 cm.), 6,800 r./min., measured in air. The depth of the irradiated cell suspension was made up to 3 half-value layer with respect to water. Care was taken to resuspend cells by frequent stirring during irradiation.

About 30 min. after irradiation, cell suspensions were incubated in conventional Warburg apparatus at 38° C. and anærobic glycolysis or uptake of oxygen determined by the conventional methods[4]. After addition of glucose and amino-acid, manometric readings were taken for 60 min. The reaction was then stopped by adding ice-cold trichloroacetic acid (final concentration 1 per cent). Proteins, purified according to Rabinowitz, Olsen and Greenberg[5], were homogenized with absolute ethanol, plated in stainless-steel planchettes and counted with a thin mica-window Geiger–Müller counter.

The results, presented in Fig. 1, show that following irradiation with increasing doses of X-rays respiration is practically unaffected whereas anærobic glycolysis appears progressively impaired. The decrease of glycolysis is directly proportional to the logarithm of the dose of X-rays administered.

Results presented in Fig. 2 clearly show that the aerobic incorporation of 1-^{14}C-leucine into protein, in the absence of glucose, is essentially unaffected even at the highest doses while the anaerobic incorporation of the amino-acid is progressively impaired. When specific radioactivity of protein and X-ray doses are plotted logarithmically, a linear relationship is obtained. The regression equation relating specific radioactivity of protein to *in vitro* X-ray dose is therefore of the form:

$$\log I_D = \log I_0 - K \log D$$

which can be rewritten in the terms of a power law relationship:

$$I_D = I_0 D^{-K}$$

where I_0 represents the value of specific radioactivity of protein at the lowest inhibition dose, taken as zero point at the origin of the co-ordinates, and I_D the value of specific radioactivity of protein corresponding to any dose D. Values of K, that is, the slope of the incorporation curve, were calculated from the dose which halves incorporation ($D_{\frac{1}{2}}$):

$$K = \frac{\log 2}{\log D_{\frac{1}{2}}}$$

The values of K, so calculated, were practically the same in all experiments performed (0·94 in my experimental conditions).

The results presented indicate that when glycolysis is linearly inhibited, incorporation of amino-acids into protein is exponentially inhibited.

The finding that incorporation of leucine is unchanged in ærobic conditions, in the absence of glucose, strongly suggests that enzyme systems involved in protein synthesis are not damaged in the irradiated cells.

The behaviour of aerobic glycolysis and incorporation in the presence of glucose are now under investigation.

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Radiation-induced Hyperpyrexia in Rabbits

The response of rabbits to 25,000–50,000 r. radiation exposure is death within 3–4 hr.[1]. Even at lower levels of radiation exposure to 600–1,000 r. a shock-like death occurs in a few animals within 24–48 hr.[2]. However, other biological responses which occur more consistently at these lower radiation dose-levels are depression of the white cell count and reduction in bone marrow activity[3]. This investigation presents evidence that a rise of body temperature is also a regular effect of acute radiation exposure in rabbits.

Virgin female Dutch rabbits weighing 1·2–2·0 kgm. were used for measurement of influence of radiation exposure on rectal temperature. These animals were housed in an animal room with temperature maintained at 70°–72° F., and they were fed a commercially available balanced rabbit diet ('Ambler Simplex

Table 1. THE EFFECT OF X-RADIATION ON RECTAL TEMPERATURE OF RABBITS DURING 4-HR. POST EXPOSURE

Temperature	No. of animals	Average* rectal temperature change (° F.)
Controls (sham radiated)	19	−0·18 ± 0·11
100 r. exposure of head	6	0·12 ± 0·20
200 r. exposure of head	6	1·08 ± 0·20
800 r. exposure of head	6	2·05 ± 0·20
800 r. exposure of head preceded by 200 mgm./kgm. of aminopyrine orally	6	0·13 ± 0·20

* Average change represents the mean difference between the average of 4-hourly post-radiation rectal temperatures and pre-radiation control temperatures and the associated standard errors.

Rabbit Pellets' prepared by the Ambler Milling Co., San Gabriel, California).

Clinical type rectal thermometers were used according to the manufacturer's specifications for accurate observation. (Apex No. 313 rectal thermometers manufactured by the Faichney Instrument Corp., Watertown, New York, were used.) Rectal temperature measurements were carried out according to methods found acceptable in regular laboratory practice[4,5].

The rectal temperatures of rabbits were determined before radiation exposure and at 4-hourly intervals thereafter in a temperature-controlled animal room. Between measurements the animals were maintained in cages without food or water. For measurement of control temperature response a group of animals received a sham radiation exposure as part of the treatment. Temperature response was determined as the change between the pre-radiation control temperature-level and the average of the four post-radiation readings.

For X-radiation of each rabbit, the subject was placed in an animal box through one end of which the animal's head was held in position. For radiation of the head alone, a piece of lead plate 1-cm. thick was placed on top of the animal box to protect the body from the direct radiation beam. A Picker 250 kVp. industrial X-ray unit, operated at 15 m.amp., provided a radiation beam having a half value layer of 1·93 mm. copper at a rate of 80 r./min.

A summary of experimental measurements is given in Table 1, while representation of fever curves are presented in Fig. 1. By variance analysis techniques[6], the results can be shown to substantiate the occurrence of a hyperpyretic response following a sufficient amount of radiation of the head alone. The consistent febrile response following radiation of the head is shown to have an apparent threshold between 100–200 r. and that oral administration of aminopyrine at an optimally effective dose[7] of 200 mgm./kgm. clocks the appearance of the fever.

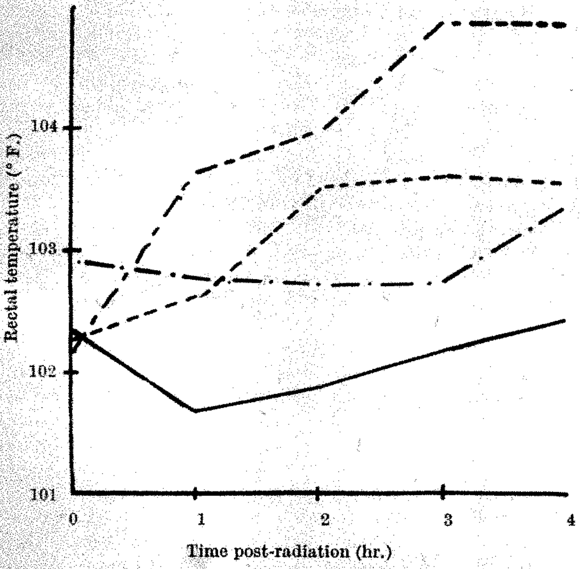

Fig. 1. Change in rectal temperature following acute radiation of the head. ——, Control; —·—, 100 r.; - - -, 200 r.; — — —, 800 r.

The results strongly indicate the susceptibility of the cephalic region of the rabbit toward radiation-induced hyperpyrexia. The significance of this finding is enhanced by the work of Ransom[8], who demonstrated that the site of temperature regulation is situated in the hypothalamus. In the instance of the rabbit, also, it has been well shown that an intact sympathetic nervous system is essential for a febrile response[9]. That the response mechanism is not incited in the effector sequence controlled by the hypothalamus is shown by the capacity of aminopyrine to block the response. This antipyretic has been established to act directly on the thermoregulatory centres[10].

Thus, the radiation-induced febrile response would seem to be evoked through the hypothalamus following an exposure as low as 200 r., but that direct radiation of the hypothalamus is not necessarily the immediate cause of the hyperpyrexic response.

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BIOLOGY

Effects of Pre-Inoculation Host Temperature on Infection of Cereal Seedlings by *Puccinia striiformis*

THERE have been several reports concerning effects of post-inoculation temperature on reaction type of wheat varieties to certain races of stripe rust (*Puccinia striiformis* West)[1,2]. On the other hand, there is very little information available on the effects of pre-inoculation host temperature. Apparently the temperature during this phase of plant growth has been held constant or has been stated to be that obtained with ordinary greenhouse conditions.

In the investigation reported here on the effects of environment on the infection process of *P. striiformis* the pre-conditioning phase was studied first as one of the initial phases in the process.

Uredospores of *P. striiformis* used in this investigation were originally collected from a field near Bozeman, Montana. Tests on the standard differential host varieties received from Dr. H. Brewer, Frankheim, Germany, indicated only one physiological race in the collection. The physiological race used in this work apparently has not been previously described either in North America or Europe[1,3–5]. Immediately following collection the uredospores were lyophilized[6] in 40-mgm. quantities, and these spores were used in all future tests. Germination generally ranged between 40 and 60 per cent when spores were hydrated[6] prior to inoculation.

Several wheat and barley varieties were grown in controlled environment chambers either at 15° or 24° ± 1° C. two weeks prior to inoculation. The photoperiod was 12 hr. a day at 2,000 ft.-candles and relative humidity was maintained near 70 per cent. Previous tests showed that the difference in saturation deficit at the two temperatures was not critical. The primary leaves of the seedlings were inoculated in a settling tower using a carbon dioxide gun[7]. About 800 spores were deposited per cm.^{2} of leaf area and distribution was fairly uniform. Following inoculation the plants were returned to a controlled environment chamber where dew was simulated in the dark at 7° ± 0·5° C. After a 24-hr. dew period, the plants were returned to a temperature of 15° C. for post-dew incubation of about 14 days. Light and humidity conditions were the same as previously stated. The plants were then rated for reaction type and relative severity of infection (Table 1).

Table 1. EFFECTS OF PRE-INOCULATION HOST TEMPERATURE ON SEEDLING INFECTION OF SELECTED CEREAL VARIETIES

	Preconditioned at 15° C.		Preconditioned at 24° C.	
Cereal variety*	Infection type†	Severity‡	Infection type	Severity
Wheat				
Westmont	4	22	4	73
Lemhi	4	50	4	30
Omar	3	68	0–2	24
Triplet	3	57	3	49
Idaed	4	44	0–2	6
Rio	0–2	21	2	5
Barley				
Manchuria	0–1	31	3	45

* Two tests for each variety, a total of at least 25 plants examined per variety.

† 0, Chlorosis or necrosis, no pustules; 1, chlorosis, necrosis, isolated very small pustules; 2, chlorosis, few small pustules; 3, chlorosis, normal pustules; 4, no chlorosis, normal pustules.

‡ Percentage of leaf area covered by rust infection.

The varieties could be separated into 4 groups on the basis of reaction type after host preconditioning. Some varieties such as Westmont and Lemhi were susceptible with both treatments, Rio was resistant with both treatments, Omar, Triplet and Idaed were resistant when preconditioned at 24° C. and susceptible when preconditioned at 15° C., Manchuria was susceptible when preconditioned at 24° C. but resistant when preconditioned at 15° C. A number of other wheat varieties were investigated, and those which were temperature-sensitive usually showed a change from susceptibility to resistance with increased temperature prior to inoculation; however, two of the differential host varieties, Holzapfels Früh and Webster, showed a shift towards greater susceptibility when preconditioned at 24° C. as compared with 15° C.

In these tests, the amount of infection or severity has varied with different host–parasite combinations, but has usually been greater when plants were grown at the lower temperature prior to inoculation. With the exception of Westmont, all varieties exhibiting susceptible type reactions when preconditioned at 15° C. showed more infection at these conditions as compared to those preconditioned at 24° C.

The results show that pre-inoculation host temperatures are also important in conditioning reaction type of wheat and barley to *P. striiformis*. Since physiological host changes conditioning reaction type may take place in the absence of the parasite, biochemical studies on the nature of resistance should be less complex. This investigation also points out that pre-inoculation conditions can influence physiological race determination. Finally, the results of this study have been helpful in interpreting field behaviour of some wheat varieties affected with stripe rust.

In future work the time–temperature period for rust reaction change will be further clarified and the effects of environment on the other phases of the infection process will be studied.

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Possible Angiosperms from Lower Permian Coal of the Transvaal

THE accompanying photograph (Fig. 1), which is natural size, represents two out of five white concretions found recently embedded in bright bituminous coal in a pillar of the underground workings on No. *C* seam of the Breyten Colliery in the eastern Transvaal, South Africa. They have every appearance of being of organic origin and suggest the mineralization of angiospermous fruits of a type far more advanced than any yet contemplated in sediments of this age, namely, lower Permian. The coal seam is horizontal and is the lowest exploitable one in the Coal Measures (Middle Ecca) of this area. *Glossopteris* leaves are known from overlying sediments. The rank of the coal is evident from the photograph, and an age not less than lower Permian is supported by spore determinations.

Each of the five objects shows constant features—a smooth outer cover, which may have been spongy and thus suggestive of an aquatic environment; an inner, possibly originally fleshy, layer which is broadest at the apex (the mineralization of these two layers differs), and a central body consisting of a number of curved, club-shaped empty compartments which radiate from the base and perhaps from a central column. The walls between these are invariably double and are very fragile. They suggest a mineral lining of each compartment at a time when the organic membrane still separated them.

The sections illustrated are natural fractures and fortunately almost true longitudinal sections.

While searching for a suitable embedding medium which would allow the cutting of transverse sections,

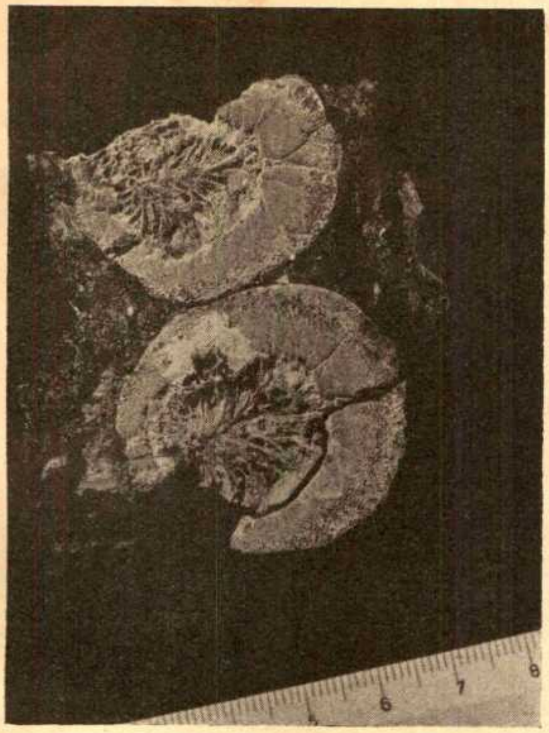

Fig. 1

and not affect the coal, the only thin section has been cut from the fractured outer portion which can be seen on the right-hand side. The whole section was yellowish in colour, remnants of cell structure could be seen and several interesting features such as the presence in one area of what appear to be small fibres and in another of oil glands. These are preliminary announcements but are submitted in support of the probable organic origin of the specimens.

Photographs have been sent to the Royal Botanic Gardens, Kew, for investigation and more details will be published later.

If these are indeed fructifications as they appear to be, their relationship is difficult to imagine. They bear no resemblance to any of the seven 'types' of fructification of Glossopteridae which have been found in South Africa[1], and which are now regarded by a number of plant morphologists as the precursors and possibly the direct ancestors of angiosperms[2,3].

Among much later fossil fruits they are somewhat reminiscent of a silicified multilocular fruit from the Deccan Intertrappean Series of India, which was described by Sahni[4] as *Enigmocarpon* but is now believed to be related to the Lythraceae.

It is possible that the mineralization might have occurred before the specimens were deposited in the swamp. This is suggested not only because no other mineralization has been seen anywhere in the seam but also because coal balls are so far unknown in South African coal deposits. This has been attributed to the complete absence of associated marine sediments from which calcium carbonate might have been derived. After many years of investigation I have found only two apparent coal balls and both proved to be boulders of silicified wood, probably introduced from an older formation.

I have for some time speculated on the higher stage of development and the degree of versatility which are apparent in the Upper Palæozoic fossil plants of the southern hemisphere and have considered the possibility that they might have provided a direct line of descent for angiosperms. The present discovery may provide further evidence.

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Respiration-rates of Bacteria, Bean Roots, and Bean Root Mitochondria as a Function of Oxygen Concentration

IT has been found that the frequencies of X-ray-induced chromosomal aberrations in the root-tips of *Vicia faba* at low oxygen tensions is much higher in the presence than in the absence of respiratory inhibitors[1,2]. The inhibitors were effective only in the presence of oxygen, and their effect appeared to consist essentially of causing the oxygen effect[3] to appear at much lower oxygen concentrations than it does in untreated roots. It was suggested that the chemicals in question enhance the X-ray sensitivity by eliminating an oxygen gradient, which is thought to exist in the roots at low oxygen tensions[2].

A possible way of demonstrating the existence of such an oxygen gradient in bean roots would be to determine the relation between oxygen consumption and oxygen concentration for excised root tips and for mitochondria isolated from the roots. The decrease of the oxygen consumption of plant roots, which is obtained when the oxygen concentration is lowered below that in air, is commonly believed to be due to the fact that the diffusion of oxygen into the roots is prevented by the oxygen consumption of the outer cell layers[4–6]. As a result, fewer and fewer of the root-tip cells are taking part in the oxygen consumption as the oxygen concentration in the surrounding solution decreases. In agreement with this explanation, no decrease of the oxygen consumption with oxygen concentration is observed in the case of unicellular organisms, such as bacteria and yeast, at oxygen concentrations higher than 0·3 per cent[7,8].

If the oxygen gradient hypothesis is correct, it would be expected that mitochondria isolated from roots should behave as unicellular organisms so far as the relation between rate of respiration and oxygen concentration is concerned. That this actually is the case is shown in Fig. 1, where the relation between oxygen consumption and oxygen concentration is illustrated for a bacterium (*Escherichia coli B*, methionine-less), for root tips of the broad bean, *Vicia faba*, and for mitochondria isolated from bean roots. It is evident from the figure that for the oxygen concentrations studied, that is, 0·1–1·8 per cent in the gas phase or 1·3–25·0 μM in solution, the oxygen uptake by *E. coli* and by bean root mitochondria is little affected by the oxygen concentration, whereas that of the root tips decreases strongly with the oxygen concentration. The small decrease of oxygen uptake in the case of mitochondria may be due to diffusion of co-factors from the mitochondria. No

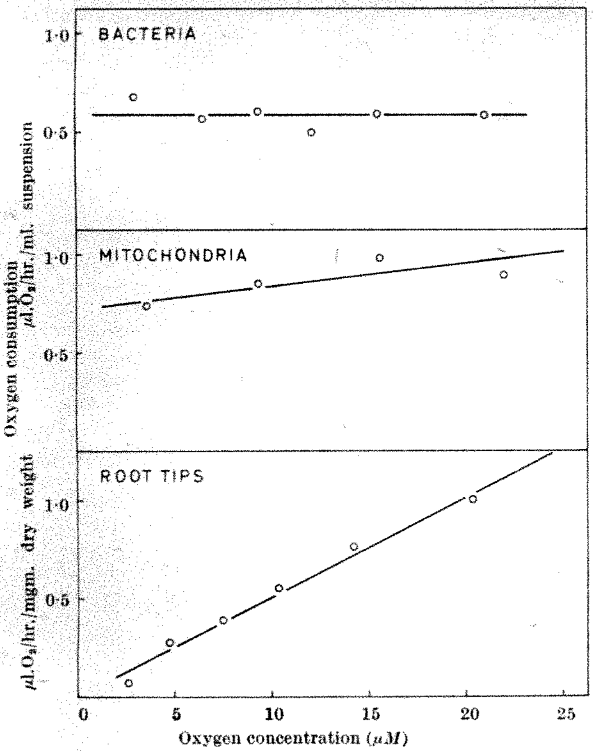

Fig. 1

such decrease in activity was found in a Warburg experiment, which was run parallel with the Hersch cell experiment in order to check the mitochondrial activity during the experimental period. The discrepancy may be explained by the fact that in the Hersch cell experiment the mitochondrial suspension was 80 times more diluted than in the Warburg experiment. This resulted in a much steeper diffusion gradient in the former.

Oxygen consumptions were determined by a method originally developed by Dewey and Gray[9]. Uptake of oxygen took place at 20° C. in a 10-ml. syringe. The syringe was completely filled (no gas phase) with the reaction medium, in which the experimental material was suspended. The composition of the medium was: (1) for *E. coli*: KH_2PO_4, 0·022 *M*; $MgSO_4$, 0·008 *M*; NaCl, 0·085 *M*; NH_4Cl, 0·187 *M*; Na_2HPO_4, 0·042 *M*; glucose, 0·022 *M*; (2) for mitochondria: phosphate buffer, 0·02 *M*, *p*H 7·1; sodium succinate, 0·03 *M*; sucrose, 0·4 *M*; $MgSO_4$, 0·001 *M*; EDTA, 0·1 m*M*: (3) for bean roots: phosphate buffer, 0·017 *M*, *p*H 7·1; glucose, 0·5 per cent.

At the beginning of the experiments the oxygen concentration of the media was adjusted to approximately 27 μ*M*. At 7–15 min. intervals a 0·8-ml. sample of the medium was introduced into a Hersch cell[10] for determination of the oxygen concentration. The oxygen uptake was calculated from the difference between the successive measurements.

In the root experiment, 25 lateral root tips, 3 mm. long, were used.

Mitochondria from roots grown under sterile conditions were prepared essentially according to the method described by Millerd[11] but with the use of ethylenediamine tetraacetic acid[12].

The number of bacteria in the mitochondrial preparation was determined by plating after the end of the experiments. It was found to be approximately 2 × 10^5 per ml. of reaction medium, which is 40 times less than the number which would give the measured oxygen uptake under the experimental conditions.

In the experiment with *Escherichia coli*, a methionine-requiring mutant was used in order to prevent multiplication during the experimental period.

This work was supported by grants from the Swedish Natural Science Research Council and the Swedish Cancer Society.

We are indebted to Dr. L. H. Gray, who suggested the experiments, for valuable discussions, and to the British Empire Cancer Campaign for assistance which enabled the possibility of this investigation to be explored by one of us (B. A. K.) in the Research Unit in Radiobiology at Mount Vernon Hospital.

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Culture and Sex Modification of Male Cucumber Buds *in vitro*

Sex expression in plants has been the subject of many investigations, which have been thoroughly reviewed by Heslop-Harrison[1]. In recent years attention has been mainly directed to the use of growth substances in the experimental modification of sex expression. Much of this work was done with cucumber (*Cucumis sativus* L.). Laibach and Kribben[2] found that application of auxins such as indolyl-3-acetic acid (IAA) and 1-naphthalene acetic acid may cause earlier occurrence of pistillate (female) flowers in cucumber plants. Application of gibberellins was found to affect their sex expression in the opposite direction[3].

In the earlier work the growth substances were applied to the intact plant. The present work was planned with a more direct approach. An attempt was made to culture very young floral buds, at a stage when they are morphologically and physiologically still bisexual[4], and to apply the growth substances directly to the floral buds. By following their morphogenetic changes induced by IAA, gibberellic acid (GA_3), or a combination of the two, it could be learned whether these growth substances exert their effect only when given to the plant, that is, in some indirect manner, or whether they can act directly on the buds, without the obligatory interference of leaves and other plant organs. Results given here tend to confirm the last possibility, at least in respect to IAA.

Plants of a pure, monœcious line of cucumber were grown in the Earhart and Campbell Plant Research Laboratories at constant conditions (23° day and 19° night temperature and 18 hr. light). Under such conditions the first 20 nodes of the main shoot bear only staminate (male) flowers. Twenty days after planting the seedlings were gathered, dissected and floral buds of the same developmental stage (about 0·7 mm. in diameter) were used for culturing. They were at the bisexual stage and borne on about the eighth node.

To eliminate contamination of the buds, the upper parts of the seedlings were immersed for 4 min. in 1 per cent sodium hypochloride, dipped in ethanol and washed in sterile water before dissection took place. The culture medium was White's basal medium as given by Henderson *et al.*[5] with the addition of 5 mgm. tryptophan, 0·1 mgm. kinetin, 2 ml. casein hydrolysate and 150 ml. coconut milk per litre. Agar concentration was 0·8 per cent and sucrose concentration 2 per cent. Only the agar and sugar part of the medium was sterilized by autoclaving. The rest of the medium, as well as the IAA and GA_3 solutions, were filter-sterilized separately and mixed before pouring the medium into the culture vials. Two buds were planted in one vial and kept at 25°–26° C., 12 hr. light (white fluorescent tubes, intensity about 160 foot-candles) for twenty days. Fig. 1 (left side) showed the vial type and the change in size of bud during culture. The buds reached a size of up to 9 mm. in length during this culture period; attached buds of this size are sexually fully differentiated, containing either a well-developed ovary and abortive staminal rudiments, or well-developed stamens and only traces of an ovary (a depression in the top part of the receptacle)[4]. At the termination of culturing, the buds were fixed, and permanent histological slides were used for the evaluation of the results.

Results are summarized in Table 1. It seems to be clear that ovaries develop only when the buds are grown in media supplemented with IAA. The most effective concentration was 0·1 mgm./l. in which 12 out of 13 cultured buds showed a fairly well-developed ovary. In addition to the concentrations shown in Table 1, lower concentrations of IAA (0·01 and 0·03 mgm./l.) were also tested but caused ovary formation in only 3 out of 6 and 2 out of 5 buds, respectively.

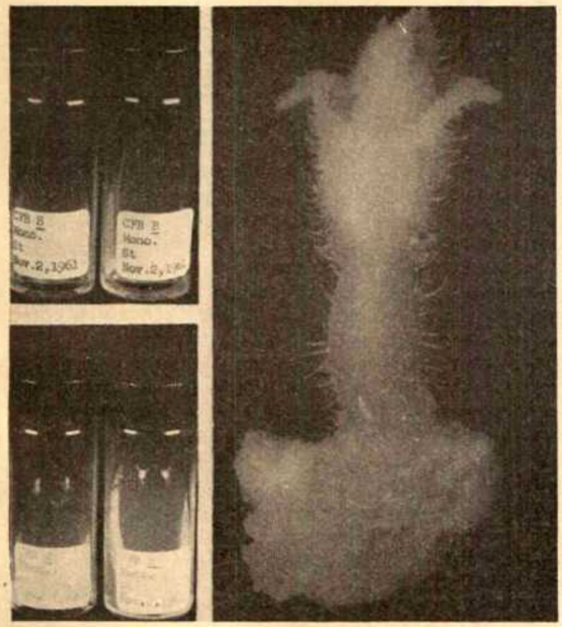

Fig. 1. Left, culture-vials, each containing two floral buds; buds planted on medium without IAA or GA_3, at the beginning (top) and termination (bottom) of culturing period (× 9/20). Right, female floral bud which developed after 20 days culture in an IAA-supplemented medium, from a potentially male bud. (× 6)

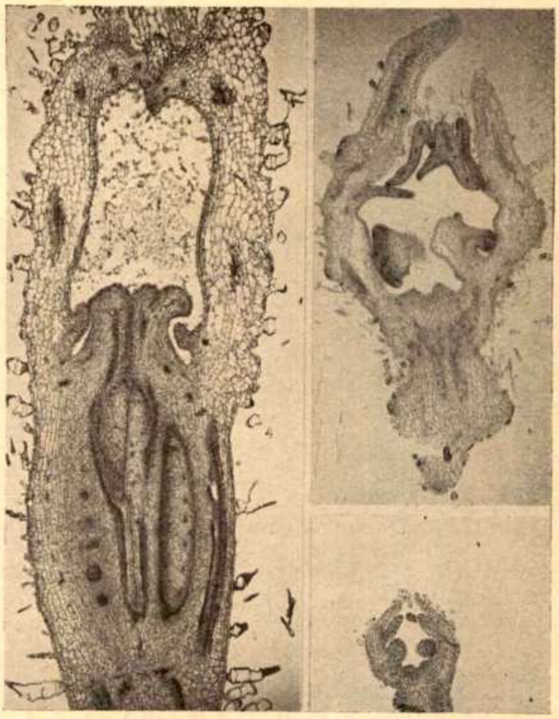

Fig. 2. Longitudinal sections of cucumber floral buds, all at the same magnification (× *c.* 21). Lower right, at the beginning of culturing. Upper right, after 20 days of culturing on medium without IAA and GA_3. Left, after same period on IAA-supplemented medium. Note the lack of appreciable change in the anther primordia in the female bud

In Fig. 2, sections of floral buds at the beginning of culture, and after 20 days of culture in either 'standard' (no addition of either IAA or GA_3) or IAA-supplemented medium, are presented. A bud grown for 20 days in an IAA medium is shown on the right side of Fig. 1. The bud was 9 mm. long and the well-developed ovary can be recognized even without dissection. The stamens in buds of this type were arrested at an early stage of development, as in female buds developing on the plant.

It should be kept in mind that all floral buds used in this work, if left *in situ*, would have become male flowers—according to the node from which they were removed. It is therefore of interest that they can be induced to develop into female flowers when grown detached from the rest of the plant. It is further remarkable that such a development occurs only in the presence of IAA. This acid thus seems to be able to affect sex when applied directly to the floral bud. As to the effect of GA_3 it can at present only be noted that it seems to reduce the effect of IAA when applied simultaneously. The stamens of buds grown in

Table 1. OVARY FORMATION IN POTENTIALLY MALE CUCUMBER FLORAL BUDS CULTURED IN DIFFERENT CONCENTRATIONS OF IAA and GA_3

	Concentration of IAA (mgm./l.)							
	none		0·1		0·3		1·0	
Concentration of GA_3 (mgm./l.)	Total buds	Buds with ovaries	Total buds	Buds with ovaries	Total buds	Buds with ovaries	Total buds	Buds with ovaries
None	11	—	13	12	5	2	7	1
0·3	14	—	8	1*	8	1*	9	—
1·0	8	—	9	—	5	—	6	—
3·0	7	—	5	—	7	—	7	—

* Slight development of ovary.

the presence of GA_3 were further developed than stamens in the female buds formed on IAA media.

The present is the only report, to our knowledge, of a successful culture of young floral buds and a direct, differential effect on their male and female parts. Work is now in progress with potentially female and hermaphrodite floral buds, as well as with buds of still earlier developmental stages, which can exhibit a somewhat different behaviour (a greater tendency to ovary formation).

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Clonal Propagation as a Practical Means of Exploiting Hybrid Vigour in Rice

MARKED vigour has been observed in rice by a number of workers in the F_1 generation of many crosses in respect of height, number of ear-bearing tillers, number of spikelets and yield. But it has not been possible to exploit this phenomenon, as hybrid seed production is a limiting factor in this crop. One method of taking advantage of the hybrid vigour might be to multiply the F_1 plants vegetatively. The technique of vegetative propagation in rice varieties which possess perennating habit has been studied intensively at the Central Rice Research Institute from 1959 onwards. Its value has now been established as an aid to increased rice production. The great potentiality of this technique is shown by the fact that all the clones to be planted in an acre have been derived by repeated multiplication of the tillers produced from two to three stubbles (clones) of the preceding crop. The number of repeated multiplication (3–6 times) depends on the nature of rice variety utilized. The increased yield, obtained by this procedure, ranged from 17 to 61 per cent, as compared with crops raised from normal rice seedlings.

Experiments were continued during 1961 involving a number of improved varieties together with their hybrids in F_1 and F_2 generations in order to obtain more information on the usefulness of this practice.

In six cross-combinations, it has been found that a sufficiently good crop could be obtained from F_1 plants, multiplied vegetatively (Table 1).

To test the effectiveness of this technique at the F_2 generation, stubbles of eleven F_2 plants were propagated vegetatively from a cross between *J*.192 (a selection from a fine-grained variety of Orissa) and *GEB*.24 (a popular variety of Madras). The fourth tiller-separation stage was the final planting of the clones as a field crop in the main season and the results are presented in Table 2.

Table 1. F_1 PLANTS OF DIFFERENT CROSS-COMBINATIONS OF RICE VARIETIES, MULTIPLIED VEGETATIVELY AND FINALLY PLANTED DURING THE MAIN SEASON OF 1961

Cross-combination F_1	Total No. of plants at final planting (12 in. × 6 in.)	Area occupied (acres)
W.114 × *GEB*.24	36,657	0·42
GEB.24 × *G.S*.137	25,520	0·29
PTB.10 × *W*.112	2,532	0·03
CH.45 × *W*.135	17,888	0·20
W.140 × *T*.412	61,080	0·70
JBS.120 × *T*.141	17,482	0·20

Table 2. VEGETATIVE MULTIPLICATION OF F_2 PLANTS AND PARENTS FROM A CROSS, *J*.192 × *GEB*.24, CARRIED OUT DURING 1961

Material	No. of tillers first planting	No. of tillers second planting	No. of tillers third planting	No. of tillers fourth planting (final planting 12 in. × 9 in.)	Area occupied (acre)
J.192 (parent)	15	76	430	6,703	0·12
GEB.24 (parent)	27	81	325	3,433	0·06
F_2 plants					
1	18	123	940	10,020	0·17
2	14	87	675	9,440	0·16
3	15	202	1,832	22,832	0·39
4	17	157	1,425	13,480	0·23
5	20	113	800	8,738	0·15
6	15	142	1,366	13,250	0·23
7	15	167	1,318	13,140	0·23
8	13	48	369	5,026	0·09
9	23	109	1,050	9,848	0·17
10	16	88	950	7,655	0·13
11	24	148	1,350	7,740	0·13

Considerable variability was observed in the individual F_2 populations in respect of multiplication of plants (tillering potential) and all the F_2 plants had shown higher production capacity except one (No. 8), as compared with the parents. These observations indicate the possibility of exploitation of hybrid vigour at the F_2 stage as well, with an added advantage that F_2 plants with desirable recombinations can be picked up.

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Intercellular Cohesion and Expansion Growth in Higher Plants

FREQUENTLY the growth of plant organs such as the root or coleoptile is considered in terms of the changing number, or size, of the component cells, and the effect of experimental treatments (or environmental changes) on the overall growth is considered in relation to the primary metabolism of the average component cell. Such an approach must fall short of describing in full the growth of the organ if it assumes that tissue behaviour is no more than the summation of the behaviour of individual isolated cells. In a healthy tissue the constituent cells are always firmly attached to each other, and this attachment and any change in it are likely to be of great importance to the growth of the tissue.

Cohesion between parenchymatous cells can be considered in terms of the linking of primary walls, across the region occupied by the middle lamella. Two versions of this system are illustrated in the models 1 and 2 of Fig. 1, where the primary walls of adjacent cells are indicated by the lines *AA* and *BB*, and the intervening space represents the middle lamella, which is traversed by linkages. In model 1 the arrangement of the links is regular, at right angles to the two walls, and in model 2, the arrangement of the links is random. When the two walls expand the increase in area of the two surfaces is the same and would be represented by an increase in *AA* identical with that of *BB*. This increase could be brought about either by uniform expansion of the whole areas, or by an identical gradient of expansion in *AA* and *BB*. In model 1, the consequence of expansion will be to enlarge the spacing between adjacent links, but otherwise produce no disturbance to the linkage system; in model 2, the individual link will be stretched to the elastic limit. Extension growth beyond this point must rupture the link; but the alternative possibility arises that if the summation of the mechanical strength of the individual links exceeds the forces of expansion then extension growth must cease. The extension growth of a cell normally produces a ten-fold increase in length, and frequently a value far greater than this. It is inconceivable that this increase lies within the elastic limit of the materials involved and therefore quite certain that with randomly disposed linkages extension growth must be accompanied by rupture of some linkages in the middle lamella.

In a plant tissue the linkages between the cells cannot be regular as in model 1 without scattering incident energy as a detectably regular pattern, and in parenchyma sliding growth in the sense of a given cell being displaced in the direction of growth relative to adjacent cells does not occur; the extension growth of adjacent cells is simultaneous and to the same extent[1], and so the conditions of model 2 prevail. The conclusion appears inescapable, therefore, that accompanying growth in this tissue, linkages between adjacent cells must be broken. Since cohesion is nevertheless maintained in the tissue, it is clear that accompanying the breaking of linkages is a complementary process of linkage formation.

The inference that growth must be accompanied by the rupture of lateral linkages does not show that the breakage is necessarily a direct consequence of the mechanical stresses of growth, and it is conceivable that the rupture occurs by an independent metabolic process that is integrated into the overall phenomenon of extension growth. But nevertheless the significance of inter-cellular cohesion in the tissue is that it may act as a resistance to expansion, and therefore regulate growth.

If the linkage between cells is provided by polymeric macromolecules with a degree of cross-linking (they may not be polysaccharide in nature) then the processes of rupture and formation of linkages become one of covalent bond-breaking and formation, and the general nature of the bonds by which cohesion is maintained is a matter of some importance. In a recent series of observations[2,3], cohesion has been studied by measuring the area to which a fragment of tissue can be compressed under standard conditions after a standard treatment with hydrochloric acid. This technique provides data from which two types of bonding can be distinguished in the middle lamella, one acid-labile, and the other acid-resistant. Under the mild conditions used to rupture the acid-labile bonds it is quite certain that these are electro-valent in character; it has been suggested that the acid-resistant bonds may be covalent, but to establish this with equal certainty would be difficult. The distinction is an important one because of the different properties of the two types, for a system of random covalent bonding in the middle lamella would permit only very limited shear displacement of the contiguous cell walls, and the elastic limit of the bonds would most probably be low, giving some rigidity to the intercellular cohesion, whereas although a multiplicity of electro-valent bonds could produce as strong a linking, the intercellular cohesion would have greater fluidity, the bonds permitting some slight relative movements between the cell walls.

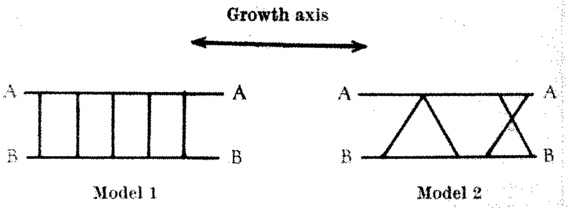

Fig. 1. Alternative models of the linkage system in the middle lamella. *AA* and *BB* represent the primary walls of adjacent cells, separated by the middle lamella which is traversed by linkages. In model 1 these are regularly spaced and perpendicular to the primary walls; in model 2 the arrangement of the linkages is random

The relative contribution of the two bonding systems varies with metabolic conditions. It has been found with root segments that the acid-resistant component is developed most intensively when metabolic activity is depressed, it increases markedly when the tissue is incubated under nitrogen, or with inhibitors such as cyanide, azide, iodoacetate, and *N*-ethyl-maleimide. Evidently the acid-resistant bond is the product of a spontaneous exothermic reaction, which is not linked to an energy-yielding process. The acid-resistant component is poorly developed when the tissue is aerated and freely supplied with sugar, and the data available suggest that the aerobic metabolism of sugar leads to the accumulation of substances in the middle lamella which are active in rupturing acid-resistant bonds.

It does seem significant that the conditions which lead to the formation of acid-resistant bonds are also those which check growth, and that the conditions which weaken the acid-resistant bonding are those which sustain vigorous growth. The restricted growth characteristic of depressed metabolism in anaerobic conditions is not uniquely conditioned by the formation of covalent bonding in the middle lamella. Effects other than those of cohesion are certainly involved in the inhibition and stimulation of growth, but it is possible at least that cohesion is a contributory factor.

Finally, if growth is influenced by the degree of cohesion then changes in the bonding system of the middle lamella might be expected as the tissue develops from a meristematic to a fully expanded state. Such changes have indeed been noted in the root, where the acid-resistant component contributes more to the intercellular cohesion in the meristematic region and in the region of fully expanded cells than it does in the intervening region of cell extension. This suggests that the development from the meristematic to the expanding state involves an enhancement of the metabolic activity through which the acid-resistant bonds are broken, and that subsequent development to the mature state is accompanied by a decay of this system. In terms of the interpretation proposed here, the more vigorous growth that occurs with development from the meristematic state is perhaps partly referable to a decreased restraint imposed by intercellular cohesion, and the cessation of growth may be due in part to this restraint being imposed again.

I thank Prof. R. Brown, in whose Department this work was carried out, for his interest and encouragement.

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Purification of Chlorophyll *c* from *Sargassum flavicans*

CHLOROPHYLL *c* occurs in many types of marine algae[1]. Little is known of its chemistry or function, and methods used for its quantitative estimation are indirect[2]. The present work describes a method for the crystallization of chlorophyll *c* from the marine alga, *Sargassum flavicans*.

Fresh *Sargassum* weed was macerated several times with acetone/methanol (50 : 50, v/v). The pigment extract was filtered, and the pigments were transferred to ethyl ether by adding an equal volume of ether and washing with 5 volumes of 10 per cent sodium chloride. The ether extract was concentrated to a small volume by evaporation under a stream of air. Chlorophyll *c* was separated from chlorophyll *a* and carotenoids by chromatographing the ethyl ether extract on cellulose powder columns using 0·5 per cent (v/v) *n*-propanol in light petroleum (60°–80°) as developing solvent[3]. Chlorophyll *c* remained as a light green zone at the top of the column. The zones of cellulose containing adsorbed chlorophyll *c* were collected and repacked into a fresh column, and the chlorophyll *c* was eluted with 5 per cent (v/v) methanol in ethyl ether.

The extinction coefficient of this material (chlorophyll *c*, stage 1) at 630 mμ in acetone was 2·24 l./gm. cm. Although free from other pigments, the chlorophyll *c* was heavily contaminated with phospholipid. This was shown by chromatographing an aliquot on paper impregnated with silicic acid according to the method of Marinetti and Stotz[4]. Six lipid fractions were present, and total phosphorus determinations gave values of 1·18 mgm. phosphorus/100 mgm. chlorophyll *c*.

Chlorophyll *c* (stage 1) was partially freed from lipid by chromatography on columns of silicic acid which had been mixed with 'Hyflo Supercel' (2·5 : 1, w/w) in chloroform. Chlorophyll *c* was eluted with 5 per cent methanol in chloroform (stage 2). The extinction coefficient of this material in acetone at 630 mμ was 9·91 l./gm. cm. Four lipid fractions were removed, and total phosphorus determinations gave values of 0·1 mgm. phosphorus/100 mgm. chlorophyll *c*.

The final purification of chlorophyll *c* was achieved by chromatography on columns of neutral aluminium oxide. Chlorophyll *c* was strongly adsorbed at the top of the column. Traces of carotenoid and one lipid fraction were removed with methanol/chloroform (9 : 1, v/v). The remaining lipid fraction was removed with ethanol/chloroform/water, 5 : 2 : 1 (v/v/v). Chlorophyll *c* was finally eluted with ethanol/chloroform/water, 5 : 2 : 2·5 (v/v/v). The emerald-green solution (stage 3) was concentrated by evaporation under nitrogen, until the solution changed colour and a deep copper-red precipitate appeared.

Crystallization was achieved by redissolving the precipitate in the minimum quantity of ethanol, adding water to about 5–10 per cent, and leaving in the dark at room temperature. After 24 hr., reddish-black crystals had separated from the mother liquor. Large crystals (100μ) assumed the habit of parallel-sided plates (Fig. 1) and minute crystals (3–5μ) appeared as hexagonal platelets.

The extinction coefficient of chlorophyll *c* (stage 3) in 100 per cent acetone at 630 mμ was 15·8 l./gm. cm., and the absorption maxima in acetone were at 628, 580, and 442 mμ. Magnesium estimations (atomic absorption spectroscopy) gave a mean value of 2·38 mgm. magnesium/100 mgm. chlorophyll *c* (range 2·13–2·60). Assuming one magnesium atom per molecule, and using the mean value of 2·38 per cent magnesium, the molecular weight of chlorophyll *c* would be 1,020. This is the same order of magnitude as chlorophyll *a* (893) and chlorophyll *b* (907). Granick[5] suggested that chlorophyll *c* was a phytol-free chlorophyll (mol. wt. about 600) on the basis of its low hydrochloric acid number. If phytol is indeed absent, then chlorophyll *c* must possess alternative groups attached to the chlorophyll nucleus.

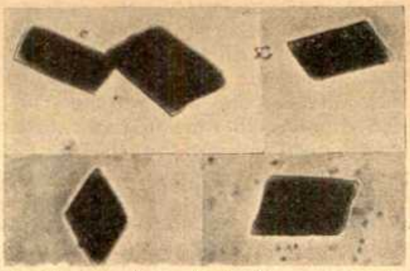

Fig. 1. Chlorophyll *c* crystallized from aqueous ethanol

Detailed descriptions of the methods used and the properties of the crystalline chlorophyll *c* will be published elsewhere.

I thank Dr. B. G. Davey, Faculty of Agriculture, University of Sydney, for the magnesium determinations.

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Spontaneous Electrical Activity of Cutaneous Receptors in Mormyrids

Lissmann and Machin[1-3] have demonstrated that the electric fish, *Gymnarchus niloticus*, is several thousand times more sensitive to a moving electrostatic charge or a moving magnet than are other fish. The electric receptors were assumed to be the 'mormyromasts' (special cutaneous organs known to exist in Mormyrids and first described by Stendell[4]); no experimental proof, however, has been presented to show that the electric stimuli indeed act on these organs.

In 1959, while examining the electrical behaviour of very young Mormyrids (*Mormyrops nigricans*), the very feeble discharge of which necessitates the use of strong amplification, I observed that the receiving electrodes dipped into the water and in contact with the fish captured rhythmic electric signals of relatively high frequency (300 impulses/sec.). The spontaneous electric discharge, generated by the electric organs of the fish, did not, in any way, affect the characteristics of these signals (Fig. 1*A*).

In 1961 these experiments were repeated: previous results were confirmed, and it could be definitely proved that on the skin of Mormyrids electric phenomena of different characteristics and location can be found. Briefly described they are as follows:

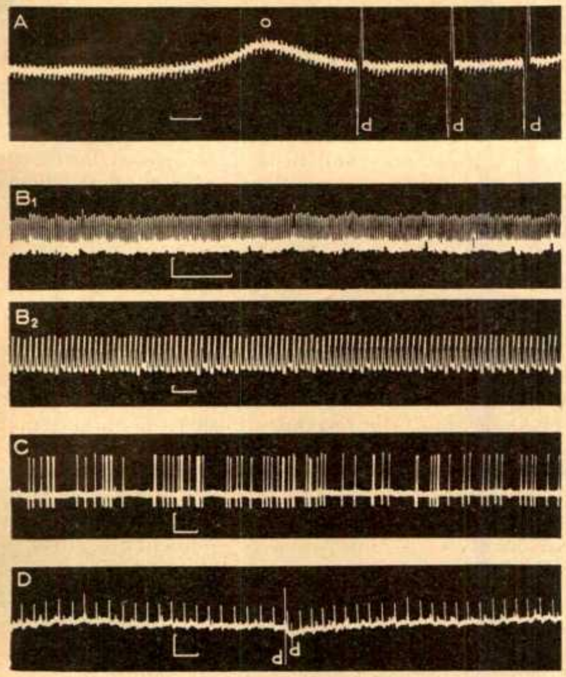

Fig. 1. Different types of spontaneous rhythmic activities recorded on the skin of various Mormyrids. *A*, Young *Mormyrops nigricans*, recording using monopolar electrode placed immediately in front of the operculum. ○, Opercular movement; *d*, discharge of the electric organ. B_1, *C*, *D*, *Hyperopisus bebe*, monopolar recording at three points of the dorsal surface of the head; the fish was kept out of water, respiration being maintained by buccal water-jet. B_2, *Mormyrus hasselquisti*, same experimental conditions as in B_1; recording point 10 mm. dorsolaterally to the eye. Calibration, 1 mV.; time, 20 msec.

(1) Rhythmic discharges of very high frequencies Fig. 1 (B_1 and B_2) up to 2,200 imp./sec. which are limited to certain regions of the head, the dorsal surface of the body and to the base of the dorsal and anal fins. In each of these regions there exist several emitting points. The frequency and amplitude (2–12 mV.) of the impulses are invariable for any given point, but they are different from one point to another. The range of frequencies is around 300 imp./sec. in the case of *Mormyrus hasselquisti* and between 1,100 and 2,200 imp./sec. in the case of *Gnathonemus*, *Hyperopisus*, *Marcusenius* and *Genyomyrus* spp. (Results of preliminary anatomical examination suggest that the particular organization of the bulbar organs of the *Mormyrus* genus may furnish some explanation for the observed differences.)

(2) Spikes of identical amplitude but of irregular frequency the emission of which takes place approximately in the same regions (Fig. 1 *C*).

(3) Exceptional spikes of low (40–140 imp./sec.) and regular frequency (Fig. 1 *D*) the emission of which is situated on the dorsal surface of the head near to the midline.

Similar electric phenomena can be demonstrated on the skin of *Gymnarchus niloticus* after suppression of the functioning of the electric organ either through spinalization of the animal or by blocking electrically the bulbar command of the electric organs[5].

These phenomena are of cutaneous origin. They persist at the level of the dorsal fin of a decapitated fish, but disappear if the skin is crushed locally. A precise localization of the emission points by means of a fine electrode, followed by histological control, showed that the rhythmic discharges of high frequency originate from the "Knollenorgane" (tuberous organs) of Franz[6]. The irregular spikes probably come from some of them; it remains to be determined, however, whether they represent a particular state of functioning of the same organs or the functioning of particular tuberous organs.

These cutaneous discharges are sensitive to electric current and also to changes in temperature[7].

A clue to the role of these organs can be found in my demonstration that the electrical stimulation of the lateral nerve influences the activity of the electric organs: as the tuberous organs form part of the lateral line system[6,8], their activity may be expected to be in close relationship with that of the electric organs.

Thus in addition to their well-known electric organ, the Mormyrids (and *Gymnarchus*) possess in their skin autoactive cells, situated predominantly on the head and at the base of the dorsal and anal fins. They may represent electroreceptors, having an auto-rhythmicity of high frequency the modulation of which would control the activity of the electric organs, via the central nervous system. It is obvious that this permanent activity of high frequency could offer large possibility of modulation, which in fact would explain the extraordinary sensitivity of these fish to electric current, observed by Lissmann and Machin.

I thank J.-P. Gosse, of the Institut national pour l'Etude agronomique du Congo belge of Yangambi, and Dr. R. Paulian, director of the Institut d'Etudes centrafricaines of Brazzaville, for their generous help.

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A Possible Ultra-Micro Biodetection Method for Androgens

VERY young guppies (*Lebistes reticulatus*) will give a visible morphological reaction to extremely low concentrations of methyl testosterone in the water in an aquarium. Observations indicate that individuals 1–5 days of age are sensitive to concentrations of this steroid in the order of 10^{-12} *M*, and thus to quantities in the μμgm. range, if not lower.

The indicating reaction is the elongation of the anal fin, which is normally a secondary sexual development in maturing males. The hormone-induced change is first detected after 3 days and is very obvious after 5 days.

The stock solution of the steroid was prepared by dissolving crystalline commercially obtained hormone in ethanol and diluting with distilled water, to produce a 10 per cent by volume ethanol solution, $3 \cdot 5 \times 10^{-4}$ *M* in methyl testosterone. A preliminary investigation with methanol as the solvent, however, resulting in more extensive morphological changes in mature female guppies than from later tests with ethanol, suggest that young guppies may be even more sensitive with methanol.

These observations indicate that the phenomenon may be useful for the detection, and possibly also the measurement, of very low concentrations of natural androgens in biological fluids.

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Failure of Histamine to induce Deciduomata in the Rat

THE uterus of the pseudo-pregnant rat responds to trauma on the fifth day of pseudo-pregnancy by producing a massive growth of decidual cells. The production of artificial deciduomata was first demonstrated by Loeb in 1908[1] and is analogous to the decidual response of the pregnant uterus to an implanting blastocyst. Why trauma should cause this response, which can result in a four-fold increase in the weight of a uterine horn in 70 hr., is still little understood. Shelesnyak has postulated that the final stimulus to the uterus is histamine, released by trauma or, in pregnancy, by the presence of the egg. The most convincing and probably most crucial evidence is that intra-uterine injections of histamine initiate a decidual response. In these experiments Shelesnyak[2] used a dose of 500 μgm. of histamine dihydrochloride, although in a subsequent experiment he found from bioassay of whole uteri that the total quantity of histamine present in the uterus was approximately 4 μgm. (ref. 3). In addition Kahlson has shown that the urinary output of endogenous histamine is of the order of 100 μgm. a day[4]. In view of these findings 500 μgm. would seem to be a rather large amount of histamine to introduce into one horn of the uterus. Hence it seemed desirable to repeat the experiments with the view of ascertaining whether smaller, more physiological amounts would elicit the response and, if possible, to determine a dose response relationship.

Rats were made pseudo-pregnant by electrical stimulation of the cervix during œstrus. Operation was performed under 'Avertin' anæsthesia 100 hr. later. The uterine horn was approached through a small incision in the flank. A number 30 standard wire gauge needle (0·3 mm. outside diam.) was passed through the wall of the uterus at the utero-tubal junction and into the lumen of the uterus for a distance of ½ in. During the injection the bottom of the oviduct was held between forceps so that the only trauma to the uterus was that due to insertion of the needle. In each animal histamine in 0·05 ml. physiological saline was injected into one horn and 0·05 ml. physiological saline into the other. Solutions were randomly allocated to right and left horns and the operator himself did not at the time of the injection know which syringe contained saline and which histamine. After 72 hr. from the time of the injections, the animals were killed and the uterine horns dissected out and weighed separately. It was hoped that the 'blind' injection technique together with the objective measurement of response by uterine weight would eliminate any unconscious bias. The doses of histamine acid phosphate used were 10, 125, and 500 μgm., with 5 animals per group. For comparison a further group of 10 animals had one horn of the uterus crushed with artery forceps, the other horn being left completely untreated.

As was to be expected, crushing caused a considerable decidual response and consequent increase in weight of the uterine horn (Table 1). The untreated horns in this group contained no deciduomata and provide an estimate of the control weight of the pseudo-pregnant uterine horn. The mean weights of both the saline- and histamine-injected horns, as shown in Table 2, were significantly ($P = 0\cdot05$) greater than the mean weight of the untreated horns. Macroscopically deciduomata were seen in many of the injected horns, especially adjacent to the site of injection. This is not surprising since injections into the lumen of the uterus must always be attended by a certain amount of trauma. However, it is clear that not only is there no increase in response with increasing dose of histamine, but in no group was the response to histamine greater than that to saline. At the same time, the responses both to histamine and to saline were significantly ($P = 0\cdot05$) less than the response to crushing. If this difference were due to the fact that crushing liberates more histamine than does the trauma of intra-uterine injection, one might reasonably expect histamine to augment the effect of saline. But this did not occur.

Table 1

Mean weights and standard errors of uterine horns (mgm.)

After crushing	No interference
341 ± 44·9	141 ± 7·6

Group size = 10

Table 2

Mean weights and standard errors of uterine horns (mgm.)
Group size = 5

Dose (μgm.)	After injection of histamine acid phosphate in saline	After injection of 0·05 ml. 0·9 per cent saline
10	240 ± 42·9	287 ± 72·6
125	167 ± 25·4	211 ± 44·3
500	198 ± 21·7	209 ± 31·3
Overall result:	Histamine injected	201·5 ± 18·6
	Saline injected	235·9 ± 29·5

The structure of the rat uterus is such that passage of fluid from one horn to the other seems unlikely. However, to check this, 5 rats were given saline into one horn and the other left untreated. The results are given in Table 3.

Table 3

Mean weights of uterine horns as before

Injection of saline	Uninjected
244 ± 41·6	155 ± 6·7

The weights of the uninjected horns are not significantly different from those of the uncrushed horns in Table 1 and macroscopically no deciduomata were seen.

After the experiment had been started we noted that histamine dihydrochloride, as used by Shelesnyak, contains a higher percentage of histamine base than does the acid phosphate salt used by us. We therefore injected a further group of 5 rats with 500 μgm. histamine dihydrochloride. The results are shown in Table 4.

Table 4

Mean weights of uterine horns as before

Injection of 500 μgm. histamine dihydrochloride in 0·05 ml. saline	Injection of 0·05 ml. saline
201 ± 21·8	176 ± 12·9

Again, there is no significant difference between the response to histamine and that to saline or between the response to this salt of histamine and the response to the acid phosphate (Table 2). Thus we have been unable to show that intra-uterine injection of histamine in saline causes a significantly greater decidual reaction than intra-uterine injection of saline alone.

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MICROBIOLOGY

Spherical Pseudomorphs in Aerosols of *B. lactis aerogenes*

During an investigation of bacterial aerosols formed by spraying from a suspension, a striking effect was observed which does not appear to have been reported previously. A small quantity of washed *B. lactis aerogenes* in distilled water was sprayed from an atomizer and the resulting aerosol passed through an evaporation tube to dry the organisms. Samples for electron microscopy were obtained with a thermal precipitator by direct deposition on to collodion-covered specimen grids. Instead of elongated bodies, spherical particles, the shape of which was verified by shadowing at 45°, were found (Fig. 1, above). The diameter of many of the particles was two or three times that of a sphere of the same volume as a given organism, and all particles were uniformly opaque with no sign of a cell wall. Similar shapes were found when sedimentation samples were taken, so the effect could not have been caused by heating during thermal precipitation.

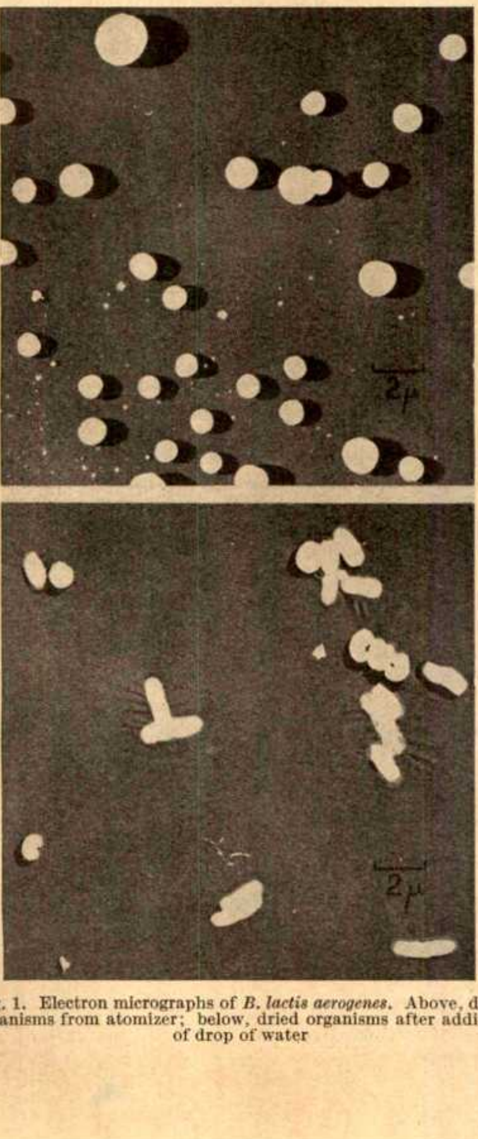

Fig. 1. Electron micrographs of *B. lactis aerogenes*. Above, dried organisms from atomizer; below, dried organisms after addition of drop of water

Attempts were made to regenerate the normal shape of the bacteria by exposure to a high concentration of water vapour; but these proved unsuccessful. However, when a small drop of water was placed on the particles on a grid, which was then allowed to dry before examination, particles in various stages of reversion to their normal shape could be seen (Fig. 1, below). It is apparent that the bacteria folded end-to-end when reaching the spherical shape, an effect which is almost certainly due to the predominant action of surface tension forces in the last stages of evaporation of the spray droplets. The larger spheres shown in Fig. 1 (above) are undoubtedly aggregates of organisms.

In another experiment, organisms were sprayed on to prepared grids so that the droplets impacted before much evaporation took place. Single organisms and closely packed aggregates were found, similar in appearance to bacteria dried on a surface from an aqueous suspension, but no spherical particles were present. It appears that complete evaporation before deposition is necessary for the bacteria to attain the spherical form.

Further experiments, in which spores (*B. globigii*) were sprayed, failed to produce any abnormal shapes.

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A Medium for the Axenic Cultivation of *Entamoeba invadens*

Cultures of *Entamoeba invadens* Rodhain, 1934, growing without other micro-organisms, were first obtained in media containing chick embryo or liver tissue[1-4]; these media were prepared under aseptic conditions as they could not be sterilized by heat or filtration. The presence of tissue cells raises some doubts as to whether these cultures should be designated axenic, as defined by Dougherty[5,6], and Diamond[7] attributes the first axenic cultivation of *E. invadens* to Stoll[8,9], whose medium contained particulate matter, but not living cells. Stoll's medium comprised a heat-sterilized liver extract together with sodium chloride, peptone and mucin, to which a Seitz-filtered liver extract was added aseptically. Diamond[7] has developed a medium free of gross particulate matter, but containing inactivated serum, presumably sterilized by filtration, which was added aseptically to a heat-sterilized, buffered solution of Trypticase, yeast extract, maltose and reducing agents. This medium supported the axenic growth of both *E. invadens* and *E. terrapinae* Sanders and Cleveland, 1930.

It was felt that a medium containing only heat-stable ingredients was desirable, as it could be sterilized in one stage, thus simplifying preparatory procedures and reducing the risk of accidental contamination. By progressive modification of Stoll's medium, I have obtained such a medium, which is also free of gross particulate matter. The following substances are dissolved in 100 ml. glass distilled water: 1·0 gm. Trypticase (*BBL*); 2·5 gm. 'Panmede' (a dried ox liver digest manufactured by Paines and Byrne); 0·5 gm. sodium chloride; 0·5 gm. glucose. After adjusting the pH with N sodium hydroxide, the medium is dispensed in 'Pyrex' test-tubes, and autoclaved for 20 min. at 15 lb., 121° C. As soon as possible after removal from the autoclave, preferably while still warm, the surface of the medium in each tube is covered with a layer of sterile 'Vaseline', to prevent aeration of the medium. The tubes are stored at 8° C. until required.

Strain N of *E. invadens*[10] has been growing without other micro-organisms since 1954, and has been maintained in the above medium for more than a year, by sub-inoculation at intervals of 3–4 weeks; the cultures are incubated at 24° C. Although growth in these cultures has been maintained at a consistent level, this, as in other axenic media for *E. invadens*, is very much less than in cultures of the amœbæ growing with bacteria. When 2,500 axenic amœbæ, in 0·5 ml. of 'Panmede'-trypticase medium, are inoculated into 5 ml. of medium (pH 6·0–6·3), optimum growth is attained at 2 weeks, the numbers of amœbæ multiplying approximately ten-fold. A similar number of amœbæ, from a culture of *E. invadens* + *Escherichia coli*, inoculated into a diphasic medium (horse serum slope covered with 5 ml. of Ringer–egg-white + starch) reach optimum growth at 3 weeks, with a 200-fold increase in numbers.

The intensity of growth in this axenic medium appears similar over the pH range 6·0–7·5, with little or no growth below 5·5 or above 8·0. Amœbic growth is not stimulated by the addition of gastric mucin or starch, and in the axenic cultures the avidity of the amœbæ for starch is much less than in mixed cultures of amœbæ and bacteria. In contrast to the readiness with which mass encystation occurs in many mono- and poly-bacterial cultures of *Entamoeba invadens*, sporadic cysts have been found only occasionally in some axenic cultures.

Work on modifications of this medium is in progress, and a more detailed account will be published elsewhere.

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A New Species of *Derxia*

A NEW nitrogen-fixing bacterium has been isolated and purified from a sample of partially retted jute plant (*Corchorus olitorius* L.) collected from Gograghat (Uttar Pradesh, India) in nitrogen-deficient mannitol-agar medium. The raised, smooth, capitate, translucent, areolate colonies, formed with a circular or oval depression at the centre having a well-defined margin, are usually hard in consistency when young but gradually become soft and slimy with age. The 5-day-old colonies measure on an average 10·95 mm. × 9·7 mm. The pale yellow colour of the colonies appearing after 3–4 days incubation at 33–34° C. in nitrogen-deficient mannitol-agar gradually changes from light to dark brown. Nitrogen-free liquid medium becomes jelly-like in consistency with change of colour of the pellicules from yellow to chocolate brown. Heavy slimy growth with chocolate brown colour appears in dextrose-nutrient broth. Stab culture in nitrogen-free mannitol-agar always shows surface growth. On streaking in the same medium, colonies are distinct but not separated. In potato slants, slimy rust-brown growth appears after 3–4 weeks at 32–33° C.

The bacterium grows fast, and efficiently utilizes glucose, alcohol, glycerol, mannitol, sucrose, lævulose and sorbitol. Growth is, however, feeble in mannose and maltose, while there is no growth in starch, arabinose, lactose, xylose, galactose, rhamnose, and a number of sodium salts of lower and higher aliphatic acids.

Growth of the bacterium is faster in combined nitrogen than in nitrogen-deficient medium. It effectively utilizes aspartic acid, glutamic acid, asparagine, ammonium acetate, sodium nitrate, peptone, alanine and urea; and shows very feeble and belated growth in ammonium sulphate and in glycine respectively. It reduces nitrate, changes litmus milk alkaline and semi-transparent on incubation. The bacterium does not hydrolyse starch or liquefy gelatine. Indole formation and catalase activity are found to be negative.

The bacterium grows with or without calcium. Molybdenum was found to be essential for fixation of nitrogen. Nitrogen fixation reaches a maximum of 30 mgm. in nitrogen-free liquid medium with 2 per cent glucose on 30 days of incubation at 31°–32° C.

The organism grows at a range of pH 5–9. Growth performance is better in alkaline range which, however, reaches 4·4 during the period of long incubation due to formation of acid.

Three distinct morphological stages are observed in the life-cycle of the aerobic, Gram-negative organism. In nitrogen-deficient mannitol-agar or glucose-agar (inc. at 34°–35° C.) banded cells are found (Fig. 1*A*) with discoid or partially annular chromatic bands in 24-hr.-old culture. This form soon changes to alveolar structure (Fig. 1*B*) within 3–4 days, and finally appeared as rods measuring on average 0·79–4·32 × 0·53–1·16μ with rounded ends (Fig. 1*C*) in old cultures. On staining, the refractive bodies in banded and alveolar cells are found to contain fat and volutin-like inclusions. Cells in young culture are embedded with slimy capsule of variable thickness. Various involution forms generally arise in dextrose-

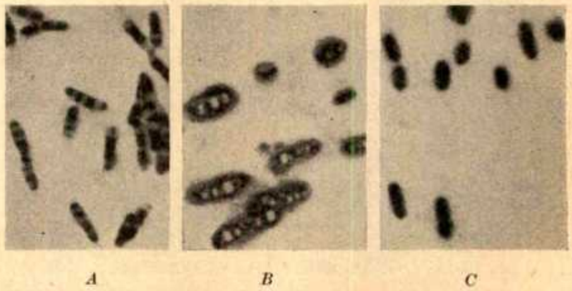

Fig. 1. *A*, Banded cells in 24-hr.-old culture; *B*, alveolar cells in 4-day-old culture; *C*, rod-shaped cells in 12-day-old culture. (× *c*. 1,500; stained in 0·1 per cent crystal violet)

nutrient agar medium. Motility is observed in glucose-ammonium acetate solution after five days of incubation at 34°–35° C. Endospores and microcysts are not formed.

The bacterium differs from *Azotobacter*, *Beijerinckia* and also from the previously reported organisms[1,2] in cultural, physiological and morphological characters. It differs distinctly from *Derxia gummosa* (Jensen *et al*)[3], in its morphological life-cycle, having cells of smaller size, for assimilation of carbon from sucrose and development of feeble growth in ammonium sulphate glucose-agar. In view of its general resemblance culturally and physiologically to the genus *Derxia*, this new organism can be included in the same under a new specific name *Derxia indica* sp. nov.

We thank Dr. K. T. Jacob and Dr. M. K. Mukherjee for their interest and encouragement.

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ENTOMOLOGY

Mating of Virgin Queen Honey Bees (*Apis mellifera* L.) following Mandibular Gland Extirpation

Mandibular glands have been suggested as a possible source of an odour that attracts drones to virgin queens for mating[1]. The experiments in this report concern attempts to determine if virgin queens without mandibular glands can mate. Data discussed here are made more significant by the discovery, made after these experiments were conducted, that the mandibular glands are indeed the primary source of mating attractant odours[2].

Nedel[3] first extirpated mandibular glands from a living queen. One of these queens did not mate when given the opportunity, and on this basis he suggested that the mandibular gland was necessary for mating to occur. Gary and Morse[4] and Gary[5] independently developed another technique for mandibular gland extirpation that was used in the following experiments conducted during 1961.

A mature queen cell within hours of emergence was placed in each of five nucleus colonies containing approximately 3,000 bees on June 27. Virgin queen flight was prevented by queen excluder material placed over the colony entrances. On June 30 the mandibular glands were extirpated from the virgin queens. During the gland extirpation, the queens were out of the colonies for 52–77 min. Queen excluders were removed when the queens were returned to their respective colonies. Dead bee traps[6] were attached to colony entrances to collect the bodies of any queens cast from the colonies.

One queen was found dead in the trap 24 hr. after gland removal. Two queens did not mate and were superseded within two weeks. The remaining two queens mated and produced normal worker brood until they were killed two weeks later for a chemical analysis.

In a similar experiment the mandibular glands were removed on July 28 from six virgin queens three days old. In this experiment colonies containing approximately 30,000 bees were used, and dead bee traps were not used. One month after gland removal two of these queens had mated and were laying normally. Incision scars confirmed previous gland removal on both queens.

The extirpation of mandibular glands does not necessarily render a virgin queen incapable of mating. Since some virgin queens without mandibular glands duly mated, it is indicated that drones utilize supplementary stimuli such as vision to locate virgin queens in flight.

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A Diuretic Hormone in *Rhodnius prolixus* Stål

The fifth-stage larva of *Rhodnius* takes very large blood meals[1], and a rapid diuresis follows[2]. The experiments reported here demonstrate that the diuresis is caused by a hormone released into the hæmolymph from the ganglionic mass in the mesothorax.

When the Malpighian tubules, attached to the top part of the rectum, are isolated from a recently fed insect and placed in a drop of hæmolymph under liquid paraffin, they produce urine; and the rate of urine production can be estimated by measuring the size of the drop which appears at the rectum. The rate is at first high, but soon falls to a very low level. If hæmolymph from a freshly fed insect is now added to the hæmolymph bathing the tubules, a further burst of urine production follows. Hæmolymph from an unfed insect has no effect.

Several lines of evidence show that the factor responsible is a hormone liberated from the large fused ganglionic mass situated in the mesothorax (which comprises the meso- and meta-thoracic and all the abdominal ganglia).

The activity of breis of various tissues in insect Ringer was tested by adding them to the tubule preparations. The central nervous system possesses considerable activity; all other tissues tested have no activity. By determining the dilution at which each of the active breis produces a standard effect on the production of urine by tubule preparations, it is possible to measure the activity of each of the parts of the central nervous system. Thus, in arbitrary units per unit of dry weight, the activity of the brain is 6, that of the subœsophageal ganglion 3, the prothoracic ganglion 1, and of the fused ganglionic mass 50.

The fact that ligating the fed insect anterior to the ganglionic mass has no effect on urine production demonstrates that structures anterior to the ganglionic mass are not involved. Thus decapitation is without effect on the normal response. On the other hand, ligating the fed insect behind the ganglionic mass soon causes the flow of urine to cease.

In fed animals which have been ligated at various points, only those portions which include the ganglionic mass contain active hæmolymph (as assayed on isolated tubules).

The ganglionic mass in the mesothorax contains several groups of neurosecretory cells. The diuretic activity, as estimated with tubule preparations, is confined to the cells of the two hindmost groups.

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CYTOLOGY

Chromosomes of the Fungus *Cyathus*

Although records of chromosome counts in fungi are fairly numerous, few of these can be considered completely reliable. Indeed the small size of fungus chromosomes and the limitations of techniques of staining make difficult the unequivocal identification of chromosomes as such in many published records.

An extensive list of chromosome numbers can be found in the review of our knowledge of fungus nuclei published by L. S. Olive[1]. Referring to Basidiomycetes (especially members of the Agaricales), Olive notes (p. 548): "reports of odd numbers of chromosomes are rare, as are reports of haploid numbers greater than 8".

Among the Gasteromycetes (to which group the genus *Cyathus* belongs) about a dozen species appear to have been examined, and in these the haploid number given is either 2 or 4 (in one instance a possible 3).

This brief reference is sufficient to indicate a probable basis for the assumption, apparently widespread among botanists, that the haploid chromosome number for common fleshy fungi is 3, 4 or occasionally 6.

That larger chromosome numbers may occur among members of the Agaricales is shown by the work of D. T. Hughes[2] on the wild and cultivated varieties of the common mushroom. The photographs published by Hughes establish the haploid number of 12 for both wild- and cultivated-types.

The accompanying photograph shows that the chromosome number for the fungus *Cyathus stercoreus* (Schw.) De Toni is $n = 12$. So far as we are aware, this is the largest number reported to date for a member of the Gasteromycetes. The demonstration was made possible by the use of a special propiono-carmine staining technique developed by one of us (B. L.). The new technique will be described elsewhere.

From young fruit-bodies of *Cyathus stercoreus* grown on nutrient agar in the laboratory, periodioles were removed and fixed. By careful dissection, young basidia were teased out on to slides and squashed gently.

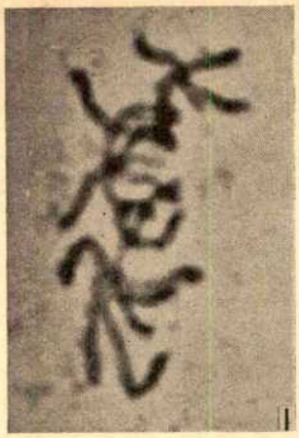
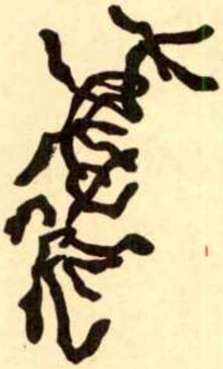


Fig. 1. Photograph of twelve bivalent chromosomes of *Cyathus stercoreus* as seen in a young basidium. (× *c.* 3,200)



2

Fig. 2. Drawing representing the same chromosomes as seen by focusing through the preparation. (× *c.* 3,200)

The photograph reproduced as Fig. 1 shows 12 bivalent chromosomes at prophase of the first meiotic division in a young basidium. In the actual preparations each chromosome can be seen in its entirety, and considerable difference in size and shape of chromosomes is observable. Fig. 2 is a drawing based on the chromosomes as seen in the several focal planes not shown by the photograph. Other similar preparations confirm the count given. Work in progress indicates that chromosome numbers of other species of *Cyathus* are also of the same order and clearly not less than $n = 8$.

Apparently the chromosomes of *Cyathus* are quite large (at least in the stage shown) in comparison with those of many fungi. It may be that further study will reveal distinctive morphology by which they may be separately identified.

The possibility of determining chromosome number in the various species and races of *Cyathus* will facilitate investigations of genetic mechanisms and of the problems of species relationships in these interesting fungi[3].

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VIROLOGY

Use of Formalin-treated Antigen in the Production of Antiserum to a Plant Virus

TOMATO black ring virus, first described by Smith[1], received little further attention until the beet ringspot virus, reported by Harrison[2], was found to be serologically related to it and to potato bouquet virus[3,4]. Smith and Short[5] later isolated a strain of tomato black ring virus from lettuce and named it lettuce ringspot.

Celery yellow vein virus, isolated from celery in 1957[6], also proved to be serologically related to strains of tomato black ring virus, and partly protected against them in plants. Celery yellow vein virus differed from other strains of tomato black ring viruses in host-range and symptoms, and in its antigenic behaviour *in vivo*, as reported here.

Celery yellow vein, tomato black ring and beet ringspot viruses were cultured in *Nicotiana clevelandii* Gray, in which they reach a high concentration within 14 days[7], and were assayed in *Gomphrena globosa* L., which produces local lesions within 7 days[8].

All three viruses could be partially purified and concentrated in several ways: by precipitation with ammonium sulphate or ethanol, by differential centrifugation, by the butanol-chloroform method of Steere[9] or a modification of it[10]. Each method yielded preparations which were highly infective, contained many 'spherical' particles (about 30 mμ diameter when examined in the electron microscope), produced clearly defined light-scattering zones in density-gradient centrifugation in sucrose columns, and reacted specifically with antiserum to beet ringspot virus.

Whereas all preparations of beet ringspot virus were highly antigenic when injected into rabbits (serum titres up to 1/2,500), no celery yellow vein virus preparation induced any specific antibody formation. Injection schedules included six graded intravenous injections at 4-day intervals, and two to four intramuscular injections at 2-week intervals using Freund's complete adjuvant.

The modification[10] of Steere's method of purification gave preparations with the greatest infectivity, particle concentration, density-gradient zone intensity, and antigenicity *in vitro*: leaves (80–120 gm.) of *N. clevelandii* were gathered 14 days after infection and minced with 1·25 × wt./vol. of $M/20$ buffer (KH_2PO_4–Na_2HPO_4) pH 7·6, containing 0·1 per cent thioglycollic acid, and n-butanol added drop by drop to the expressed sap to give a final concentration of 8·5 per cent. The mixture was clarified by centrifuging for 25 min. at 12,500 r.p.m. in the $R30$ rotor of a Spinco model L preparative centrifuge. The supernatant fluid was filtered, and the virus sedimented for 2 hr. at 30,000 r.p.m. The pellets were re-suspended in about one-twentieth of the original sap volume of $M/30$ phosphate buffer, insoluble matter removed by brief centrifugation, and the colourless, slightly opalescent fluid used for the preparation of antiserum.

Preparations of some animal viruses have been treated with formalin to destroy infectivity, without apparently affecting their antigenicity when used as vaccines. Formalin was added to freshly prepared celery yellow vein virus preparations to give 0·2 per cent concentration, before injection into rabbits. Two intramuscular injections of 0·6 ml. and 1·25 ml. respectively of virus preparation, each emulsified with 1 ml. of Freund's complete adjuvant (Bacto), were followed 3 weeks later by an intravenous injection of 1·25 ml. of formalin-treated preparation. The serum obtained 4 weeks later, reacted specifically with purified preparations of celery yellow vein, tomato black ring and beet ringspot viruses, but not with control preparations from uninfected plants. The serum titre in precipitin tube tests against the homologous antigen was 1/1,520.

This difference in antigenic behaviour *in vivo* between strains of a plant virus has not apparently been reported before, and could not be correlated with any differences in virus concentration or *in vitro* stability.

This use of formalin-treated antigen does not appear to have been previously recorded, and may enable antisera to be prepared against some other plant viruses for which the standard methods have failed.

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GENETICS

Controller Genes in Synthesis of Human Hæmoglobin

MOST current accounts of gene action stress protein production as the primary gene function. Structural and functional proteins alone, however, cannot account adequately for embryological differentiation and specialized cell function. Since all cells derived from one fertilized ovum share identical genetic material, control systems must exist which govern activation and inhibition of gene action to allow complex cell functions.

The chemical structure of normal adult hæmoglobin (Hb A) is $\alpha_2\beta_2$: 2α polypeptide chains and 2β polypeptide chains. A normal, electrophoretically slow hæmoglobin component, Hb A_2, is $\alpha_2\delta_2$, the δ-chain being a slightly modified β-chain. Normal fœtal hæmoglobin is $\alpha_2\gamma_2$, the γ-chain differing from the adult-type β-chain. The α-chain is identical in all these three normal hæmoglobins. Each of these polypeptide chains has been shown to be under independent genetic control, and mutations affecting their chemical structure have been found.

Recent observations on a human hæmoglobin mutant which apparently does not affect polypeptide structure sheds some light on the control of gene function and protein synthesis. This mutation manifests as the persistence of large amounts (15–35 per cent) of fœtal hæmoglobin[1,2] in otherwise normal adults, heterozygous for this trait. Fœtal hæmoglobin in these subjects is physicochemically identical to fœtal hæmoglobin of the new-born[3]. The condition is inherited as an autosomal trait. The term 'non-microcythæmic thalassæmia' has been applied to it by some investigators. Since other types of thalassæmia unrelated to the high fœtal hæmoglobin trait may be non-microcythæmic and since this trait appears to be different from thalassæmia (see below), this designation should be abandoned.

Children of individuals doubly heterozygous for (*a*) the high fœtal hæmoglobin trait and (*b*) the sickle cell (Hb S) trait[1,2] (or Hb C trait)[4] always inherit one or the other abnormality, but not both. Since the Hb S and Hb C gene are known to be mutations of the Hb β locus, the locus for the 'high fœtal hæmoglobin trait' can be assumed to be allelic or closely linked to the Hb β locus. Close linkage between the Hb β and the Hb δ loci has also been indicated, since children of individuals possessing (*a*) the sickling trait[5,6] (or high Hb F trait)[2] and (*b*) a mutant of the δ locus, Hb A_2 ($\alpha_2\delta_2{}^{A_2'}$—also known as Hb B_2), similarly inherit one or the other but not both abnormalities. The Hb α locus appears to be assorted independently from the β locus. No information on the linkage relations of the γ locus is available.

These data allow the construction of a tentative genetic map of the Hb β locus and related sites which aids in the explanation of gene action at this complex locus (Fig. 1). It is suggested that hæmoglobin synthesis by the linked and closely related Hb β and δ loci is regulated by an operator gene at an adjacent chromosomal site. The operator gene is inactive during early fœtal life, and no β- and δ-chains are synthesized. During fœtal development, the operator gene activates the expression of the adult-type β and δ-chains and, as a secondary effect, suppresses the production of fœtal type (γ) chains in a reciprocal relationship. The persistent fœtal hæmoglobin trait is considered a mutation of the operator gene, causing failure of normal activation of the β- and δ-chains. Continued production of fœtal γ-chains ensues. A patient homozygous for the Hb F syndrome had only Hb F ($\alpha_2\gamma_2$) and no Hb A ($\alpha_2\beta_2$)[8,10]. Absence of β-chain synthesis under control of the mutant chromosome is also clearly demonstrated in individuals doubly heterozygous for the high fœtal Hb trait and Hb β locus mutations (such as Hb S or Hb C). In such patients, hæmoglobin analysis shows approximately 70 per cent Hb S ($\alpha_2\beta_2{}^S$)[1,2,4] or 70 per cent Hb C ($\alpha_2\beta_2{}^C$)[2,4] and 30 per cent Hb F ($\alpha_2\gamma_2$). No normal hæmoglobin is found, indicating that no β-chains are produced under control of the chromosome with the 'operator' mutation. Evidence for suppression of the δ locus by the operator gene is suggested by low levels of Hb A_2 in the blood of newborns[9], decreased levels of Hb A_2 in high fœtal hæmoglobin heterozygotes[2,3], and by the complete absence of Hb A_2 from the blood of the high fœtal hæmoglobin homozygote[10]. The operator gene affects only the immediately adjacent linked β and δ loci of its own (*cis*) chromosome. Expression of the β and δ loci of the other non-mutant (*trans*) member of the chromosome pair is normal in heterozygotes with the high fœtal hæmoglobin trait, since Hb A ($\alpha_2\beta_2$) and A_2($\alpha_2\delta_2$) are both formed by such individuals.

Family data indicate that the most frequently encountered form of thalassæmia (β-thalassæmia) also is linked to the β locus[5]. In this type of thalassæmia there again is marked suppression of β-chain production. This β-chain suppression, however, is usually less complete than in the high fœtal hæmoglobin trait since thalassæmia homozygotes, sickle-thalassæmia double-heterozygotes, and high Hb F-β-thalassæmia double heterozygotes[2,10] frequently produce some normal hæmoglobin ($\alpha_2\beta_2$) with β-chains. In contrast to the high fœtal hæmoglobin trait, Hb A_2 is relatively elevated in β-thalassæmia heterozygotes, indicating that δ-chain formation is not suppressed and may even be absolutely increased. β-thalassæmia, however, severely interferes with total effective hæmoglobin formation by the erythropoietic cell and the relative failure of β-chain production is only partially and unsuccessfully compensated by γ-chain formation. In contrast, in the high Hb F trait total effective Hb synthesis is only slightly impaired since this condition represents failure of normal activation of adult type hæmoglobin chains with persistence of the normal physiological mechanism for fœtal chain production. The one known homozygote for the high Hb F trait was clinically normal with normal total hæmoglobin even though all the hæmoglobin was of the fœtal ($\alpha_2\gamma_2$) variety[10]. In β-thalassæmia, the marked depression of β-chain synthesis with unsuccessful compensation by γ-chain production and the sparing of the δ locus indicate a different kind of mutation. Several authors suggested that β-thalassæmia is caused by an electrophoretically undetectable mutation[11] of the β locus, but such a theory has not yet been proved[12]. As an alternative hypothesis, Ingram[13] suggested the existence of a 'tap', a chromosomal region linked to the Hb β locus, controlling the rate of β chain production. In β-thalassæmia, mutations of the 'tap' would lead to relative failure of β-chain production. The biochemical and functional relationship of the postulated 'tap' gene to the 'operator' gene remains unknown. Whatever the exact mechanism in β-thalassæmia, the findings in the high fœtal Hb trait are a clear indication of the operation of a discrete control system in mammalian protein synthesis.

	Operon			
	Controller genes		Structural genes	
	'Operator' gene	'Tap' gene	Hb β gene	Hb δ gene
Normal function	Activates β and δ chain formation during later fœtal life and, as a secondary effect, inhibits γ-chain formation in a reciprocal relationship.	Controls rate of β-chain synthesis.	β_2 chain production	δ_2 chain production
Mutants :	High Hb F trait : Mutation of the operator gene causes failure of Hb β and δ activation and, therefore persistent production of γ chains.	β-thal-assemia	Hb S,C,E etc.	Hb A_2' (Hb B_2)

Fig. 1. Tentative genetic map of the Hb β gene and related sites. Family data indicate linkage of the β locus to the loci for Hbδ, high fœtal Hb trait and β-thalassæmia. The linkage relationship of the various sites of this gene complex to each other is postulated. Evidence for the existence of the 'operator' gene is stronger than that for the 'tap' gene

Certain analogies between the control mechanisms of hæmoglobin synthesis and current concepts of the control of protein synthesis in microbial systems are striking. Data on mutants affecting enzyme production and repression in *E. coli* have led Jacob *et al.* to formulate a concept of structural genes concerned with protein synthesis and controller genes modulating such protein synthesis[14]. The controller genes in the microbial system are not affected by agents which block protein synthesis and their gene products presumably are not proteins. Various data suggest that the genetic control system comprises 'regulator' genes producing repressor substances and 'operator' genes which switch on and off the expression of several linked and functionally related genes to which the operator gene is also linked. The group of functionally related genes affecting a given sequence of metabolism has been called the 'operon'. The regulator genes may or may not be linked to the 'operon'.

Applying such a concept to the above data, a hæmoglobin operon may be postulated which is made up of the operator gene linked to, and controlling, the adjacent structural Hb β and δ loci (Fig. 1). In both the microbial and hæmoglobin systems the data indicate that expression of the immediately adjacent (*cis*) operon only, and not of the operon on the other (*trans*) member of the chromosome pair, is affected. An additional feature of the hæmoglobin control mechanism is the role of the Hb operator gene during embryonal development in activating the synthesis of a different type of adult protein chain. The functional need for this activation is not obvious since the only homozygote for the persistent fœtal hæmoglobin trait is apparently in good health. Furthermore, a successful mammalian species, the horse, lacks the fœtal-adult hæmoglobin switch[15]. Continued study of these and similar control systems of protein synthesis should help in understanding the integrated gene function necessary for differentiation and cell function in complex organisms such as man.

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Gene Expression in Heterozygotes

IN many cases a gene appears to function only by synthesizing a polypeptide chain through the mediation of ribonucleic acid (RNA), and hence controlling the synthesis of a protein. A mutation may cause a different polypeptide chain to be synthesized, for example, in the hæmoglobinopathies, or may result in failure to produce a polypeptide chain at all. In man there is little variation, in most individuals, of the concentration of each of the various proteins in the body fluids. This suggests that the rate of synthesis of each protein is controlled by the concentration already present. If this is so, a process of feed-back repression must operate. This may be at gene-level, perhaps resembling the regulator gene and repressor mechanism of bacteria[1], or at RNA-level. It is suggested that, in suitable cases, a study of the concentration of a protein or enzyme in the heterozygote may help to decide which type of mutation is involved.

An example is provided by analbuminæmia and bisalbuminæmia. In analbuminæmia the abnormal homozygote has no albumin or albumin-like protein in the blood, but the heterozygotes have normal concentrations of normal albumin[2]. In bisalbuminæmia the abnormal homozygote has not as yet been detected; the heterozygote has roughly equal concentrations of typical and atypical albumins in the blood, the sum of the two being equal to the normal concentration of albumin[3]. In analbuminæmia the heterozygote has one silent gene and one normal gene; it is suggested that the feed-back repression of albumin synthesis responds to the concentration of albumin in the blood and, in consequence, synthesis of albumin by the normal gene is not inhibited until this concentration is normal. On the other hand, in the heterozygote for bisalbuminæmia, the two albumins are so similar that feed-back repression responds to both typical and atypical albumins and, in consequence, the activity of both the normal and the mutant genes is inhibited when the concentration of typical albumin is only about half the normal concentration.

A second example is provided by cases of hæmophilia studied by Graham *et al.*[4,5]. Hæmophilia (hæmophilia *A*) can be severe or mild; in severe hæmophilia virtually no antihæmophilic globulin (*AHG*) activity can be detected in the blood; in mild hæmophilia *AHG* activity is far below normal but is measurable. The mothers of severe hæmophilics were found to have normal *AHG* activity in their blood, the mothers of mild hæmophilics to have *AHG* activity between the normal and hæmophilic levels. In both cases the mothers have one normal gene and one gene for hæmophilia.

It is suggested that, in these cases of mild hæmophilia, the mutant gene causes the synthesis of some protein only slightly different from antihæmophilic globulin and that this new protein has relatively slight *AHG* activity. Heterozygotes for mild hæmophilia would then synthesize normal *AHG* and some *AHG*-like protein in roughly equal amounts. If the rate of production of *AHG* in the homozygous normal is controlled in some way depending on the concentration of *AHG* in the blood, by a process of feed-back repression, and the regulating mechanism cannot distinguish between *AHG* and the *AHG*-like protein, only half the normal amount of *AHG* will be synthesized.

By an extension of this argument, it is suggested that in severe hæmophilia the mutant gene is either silent, that is, produces no protein at all, or produces a protein so different from *AHG* that the regulatory mechanism does not respond to it. In heterozygotes the feed-back repression of *AHG* synthesis would respond only to the *AHG* produced by the normal gene, therefore synthesis of *AHG* would not be inhibited until its concentration in the blood reached normal levels. This mechanism would also explain why the hemizygous male has *AHG* activity in his blood equal to that of the normal female[6].

In some cases of severe hæmophilia the carriers have reduced *AHG* activity in their blood[7-9]. Possible explanations are: (1) that the mutant gene in these cases causes the synthesis of a protein devoid of *AHG* activity but sufficiently similar to *AHG* to affect the regulatory mechanism; or (2) that the regulatory mechanism itself is at fault; or (3) that present methods of determining *AHG* activity do not measure the true concentration of a single molecular species. There is some immunological support for alternative (1)[10].

Yet a third example is pseudocholinesterase deficiency. Kalow[11] has demonstrated that, in most cases of pseudocholinesterase deficiency, the homozygote has an atypical enzyme of low activity in his plasma while the heterozygotes have pseudocholinesterase activities between the normal and homozygote levels, with a mixture of typical and atypical enzymes. Recently a family has been found with a silent gene[12]; the homozygote has neither typical nor atypical pseudocholinesterase in his plasma, but the heterozygote has pseudocholinesterase activity in the normal range. This suggests that the silent gene is producing nothing that can cause feed-back repression of pseudocholinesterase synthesis, hence the normal gene in the heterozygote produces about as much pseudocholinesterase as is present in the homozygous normal. On the other hand, atypical pseudocholinesterase produced by the more usual type of mutant gene affects the regulatory mechanism, causing the heterozygote to have low concentration of typical pseudocholinesterase.

Apart from circulating proteins, there is some evidence that a similar state of affairs may exist in galactosæmia[13]. Phenylketonuria exists in 'mild' and 'severe' forms[14], and family studies are being carried out to see whether the hypothesis advanced here can apply to phenylketonuria.

I thank Sir George Pickering for a helpful discussion, and Dr. H. Lehman for unpublished information on cholinesterase deficiency.

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ANTHROPOLOGY

Age of Basalt underlying Bed I, Olduvai

I have received a copy of the communication by Drs. von Koenigswald, Gentner and Lippolt, on the age of the basalt flow at Olduvai[1]. This article contains certain mis-statements, which seem to be the result of overlooking reports between 1951 and 1960 and quoting from out-of-date chapters of my book on Olduvai[2].

Drs. Gentner and Lippolt have apparently used three specimens.

(*a*) A specimen collected by Oakley in 1947, which they use without any indication of whereabouts in the Gorge it was collected. It must, I think, represent a specimen from the weathered and exposed surfaces of the basalt on the floor of the Gorge, since in 1947, when Oakley visited Olduvai for a few hours, he had no means of access to the underlying lava at places where it is sealed in by the overlying beds.

(*b*) The other two specimens used were tools of Oldowan type collected when von Koenigswald visited Olduvai with me in 1951. I made it quite clear to him at the time that these were surface specimens and might have come from Bed I, but equally from anywhere up to the top of Bed IV, since, typologically, Oldowan tools continued to be used right to the top of the sequence, although in Bed I they are characteristic, and in Bed II, at the base, predominate over all other types.

These two specimens were only given to von Koenigswald as representative examples of the shape and form of the Oldowan type of tool and certainly not as representing *in situ* Bed I material for dating purposes; so far as Bed I is concerned, they are irrelevant, since they may have been tools used in mid-Pleistocene times and made from mid-Pleistocene lavas, or anything earlier.

The three authors also discuss the age of Bed I on the basis of the fossil fauna, but, in this context, quote nothing more recent than Hopwood's out-dated lists in my 1951 volume on Olduvai. I have made it clear on a number of occasions, since then, that this is out of date and inaccurate.

The three authors correctly state "after the discovery of *Zinjanthropus*, however, Leakey included Bed I in the Lower Pleistocene", but they ignore the fact that I have given reasons in various places for doing so, and that, in fact, I suggested a Lower Pleistocene date as far back as 1935, although I temporarily withdrew this in 1951 because Hopwood had wrongly identified some of the fauna and so misled me.

A lengthy three-volume report on the fauna and other aspects of the Olduvai work will shortly be going to press. I must state, however, that there is no trace whatsoever of *Elephas* (*antiquus*) *recki* in Bed I where the characteristic elephant is *Elephas* cf. *africanavus* of the Villafranchian of North Africa. Even at the base of Bed II I have not, so far, found *Elephas* (*antiquus*) *recki in situ* but only *Elephas exoptatus* (or cf. *meridionalis* of Arambourg).

Indeed, far from what Hopwood claimed, the fauna of Bed I, in so far as its very numerous large mammals are concerned, is more than 95 per cent extinct.

There is abundant faunal as well as geological evidence to indicate a time-break between Bed I and Bed II and, moreover, I have indications that the base of Bed II is different from the top of Bed II; with the base of Bed II probably of the same age as the Omo fossil beds. There is also a distinct difference between the fauna of the lower and middle part of Bed I and the top of Bed I.

Bed I is not "30 metres thick". It has such a thickness in places and varies from as little as 18 ft. to more than 100 ft. In the area of the *Zinjanthropus* sites it is about 40 ft. thick. The authors repeatedly restate the wholly out-dated suggestion that the fauna of Bed I and Bed II is roughly the same, which is quite incorrect.

Samples of the lava, which underlies the Olduvai deposits at site *FLK* I and other sites, are being studied by Curtis and Evernden and I am asking them to reply independently to this aspect of the communication by von Koenigswald, Gentner and Lippolt.

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[1] *Nature*, 192, 720 (1961).

[2] Leakey, L. S. B., *Olduvai Gorge* (Camb. Univ. Press, 1957).

In Dr. Leakey's reply to our communication I miss his answer to our main question: What is his date for the basalt of Olduvai ?

After Dr. Curtis's cautious remarks early in 1961[1] about the age determinations from Olduvai—"However much of that section is contaminated . . . I think the thing to do is to get a sequence of dates and throw out those that are vastly anomalous"—I did not expect such categorical statements shortly afterwards. I am fully aware of the difficulties, but just as the results seem to indicate a so much greater age than expected I feel that all sources of possible errors and the sedimentological implications should be fully discussed before they are accepted.

So far as the fauna is concerned, I am surprised that Dr. Leakey now rejects Dr. Hopwood's determinations which he included in 1951 in his book *Olduvai Gorge*. Dr. Leakey stated that certain species are restricted to Bed I; Beds I and II still have in common such archaic genera as *Dinotherium*, *Mastodon* and even *Australopithecus*[2]; this could scarcely be expected if such a major break existed between Bed I and Bed II as suggested by Dr. Leakey.

It is, in connexion with the time-factor, interesting to learn the Oldowan tools, typologically, continue to the top of the sequence. But who is responsible for the Oldowan culture *s. str.* ? Quoting Dr. Leakey[3]: "In my report to *Nature* on the discovery of the skull of *Zinjanthropus boisei* at Olduvai Gorge in 1959, I made it clear that I believed that the very close association of quantities of primitive Oldowan stone tools with the skull of *Zinjanthropus* . . . suggested that it represented the makers of this culture. I admit, that I am no longer quite so certain, but I still think that in all probability . . . "

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[1] *Ann. New York Acad. Sci.*, 91, 349 (1961).

[2] Dahlberg, *Nature*, 188, 962 (1960). Robinson, *ibid.*, 185, 407 (1960). v. Koenigswald, *Proc. Kon. Nederl. Acad. Wetensch. Amsterdam*, 63, Ser. B, 20 (1960).

[3] Leakey, L. S. B., *Progress and Evolution of Man in Africa*, 44 (Oxford Univ. Press, 1961).

The age of 1·75 million years obtained by us for early man at Olduvai Gorge has been questioned by von Koenigswald, Gentner and Lippolt on the basis of their determination of an age of 1·3 million years for the basalt underlying the strata containing the hominid remains.

While at the Gorge in April 1961, one of us (G. H. C.) examined the basalt carefully for dating purposes. In most exposures, however, the basalt shows effects of chemical alteration and is clearly not suitable for obtaining reliable ages. Nevertheless, three specimens were sent here for examination. Thin-section examination revealed that none of these was suitable for dating; but with the appearance of the above-mentioned communication we chose the best two specimens and made age determinations in order to compare the numbers.

Specimen *KA* 927 was collected by Leakey at Site *FLK* I and specimen *KA* 933 was collected by Curtis at the third vault. *KA* 927 is an olivine basalt exhibiting subophitic or intergranular texture with numerous phenocrysts of olivine up to 1 mm. in length in a groundmass of titanaugite and plagioclase. The plagioclase laths average about 0·4 × 0·1 mm. but vary considerably, while the titanaugite is more nearly equidimensional and averages about 0·2 mm. in diameter. Grains of ilmenite compose about 5 per cent of the rock. Some of the sparse vesicles are filled with calcite, and a carbonate is intergrown with plagioclase and titanaugite in the groundmass in a manner suggesting that it is a primary mineral. Most of the margins of the titanaugite are chloritized, and chlorite or a serpentine mineral replaces the margins and cleavage cracks in most of the olivine[1]. Even some plagioclase grains are extensively chloritized.

Specimen *KA* 933 was probably an olivine basalt or olivine trachyte, but the few phenocrysts of olivine have since been completely converted to iddingsite. In other respects, however, the specimen appears less altered than *KA* 927 and no chlorite is present. The rock is exceedingly fine-grained though holocrystalline. The average plagioclase is approximately 0·05 mm. in length and less than 0·01 mm. in width. A few plagioclase phenocrysts attain 1·5 mm. Intergranular clinopyroxene averages approximately 0·01 mm. in diameter, but a few grains reach 0·2 mm. Dark brown and black equidimensional grains of magnetite and hæmatite averaging 0·01 mm. in diameter are so abundant that they render the thin-section opaque in 30μ thickness. Only thin edges of 10μ or less in thickness can be examined in transmitted light. The grains of magnetite and hæmatite occur along margins, in centres, and in zones within the plagioclase. A striking feature of the rock is the presence of biotite both in the groundmass and extending out from the sides of very numerous micro-vesicles. Some biotite flakes are up to 0·5 mm. in length. Many vesicles are partly coated with calcite.

The proportions and types of minerals are different in the two specimens, and it appears unlikely that they are specimens of the same flow. The difference in potassium contents supports this view. As two superimposed flows can be seen at one of the exposed faults in the Gorge, the occurrence of two different types at widely spaced localities is not surprising.

The data that we have obtained on these two specimens are given in Table 1.

We conclude that the basalts at Olduvai are at least four million years old but that they are unreliable for dating purposes. There is thus no conflict between the dates obtained on the overlying tuffs and the best estimate of the age of the underlying basalts. There are good reasons why one should question the reliability of dates obtained on these basalts. The location of potassium in basalts is not known with certainty. Some is contained within the plagioclase and trace amounts go into the pyroxene. Probably in most cases potassium is concentrated in the final intergranular hydrous siliceous fluid where it either enters the albitic rims of the plagioclase or, in the case of

Table 1

KA	Weight (gm.)	Percentage K	Percentage A^{at}_{40}	Ar_{40}/K_{40}	Age (m y.)
927	32 19	0 876	92	2 37 × 10^{-4}	4·1 ± 0 5
927 (repeat)	16·46	0 876	97	2·32	4 0 ± 1·0
927 (repeat)	18·34	0 876	80	2·56	4·4 ± 0 2
933	21·16	1 472	90	1·00	1·7 ± 0 2
933 (repeat)	18·77	1 472	89	1 00	1·7 ± 0 2

specimen *KA* 933 above, forms late potassium-bearing minerals in the interstices of other minerals or in the vesicles. Any later chemical alteration of the rock attacks these grain-boundary areas first and the resultant loss of radiogenic argon may considerably reduce the age obtained. Several basalts which we have attempted to date from elsewhere, such as the Columbia River area and the Berkeley Hills, have yielded anomalously low ages, and we can only explain them along the foregoing line of reasoning. In view of the difficulty that we had in obtaining moderately fresh basalt from Olduvai Gorge, it appears unlikely to us that Gentner's specimen, obtained from the British Museum by von Koenigswald and collected by Oakley without this objective in view, is as reliable for dating as ours.

Equally significant may be the extremely fine-grain size of *KA* 933. Though average dimensions are given above for grain sizes, much of the rock is so fine-grained that individual crystals appear only as specks of light, that is, sufficient only to attest to the crystalline rather than glassy nature of the groundmass. The effective grain-size for argon diffusion is no doubt much greater in volcanic glass than in this rock. The rock carries about 5 per cent biotite so that only about 20 per cent of the potassium of the rock is in this mineral with the remainder probably being in the albitic rims of the plagioclase crystals. These crystals have an average width of less than 0·01 mm. with the result that most of the potassium is within less than 1μ of a grain boundary. Even this unattractive situation is rendered worse by the fact that these crystals are charged with magnetite and hæmatite. It is not certain that such inclusions will adversely affect argon retention, but it is certain that they will reduce the average distance to grain boundaries to less than one micron and will introduce strains into the feldspar lattice. Quantitative treatment of the indicated diffusion problem is impossible due to the uncertainties of some of the parameters, but approximate solutions indicate that the diffusion coefficient necessary to explain the observed losses is probably reached at the surface temperatures of the Earth.

Some of the arguments used by von Koenigswald *et al.* require further discussion and clarification. Gentner raises the question of inherited argon in the samples of tuff used by us. While one cannot be absolutely certain that inherited argon is not a problem in some rocks, certain definite statements can be made. All available data on argon diffusion in feldspars indicate that argon retention in feldspar crystals suspended in molten rock is essentially zero over time spans of even a few days. We can state that the few volcanic sanidines of historic age dated by us have yielded ages consistent with the concept of zero argon content at the time of eruption. Both the 1912 eruption of Katmai and the 1304 eruption of Ischia yielded zero potassium/argon ages. Also, dates of late or post Pleistocene events have given reasonable ages. A late Gamblian tuff from Lake Naivasha in Kenya gave 28,000 years and a prehistoric post-glacial pumiceous rhyolite dome near Mono Lake, California, gave 5,600 years.

If the possibility of faulty stratigraphic correlation can be eliminated, the most probable source of error in any tuff lies in the possibility of contamination from older admixed debris, whether erosional debris or country rock torn from the sides of the conduit at the time of eruption. The Olduvai tuffs were examined carefully for such material before being used. The mineral used would virtually eliminate any possibility of significant conduit contamination, and the nature of the deposits (that is, primary tuff falls) argues strongly against any admixture of eroded debris. We see no possibility of explaining the tuff ages in terms of admixed older anorthoclase crystals.

The only sample of post-Pleistocene feldspar that has yielded an age far older than that assigned to it by field investigators is the concentrate of Laacher See sanidine sent to us by Dr. Frechen, University of Bonn. We have made several runs on this material, and all ages obtained are several hundred thousand years rather than the 11,000 years suggested by its assignment to Allerod. As we had nothing to do with the collection or concentration of this material, we are in no position to explain the gross discrepancy. However, we feel that the diffusion data and the dates from other young samples argue so positively for total argon loss at temperatures of 1,000° C. that we must conclude that the Laacher See sample is either contaminated or misplaced stratigraphically.

von Koenigswald appears concerned about the slow rate of sedimentation in Bed I and believes that every trace of bone would have disintegrated before becoming sufficiently buried. This opinion is based on a complete misconception as to the nature of sedimentation at Olduvai. Virtually all the layers in Bed I are primary tuffs, that is, ash falls directly from the air. They instantly buried everything lying on the land surface or in the shallow margins of the lake. One of these ash-fall units is 12 ft. thick.

Again in response to an objection of von Koenigswald, it is to be noted that the tops of many of these ash-fall layers show well-developed soil profiles, some of them quite deep (up to 4 ft.) and indicating protracted periods of time of development. Each of these soil profiles indicates a disconformity. The soil profiles are numerous, well-developed, and plainly visible in quarry faces though not in weathered canyon-wall views. It may come as a surprise to many to learn that the stratigraphy of such an important locality as Olduvai Gorge is very imperfectly known. The only reference describing Bed I (*Olduvai Gorge Monograph*, Leakey *et al.*) does not even mention the crystal content of the tuffs though this content may make up more than 30 per cent of the rock. This reference does not mention the existence of soil profiles and virtually implies absence of them. The large cuts made in conjunction with the *Zinjanthropus* excavations now clearly display an extensive development of soil profiles. This lack of adequate description of the Olduvai section will be remedied shortly as the tuffs are being studied by a competent volcanologist.

In conclusion, it can be stated that the basalts at Olduvai are at least four million years old, and that the result obtained by Gentner is to be interpreted as the result of using unreliable material. In addition, the soil profiles sought by von Koenigswald are present in profusion, and his bone destruction hypothesis is invalid on the grounds of inadequate understanding of the mechanism of tuff accumulation at Olduvai. All data so far available agree that there is no alternative to accepting an age for *Zinjanthropus* and for Lower Pleistocene of approximately two million years.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 14

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St Mary's Hospital Medical School, Paddington, London, W.2), at 5 p m —Dr. J. L. Gowans "The Role of Lymphocytes in Immunological Reactions".* (Second of four Almroth Wright Lectures.)

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (at the Science Museum, Exhibition Road, London, S W.7), at 5.30 p.m —Mr A. W. Badcock. "Physical Optics at the Royal Society to 1800".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Adventure in Nepal", Colour films with commentary by Mr J. B. Tyson.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m —Mr. J. Macdonald· "Forestry". (Second of three Cantor Lectures on "The Development of Natural Resources".)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.45 p.m.—Annual General Meeting, followed by Dr H. G. Taylor: "The Work of the Electrical Research Association".

Tuesday, May 15

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynæcology, Hammersmith Hospital, London, W.12), at 2 p.m —Prof. P. Polani "Human Chromosomes and their Abnormalities".*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street. London, W.1), at 5 p.m.—Mr Julian M Leonard: "Petroleum—a New Concept in the Field of Chemical Manufacture" (Presidential Address)

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C 2), at 5 30 p.m. —Mr. R. F. Purton "A Survey of Telephone Speech-Signal Statistics and their Significance in the Choice of a P.C.M. Companding Law".

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting and Chairman's Address.

Wednesday, May 16

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W 7), at 5 p m.—Dr. R. W. Riddell: "The Bacteriology of Mycobacterial Disease".*

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.15 p.m —Dr. D. J. Finney. "The Statistical Evaluation of Educational Allocation and Selection".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W C 2), at 5.30 p.m —Dr. E Eastwood: "Radar Observations on Bird Migration".

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. K. Lloyd-Williams, Mr. F. Lloyd-Williams, Mr. C. M. Cade and Mr. C. J. Hart. "Infra-Red Radiation Thermometry in Clinical Practice".

Thursday, May 17

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4 p.m.—Annual General Meeting. Mr. J. B. Simpson. "Security in the Mining Profession" (Presidential address).

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. F. G. Young, F.R.S. "On Insulin and Its Action" (The Croonian Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p m.—Annual General Meeting. 6.30 p.m.—Dr. F. T. Farmer. "Some Aspects of the Application of Electronics to Medicine".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. R. B. Heisch. "Feral Aspects of Rickettsial Infections in Kenya".

Friday, May 18

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m. —Meeting on "The Navigation of Hovercraft".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m —Dr. H Lowery "On Making Music".

Monday, May 21

INSTITUTION OF THE RUBBER INDUSTRY (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 4 p.m.—Prof. G. Stafford Whitby. "The Biogenesis of Rubber—How the Tree Makes Rubber" (Seventeenth Foundation Lecture).

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Dr. A. Isaacs: "The Antiviral Action of Interferon"* (Third of four Almroth Wright Lectures).

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. H. Cole: "Fisheries". (Last of three Cantor Lectures on "The Development of Natural Resources").

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6 15 p.m.—Mr I A. Menzies. "The Electrodeposition of the Less Common Metals"

SOCIETY FOR VISITING SCIENTISTS (at the English Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m —Discussion Meeting on "The Turkey X Disease". Chairman Prof. Alexander Haddow, F.R.S. Speakers· Dr. P. C. Spensley, Dr. J. M. Barnes and Mr. J G. Carnochan.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

RESEARCH ASSISTANTS (2) (with either a science or medical degree, and preferably a knowledge of cytology and sterile techniques), for work in connexion with cancer research at the Chromosome Unit of the Liverpool Radium Institute—The Registrar, The University, Liverpool 3, quoting Ref. CV./69/N (May 19).

HEAD (highly qualified) OF THE DEPARTMENT OF PHYSICS—The Principal, College of Advanced Technology, Gosta Green, Birmingham 4 (May 22)

ASSISTANT TRIALS OFFICER (with a degree in botany, agricultural botany or agriculture), to work at the Regional Trials Centre at Leeds University Farm, Headley Hall, Yorks—The Secretary. National Institute of Agricultural Botany, Huntingdon Road, Cambridge (May 23).

ASSISTANT LECTURER IN PHYSICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (May 25).

SENIOR LECTURER and a LECTURER (preferably with qualifications in applied mathematics or geometry) IN THE DEPARTMENT OF MATHEMATICS, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 25).

SENIOR LECTURER IN GEOGRAPHY at the Port Elizabeth Division of Rhodes University, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, May 25).

ASSISTANT CURATOR IN GEOLOGY in the Hunterian Museum—The Secretary of University Court, The University, Glasgow (May 26).

ASSISTANT IN GEOLOGY in the Hunterian Museum—The Secretary of University Court, The University, Glasgow (May 26).

ASSISTANT or a LECTURER (with qualifications in palæontology, including stratigraphical palæontology) IN GEOLOGY—Secretary of University Court, The University, Glasgow (May 26).

LECTURER or ASSISTANT LECTURER (with a knowledge of stability and control of aero-elasticity) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS—The Secretary and Registrar, The University, Southampton (May 26).

LECTURER or ASSISTANT LECTURER (with a recognized honours degree or equivalent and experience in teaching and research) IN THE DEPARTMENT OF BOTANY, University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, May 31).

LECTURER or ASSISTANT LECTURER (preferably with a knowledge of either colloid or surface phenomena) IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (May 31).

PROFESSOR (of high academic standing and specialized in some branch of pure or applied mathematics or theoretical physics) OF MATHEMATICS at the University of Natal, Durban, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, May 31).

SENIOR LECTURER (physicist with a Ph.D. degree, extensive experimental experience in electronics, and interested in plasma physics) IN THE DEPARTMENT OF NATURAL PHILOSOPHY—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C 1 (May 31).

TUTORIAL FELLOW IN PHILOSOPHY—The Senior Tutor, University College, Oxford (May 31).

FELLOW, and a RESEARCH FELLOW, IN THE DEPARTMENT OF BIOCHEMISTRY, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 4).

ASSISTANT LECTURER (preferably with a special interest in biogeography or surveying) IN THE DEPARTMENT OF GEOGRAPHY, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C 1 (June 5)

LECTURER (preferably with a special interest in micro-palæontology and/or palynology) IN GEOLOGY at the University of Canterbury, New Zealand, to assist in the teaching of palæontology and historical geology—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 15)

MASTER TO TEACH CHEMISTRY in the Sixth and Fifth Forms—The Headmaster, Bolton School, Bolton, Lancs.

MIRRLEES-NATIONAL RESEARCH FELLOW (with a good honours degree in engineering and some research experience) IN THE DEPARTMENT OF MECHANICAL ENGINEERING in the Faculty of Science, to undertake research in combustion in reciprocating engines in accordance with a prearranged programme of work—The Registrar, The University, Manchester 13 (July 1).

HEAD (with a good honours degree and teaching experience) OF THE PHYSICS DEPARTMENT; and a MATHEMATICS MASTER and a PHYSICS MASTER—The Headmaster, St. Albans School, Abbey Gateway, St. Albans, Herts.

READER IN THE DEPARTMENT OF PHYSICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

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SCIENCE AND THE NATIONAL ECONOMY

IN his Bernard Dyer Memorial Lecture to the Society of Analytical Chemistry in Manchester on March 7, Dr. D. W. Hill, dealing generally with research and the national economy, pointed out that in 1958–59, out of a total expenditure in Great Britain on civil research of some £200 million, £23 million went to the universities mainly for fundamental research, £8 million to the research associations for fundamental and applied research, as well as technical services to industry, £124 million to private industry for research and development, and £46·5 million for Government research stations for a wide range of research and other activities of general importance to the economy. The Government's contribution to this expenditure amounted to £80 million, of which £20 million went to the universities, £1·7 million to the research associations, £12 million to private industry, usually through research contracts, and £46·5 million to its own establishments.

Dr. Hill, without challenging the need to support fundamental research or considering whether the present support for such research was adequate, argued that the resources at present devoted to technological research were inadequate. Support for fundamental research was essentially an investment in the next generation, but both fundamental and applied research were vital to the scientific effort as a whole. They were also interdependent and each was liable to suffer from deficiencies in the other. With expenditure on research approaching 2·5 per cent of the gross national product, it was important to ensure that the distribution of this effort was properly balanced, particularly since the development of a project from the fundamental investigation into a commercial project was an extremely expensive undertaking.

It might be assumed that the universities were in 1958–59 spending the whole of their £23 million on fundamental research. For the research associations Dr. Hill suggested a figure of £2–3 million; for private industry about £12 million; and for Government research stations about £16 million; giving a total expenditure on fundamental research of rather more than £50 million. On Dr. Hill's figures, this leaves less than £150 million for all other forms of research and development, including technical services, an amount which, he suggested, could scarcely be considered sufficient to take full advantage of the expenditure on fundamental research, and if we were not to accept a reduction in expenditure on fundamental research, the balance could only be put right by increasing our expenditure on applied research and development.

Dr. Hill thought it was unreasonable to expect industry to increase its present contribution of £124 million a year in its own laboratories without the provision of specific incentives, whereas he believed that it would be relatively easy for direct Government assistance to be adjusted to the present needs. Directing attention to the comparatively small and steadily decreasing contribution from Government sources to the research associations, he asserted that a steady investment in research associations would result in a valuable return to the economy. Whereas in the early years the Government contribution was 70 per cent of the industrial contribution, in 1958–59 it had fallen to 28 per cent. While the income of the forty-six research associations operating in 1958–59 averaged £174,000, eight associations accounted for more than half the total expenditure; nine had incomes less than £50,000 a year, and a further fifteen incomes less than £100,000.

In concluding his lecture, Dr. Hill estimated that, with a present expenditure of some £8 million a year, the fifty-two research associations now operating were yielding returns to industry in direct and measureable benefits of more than £100 million. This aspect, however, was discussed more fully in an earlier lecture, last November, to the Manchester Statistical Society by Sir Harry Melville. Taking the expenditure on civil research and development in 1958–59 as some £243 million (out of a total expenditure on research and development of £478 million) or about 1·25 per cent of the gross national product, Sir Harry considered the Department of Scientific and Industrial Research, with an expenditure of a little more than £10 million, as the vehicle for the Government's investment in most fields of civil research and development except aircraft and nuclear energy, arguing that the Department's importance was in acting as a catalyst, inducing changes in the field of research and development which were out of all proportion to the actual expenditure.

Dealing briefly with the Department's provision of research grants and the work of its own research stations, Sir Harry Melville devoted much of his address to its support of the research associations, expressing the view that industry is prepared to finance an increasing volume of research and development within the associations. In the period 1949–58, the income of the forty-six research associations rose by 115 per cent, within which industrial contributions increased by 133 per cent, income from research contracts by 217 per cent, and the Department's own contribution by 53 per cent. In the period the amount of research work carried out by the associations is estimated as increasing by 52 per cent, while industrial production increased by 30 per cent.

Sir Harry Melville referred specifically to work of the Motor Industry Research Association and of the Research and Development Committee of the Timber Development Association to illustrate the way in which the work of the research associations pays off in returns to industry and the country's economy. He referred too, very briefly, to their educational function in the provision of technical and managerial staff, but neither he nor Dr. Hill examined the question of whether the research associations provided the most effective method of securing such results from the effort expended. It is this aspect that should be engaging the attention of the chairman of the Council of Scientific and Industrial Research in the review of the associations which the Minister for Science has requested.

That also can be said of the somewhat superficial policy statement, *Science in Government and Industry*, recently issued by the Association of Scientific Workers*. Starting from figures for expenditure on research and development in 1958, which agree with those taken by Dr. Hill and Sir Harry Melville, it urges that the Government should call for an increase in the allocation of money to research and development, strengthen the Ministry for Science and establish targets for the nationalized industries, making special grants for research where it can be shown that the revenue is insufficient to provide the money needed to reach those targets. Special measures are suggested for industries such as textiles and shipbuilding, including incentives to spend more on research, strengthening the research associations and the use of development contracts.

It is implied in the statement that at present Government expenditure is based on insufficient appreciation of the needs of the nation, though no supporting evidence is advanced, nor is there any critical examination of the decisive problem first of ensuring that research projects are well chosen and, secondly, that in the backward industries effective use is made of the results of research. It seems to be taken for granted that expenditure on research of itself will ensure development and the application of results. The possibility that research effort could be wasted or misdirected or that a point of no return could be reached does not appear to be considered; yet with Britain's limited resources, wise selection of projects, sound choice of method, are essentials to be determined before decisions are taken as to how much to spend on research.

The Association contends that the Ministry of Science is not fulfilling in some quarters the functions expected when it was first created, but with what justification it is difficult to determine in advance of the several inquiries into the deployment of our research effort which Lord Hailsham has already initiated. Apart from this, however, the statement inclines to accept without question the proposition that the State should intervene to support backward or declining industries by stimulating research or other means and not to face the alternative possibility that the healthier way for British economy as a whole would be to let the process of competition take its full course and allow such industries to decay, transferring labour to those industries in which Britain's greatest asset—the trained manpower and intelligence—are allowed full expression. We may well be paying already too high a price in supporting some industries too backward and too blind to help themselves.

The Association recognizes, nevertheless, that our last natural resource is manpower without drawing the appropriate deduction, and its assertions that in general we spend too little on research and development and that the distribution of this expenditure does not correspond to the need in various branches of industry are not supported by convincing evidence. Some sound suggestions are made, notably on the opportunity which the research association offers for organizing the most effective use of highly expensive equipment which a single institution or project could scarcely secure. The criticism of the low expenditure on research in the nationalized sector is probably well founded, more so than that of expenditure on educational research and research into health: the former is already being further encouraged by the Minister of Education, and on medical research the Association is at issue with official views that medical research suffers from lack of ideas rather than lack of funds. Undoubtedly, the statement indicates the importance of the review of the organization for the promotion of civil science by Government agencies which has now been initiated, and it also points to the urgency of the Advisory Council on Scientific Policy reaching early decision on its own inquiry into that field and ensuring that all the information required for sound decisions is available at the earliest possible moment.

ACADEMIC SCREWTAPE

Letters to a Vice-Chancellor

By James Dundonald. Pp. 109. (London: Edward Arnold (Publishers), Ltd., 1962.) 12*s*. 6*d*. net.

In *The Screwtape Letters* C. S. Lewis provides some brilliant and perceptive advice from an old experienced devil to a neophyte in devilry. In *Letters to a Vice-Chancellor* the don who hides his modesty under the name James Dundonald borrows this device (without, alas ! the brilliance or perception) to tell a newly appointed vice-chancellor to one of the newly founded universities what he ought to know.

The two books have one thing in common. If C. S. Lewis's Wormwood is as naive as he is made out to be, he will never make an efficient devil; and, similarly, if Dundonald's Sir Ronald Pellew (the new vice-chancellor) needs to be told some of the things which are included in *Letters to a Vice-Chancellor*, it is a poor prospect for the new University of Barstow which appointed him. James Dundonald's letters explain to Sir Ronald Pellew, ever so gently, that he must be patient when he presides over Senate meetings, because some professors are both prolix and irrelevant; that he must be at great pains to provide himself with an efficient registrar; that he must encourage his professors to take teaching seriously, and that he should persuade some of them to live on the 'campus' (the word has finally crossed the Atlantic), with a grant of £15 a year to pay for coffee and sherry parties for students; that he must not be too insistent on residence for all students; that he must play a sort of Russian diplomacy with the local technical college; and that over Sir Charles Snow and his two cultures the new vice-chancellor should look to Keele, and then try to improve on Keele's experiment.

All sound doctrine, no doubt. James Dundonald writes amiably and with tolerance. But he goes no deeper than the etiquette of running a university; the reader of this book might easily be left with the impression that there really are no difficult intellectual problems in university administration. In fact, there are some very difficult intellectual problems, and the future of British higher education depends on the way they are solved. Here (for Sir Ronald Pellew's benefit) are three of them. The new University will need a constitution. Will the constitution affirm that the University is a self-governing society whose members, the academic and senior administrative staff, are not employees but co-partners ? Or will it follow the example of Keele and Sussex, the constitutions of which leave everyone guessing whether a university teacher is a member of a society or an employee in a hierarchy ? Secondly, the new University will need to determine the standard and content of its degree. Everyone will assume that the standard must be identical, and the content only slightly at variance, with that in all other British universities, guaranteed by an academic advisory committee and enforced by an annual visitation of external examiners, which would mean that any really revolutionary ideas about the pattern of degrees (such as are to be found in Moscow or Chicago) would be withered by the arid scorching wind of academic public opinion. Thirdly, everyone will assume that the new University will teach for about 22 weeks out of 52, with examinations for another 4 weeks, thus perpetuating the belief that students read and reflect in the vacations, whereas in fact they pick peas or work in cafés; and that professors do research and re-write lectures, whereas some of them paint the bathroom and earn extra money by writing broadcasts. Will Sir Ronald think about these, and a dozen other equally important problems ? Or will he accept current practice on these big matters, and confine his innovations to trivialities ? "BRUCE TRUSCOT, JUN."

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* *Science in Government and Industry*: a Policy Statement by the Association of Scientific Workers. Pp. 8. (London: Association of Scientific Workers, 1962.) 1*s*.

A MODERN DIALOGUE

A Threefold Cord

Philosophy, Science, Religion. A Discussion between Viscount Samuel and Professor Herbert Dingle. Pp. 280. (London: George Allen and Unwin, Ltd., 1961.) 25*s*. net.

THIS book is an extended dialogue in which Lord Samuel and Prof. H. Dingle try to elucidate and to reconcile the points of view of a philosopher and a scientist. It begins appropriately with a chapter entitled "View-Points". Then it examines topics of special interest in three chapters on "Energy and the Ether", "Science and Mathematics" and "Life and Mind"; and it ends with a chapter of "Retrospect and Prospect", in which the authors, having re-stated their differences, hope for a new harmony in religion.

All this ought to make a fascinating progression, particularly in the hands of two men who write so clearly and so well as Lord Samuel and Prof. Dingle. Alas, the book disappoints our expectation. There is no progression; each author virtually ignores the arguments of the other; and their exchanges become a series of repetitions which throws no new light on the views of either. Perhaps the form of the dialogue, which worked so well between Socrates and his young friends, is not suited to two protagonists of equal intellectual standing. In this book it has merely encouraged the authors to digress, and to ride away from the debate on hobby-horses of their own.

But there is a deeper reason for the inconclusiveness of the book; it springs from the narrow and sectarian way in which the authors present their two disciplines. If common ground is to be found between philosophy and science, then it must surely be looked for, not in one philosophy and not in one interpretation of science, but in the basic objectives of the two studies. The writer who wants to show that philosophy has something helpful to say to scientists must be willing to speak for what is fundamental to all philosophy as a mode of inquiry, and which makes it different from other modes. He must show what philosophy is at and what it is about—above all, why the questions which philosophers ask are not foolish. Similarly, the spokesman for science must express the aims and demonstrate the methods which are shared by all scientists. He must show how the theories of science are related to the findings in the laboratory, and why; at bottom, he must explain to the philosopher why the precise questions which scientists ask are thought by them to be interesting.

The authors of this book have attempted nothing so ambitious as this. Lord Samuel does not try to speak for philosophy as such, or to present it as a method with its own unity. Instead, he states and re-states his personal philosophy, which is a kind of common-sense realism; when he refers to other philosophers, it is almost always critically. Equally, Prof. Dingle does not try to give a reasoned exposition of the principles which underlie all the sciences (including the biological sciences). Instead, he confines himself to explaining, patiently and often, his own rigid view of science as an operational technique. When he describes the outlooks of other scientists, he leaves no doubt that he thinks them illogical and even bigoted, and Lord Samuel joins him in these criticisms. Indeed, the one ground on which the authors are usually united is their distaste for the views of others.

It is not to be wondered at, then, that Lord Samuel and Prof. Dingle find little to agree on at the end of the book. Common-sense realism on one hand, and scientific operationalism on the other, have not much in common. But even if they had more, it would give no help to the philosopher in general or to the scientist in general. Most scientists think that philosophy is an artificial study, and many philosophers think that science is a pedestrian study. This book does nothing to correct their prejudices.

J. BRONOWSKI

A TEXT-BOOK OF PHYSICAL ANTHROPOLOGY

Manual of Physical Anthropology

By Prof. Juan Comas. Revised and enlarged English edition. Pp. xxi + 775. (Springfield, Ill.: Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1960.) 140*s*.

THIS manual first appeared in Spanish in 1957 and arose, as Prof. Comas states in his prologue, partly as a result of his teaching experience and partly in answer to the need for a suitable text-book in the Latin American world. It now has appeared as a revised and enlarged English translation with a preface by Prof. W. M. Krogman.

A brief outline of the contents will indicate the scope of the work. It commences with the history and objectives of the subject, followed by the elements of genetics including a section on race mixture and racism. After a general account of growth with an evaluation of measurements and indexes, there are sections on descriptive observations of the living, serology, somatometry, biotypology, osteology and palæoanthropology. Contemporary racial classifications are compared and the principal living peoples described. The final chapter is on the application and teaching of physical anthropology. In the appendixes the international agreements on anthropometry of Monaco and Geneva, and the Unesco statement on race are reproduced as well as several blanks for recording data. The bibliography deals with the principal works under subject headings and in addition there is a list of periodicals. There are separate indexes for authors and subjects, and throughout the book sources are given by means of footnotes on the relevant pages.

The author follows the classical tradition of physical anthropology and, in so far as he does so, he strikes a good balance between the different sections. However, one wonders whether this concept is not too narrow. The whole field of physical anthropology has altered so radically that to-day there is a much greater emphasis on genetics, biochemistry, physiology, pathology, statistics, experimental morphology and the study of behaviour and of populations and, unfortunately, this change is not revealed in the manual. It is a great pity, for the book stimulates thought and a healthy critical outlook in what it covers. The time has probably come when single authorship has become hazardous in attempting to encompass the whole subject in a sizeable volume. One makes this criticism with all due deference to the author, who has undoubtedly marshalled quite an array of material.

On the positive side one has to welcome the number of French, Spanish, Italian and South American references that are included and which so often have either been omitted or are even unknown to English-speaking people. The applied side of the book is the most valuable part. Thus, the measurements frequently used in studies of growth, the helpful introduction to somatology, the sections on somatometry and osteometry, and the evaluation of various methods used in the estimation of stature and sexual differentiation, as well as the practical advice on collecting, restoring and preserving material are excellent. The part on race mixture and racism is well balanced. The section on palæoanthropology is sound, the different schools of thought are fairly presented, and the final chapter on the application and teaching of physical anthropology is important and useful. The many tables and figures are clear and instructive.

Apart from the overall criticisms made, there are certain points one has to raise. Thus, the growth section does not mention the work of Brozek or Tanner and the work of the latter should also have been considered in the chapter dealing with biotypology. The photoelectric method for the evaluation of skin colour is insufficiently emphasized; this technique surely gives the most precise results and therefore should have been dealt with more fully than the more historic methods. The work of Barnicot on pigmentation, and the association of sickle-cell disease and malaria are not mentioned, and thalassæmia, the hæmoglobins and haptaglobins are omitted. When considering brachycephalization some account might well have been given of recent hypotheses instead of mere references. In the discussion on dating fossil material, the nitrogen and potassium–argon methods are not mentioned. More space could have been devoted to the important subject of population biology. Nor has population genetics been touched on. The bibliographical list of principal works should have included that of Li on population genetics and of Rensch on evolution. In the periodical section, reference ought to have been made to two important abstracting journals, *Excerpta Medica* (Section I) and *Biological Abstracts*, and to certain journals on genetics. A few authors, though referred to in the text and in the author-index, are not cited in the relevant footnotes where the references are usually given in this book, for example, Zook (p. 204) and Geissler (p. 221).

The production is of the usual high standard one has come to expect from this publisher. But the price is prohibitive.

F. P. LISOWSKI

DEVELOPMENTAL BIOLOGY AND INFORMATION THEORY

Oogenesis

The Storage of Developmental Information. By Prof. Chr. P. Raven. (International Series of Monographs on Pure and Applied Biology. Division: Zoology, Vol. 10.) Pp. viii+274. (London and New York: Pergamon Press, 1961.) 50s. net.

THIS book, which deals with some of the major problems in developmental biology, will present many biologists with their first introduction to the concept of information theory. Derived from communication engineering, information theory might seem very remote from the subject of oogenesis, but Prof. Raven, using a minimum of technical terms, quickly and convincingly explains its application. On this basis, the author examines the intricate relationship between oogenesis and the storage of developmental information in the fertilized egg at the beginning of its development, and how this information is transmitted from one generation to the next through the egg cell.

The book is divided into 10 chapters, which cover: embryonic development as a communication problem; the formation of egg cells; the egg nucleus during oogenesis; the cytoplasm and its inclusions; the relationship between the egg cell and the auxiliary cells; metabolism of the oocyte; the cortex; polarity and symmetry; the storage of developmental information, and information theory and biology. Much of the book is given to a detailed account of the mechanisms by which substances are transferred from the maternal tissues to the oocyte, and the transformations which they undergo during the growth of the latter.

Throughout the animal kingdom, there is great diversity in the structure and function of the egg cell. By selecting examples from a wide variety of species, both vertebrate and invertebrate, attention is directed to the comparative nature of the problem. This approach is particularly stimulating.

In attempting to derive estimates of the amount of developmental information carried by the nuclear genes, the cytoplasm and the cortex of the egg, it is agreed that the figures can only be crude approximations. The picture is further complicated by the existence of unknown quantities of redundant information. However, as a starting point, these estimates should provide a useful basis for further thought.

Although many controversial points are raised and some debatable assumptions have had to be made, the discussion is well balanced; where information (in the ordinary sense) is lacking, due allowance is made. By pointing out, in the appropriate places, that our knowledge is fragmentary, for example, little is known about the egg cortex, subjects for further research are suggested.

When refuting Elsasser's statement (p. 215) "that the great majority of germ cells produced by a healthy organism are viable", the author is possibly equally open to criticism in concluding "that in most animals a completely normal germ cell is rather an exception than the rule". One further minor criticism concerns the misleading impression given on p. 30 where it is stated that in mammals the egg is extruded into the abdominal cavity. Mention of the oviduct would have eliminated the ambiguity. To make these two points is in no way meant to detract from the general excellence of the discussion.

The book is amply illustrated with 44 text-figures and 4 plates, including 5 electron micrographs, which show the value of electron microscopy in the analysis of the fine structure of the egg. There is a good bibliography, containing nearly 400 references. Author, taxonomic and subject indexes complete the work.

Written in a clear style, this informative and stimulating book will appeal especially to those interested in embryology, genetics and theoretical biology; it is confidently recommended.

C. E. Adams

BIRDS OF THE AFRICAN CONTINENT

Birds of the Southern Third of Africa

By C. W. Mackworth-Praed and Captain C. H. B. Grant. (African Handbook of Birds, Series 2, Vol. 1.) Pp. xxiv + 688 + 38 plates. (London: Longmans Green and Co., Ltd., 1962.) 50*s.* net.

The two volumes (now in their second edition) of the first series of the *African Handbook* covered eastern and north-eastern Africa, from the Sahara to the Zambesi; the second series covers the continent south of the Congo–Zambesi divide, and on the western side includes most of Angola. This first volume deals with all the non-passerine birds and the first four passerine families. Captain Grant died in 1958; but the greater part of the work had been completed by then.

Their basic taxonomic studies had been published in detail by the authors in scientific journals over many years; the essential facts are now presented in didactic and summary form. Distinguishing characters and distribution are particularly stressed. The information on habits is often extremely brief, as in many instances little is available; the authors' avowed intention is to provide a sound basis of identification for the further field observations for which there remains so much scope. For that purpose this volume, with its companion to follow, should prove most useful and convenient. In addition to coloured plates by various artists, and some photographs, marginal drawings and little distribution maps are again a welcome feature.

In general, we have a picture of the bird-life of a large area, partly tropical and largely warm temperate in climate, presenting a considerable range of environments. There are mountains rising to 6,000 ft. or more, many important rivers, and a long coastline; there is not much dense forest and only one 'true' desert; woodland of different kinds, grassland, and semi-desert make up the greater part. Some habitats have been altered or destroyed by man, deforestation often being followed by erosion; elsewhere shade and water have been created where they did not exist before. This is a 'wintering' ground (in the southern summer) for many migrants from Europe and Asia; it is inhabited during part of the year by species that spend the other part in equatorial Africa; and it is the permanent home of many that are either sedentary or migrate only within the area. The breadth of the representation of different groups may be judged from the fact that the first two species to come under notice are the ostrich and the only native penguin.

Landsborough Thomson

ASPECTS OF SOLID-STATE PHYSICS

Solid State Physics

Advances in Research and Applications, Vol. 11. Edited by Frederick Seitz and David Turnbull. Pp. xvi + 438. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12.50 dollars; 89*s.* 6*d.*

A further volume of *Solid State Physics* provides us with another five authoritative review articles on various aspects of the subject: two on specially important materials (grey tin and cadmium sulphide) and three on techniques which have wide application (high pressures, piezo-resistance effects in semiconductors and cyclotron resonance).

The article on the semiconducting properties of grey tin by G. A. Busch and R. Kern is like an ingenious puzzle which must be both set and solved by the players. The game is to assign the characteristic parameters—energy gap, effective mass, etc.—so as to fit best the experimental results. What makes the game especially piquant here is the paucity of the experimental results. R. H. Bube's essay on ionization energies associated with imperfections in CdS-type materials displays (in contrast) an indigestible feast of experimental results.

Perhaps the most appealing article of the collection is C. A. Swenson's account of high-pressure physics since 1945. Like low-temperature physics, high-pressure physics is a dying subject—not indeed because it is no longer being practised, but because it is being so widely practised that the element of 'craft' has passed over to technology. This article may thus prove to be a memorable 'obituary' which will be used and enjoyed for many years.

An instructive lesson on the power of resonance techniques can be obtained by comparing the article by B. Lax and J. G. Mavroides on cyclotron resonance with that of R. W. Keyes on piezo-resistance in semiconductors. The latter, 'classical' technique yields at the best vague information about band structure. The former, however, has given an astonishing amount of new knowledge in a short space of time: the authors have no difficulty in showing that it is the most important and promising technique available.

This volume of *Solid State Physics* includes a cumulative subject-index of Volumes 1–10.

R. O. Davies

Diagnostic Methods in Veterinary Medicine

By Prof. Geo. F. Boddie. With a chapter on Diagnosis of Poultry Diseases by Dr. J. G. Campbell. Fifth edition. Pp. ix+420. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 30*s*. net.

THIS is a good book written by a clinician for clinicians. The arrangement is consequently according to the demands of the man observing a case. The general clinical everyday methods of observation and examination are given most of the space in the text, and special and elaborate systems of diagnosis are, in general, relegated to special chapters. Nine of the twenty-one chapters are concerned with body systems, and the amount of clinical detail covered is extraordinarily complete.

Specific infective processes are less completely covered and many that might lead the veterinarian to consult the book, for example, melioidosis or coccidioidomycosis, are outside its scope.

There is much more in the book than is indicated in the contents pages. Tables that are given in sequence in certain chapters might be numbered serially throughout the volume and listed as such on a preliminary page. With such a mass of essential information some greater breakdown showing where special items are to be found would help a reader.

For the practitioner on the farm or in the town the book is an essential source-book to be consulted at every contingency. The publishers' share in the work is up to the standard to be expected of them.

W. A. Pool

Synovial Joints

Their Structure and Mechanics. By C. H. Barnett, Prof. D. V. Davies and Prof. M. A. MacConaill. Pp. xi+304. (London: Longmans Green and Co., Ltd., 1961.) 50*s*. net.

THIS book sets out, at not too great a length, the modern views on most aspects of synovial joints. It is divided into four sections, the first dealing with the structure and composition of the various components of joints, and the second with such physiological topics as their nutrition, repair and development. The third section is an entirely non-mathematical treatment of the mechanics of joint movement (though adequate in its way, this section would have been improved by the addition of an extra chapter setting out some of the ideas in a rigid mathematical form). Various functional aspects are discussed in a final, shorter section.

The medically qualified reader, for whom the book is primarily intended, will find it most useful. The non-medical biologist, provided he can accept the extensive use of anatomical terms, should find that there are several chapters to interest him. The anatomical emphasis is very strong, reflecting the fact that all three authors are in anatomy departments. Other disciplines have not been ignored, but some of the biochemical asides could be misleading to the uninitiated. Although the individual authorship of certain passages is here and there detectable, the three authors have achieved a commendable degree of uniformity of style. The overall balance is good, though a few hobby horses have been ridden rather hard. The book is well documented, with more than 400 references, which appear to have been well chosen. The bibliography has been made all the more useful by the inclusion of an author index.

P. R. Lewis

Progress in Operations Research

Vol. 1. Edited by Russell L. Ackoff. (Publications in Operations Research, No. 5.) Pp. xii+505. (New York and London: John Wiley and Sons, Inc., 1961.) 92*s*.

THIS book, the first of a set of review volumes dealing with research methods, is number five in the series *Publications in Operations Research*. The set is to be a basic reference work, and, if this first volume is a guide, will undoubtedly be just that: a reference rather than an exposition. Apart from chapters of a general character, on subjects such as "The Meaning, Scope, and Methods of Operations Research", there are chapters dealing with subjects carrying specific mathematical apparatus, such as inventory theory, programming, Markov and queueing processes, sequencing theory and replacement theory. But these chapters, while they will be of value from the bibliographical point of view, are intensely unsatisfying. The reader is likely to feel insecure in that he will have been taken on a quick tour of inspection of some field, without being able to linger on detail at any point. The only exception to this seems to be the chapter by Prof. Morse on the dynamics of operational systems, which is excellent in that it combines a fine, expository power with some real mathematical technique.

For the postgraduate student contemplating entry into the field of operations research, the present book will be valuable in that it gives a quick bird's-eye view of practically all the methods so far used. There is also an ample bibliography at the end of each chapter to guide future reading, and the book, on the whole, achieves its aim of making the content of recent work in operations research readily available.

L. S. Goddard

Transformers and Generators for Power Systems

Their Behaviour, Capabilities and Rating. By R. Langlois-Berthelot. Translated and revised in collaboration with Lieut.-Colonel H. M. Clarke. Pp. 541. (London: Macdonald and Co. (Publishers), Ltd., 1960.) 65*s*. net.

THE writer is known, not only as a distinguished French engineer but also as the author of a series of four text-books which have been in use by French engineers and in the French colleges for several years. The present volume, together with the earlier work entitled *Electro-magnetic Machines*, covers the range of the four French books. They are, however, not exact translations but have been adapted to British needs with the help of Lieut.-Col. H. M. Clarke. As would be expected, the book takes full account of modern developments and, moreover, has a very valuable bibliography.

The book is of particular value to engineers interested in transformers and synchronous machines in relation to their application rather than to questions of detailed design. All the important practical operating problems are discussed, ranging from insulation and heating to steady and transient performance. It is of considerable interest to advanced students in the electrical power field, but in Britain it is of less value for undergraduate students because of the growing tendency in college courses to concentrate more on physical fundamentals than on the techniques used for exploiting them. Very little time is devoted nowadays in an undergraduate course to the subjects covered by this 540-page book.

B. Adkins

NEW SCALE OF NUCLIDIC MASSES AND ATOMIC WEIGHTS

By Dr. EDWARD WICHERS
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IF physicists and chemists accept the recommendation of their principal international bodies, the International Union of Pure and Applied Physics and the International Union of Pure and Applied Chemistry, they once again will be using a common scale for expressing the relative masses of atomic particles. For about thirty years two scales have been in use, both based on the integral number 16. On one scale, commonly called the chemical scale, this number was used as the relative atomic weight of the element oxygen. On the other, the physical scale, the same number was used as the atomic or nuclidic mass of the principal isotope of oxygen. If expressed on the physical scale the atomic weight of the element oxygen would be approximately 16·004 instead of 16. The new, unified, scale has as its denominator the integral number 12 as the relative mass of the atom of the principal isotope of carbon, ^{12}C. On this scale the atomic weight of oxygen becomes 15·9994. For those whose interest may go beyond this brief statement of a completed interdisciplinary agreement the following discussion will describe the situation that led to the existence of the two scales and the considerations that have now led to their replacement by the new unified scale.

An inescapable consequence of the atomic theory of matter, as first clearly enunciated by Dalton, is that every atomic particle of a given species of matter must be identical in all respects with all other particles of the same species and must differ characteristically from the particles of all other species. Thus, the masses of atoms were early regarded as natural constants of fundamental importance. For a long time the absolute magnitude of atomic masses could only be estimated; but most purposes of physical science were served by knowing the relative masses of the different kinds of atoms. This knowledge, combined with the discovery of the laws of simple and multiple proportions, made it possible for chemistry to evolve from a merely descriptive to a quantitative science.

A very large part of chemical research during the nineteenth century was concerned with the discovery of what were then regarded as the fundamental species of matter—the chemical elements—and with the determination of their relative atomic masses. At first the only available way to determine atomic masses was to measure the weights of the various elements that were equivalent to one another in selected chemical reactions or that combined to form simple molecules. Thus, the term 'atomic weight' came to be used to express the more fundamental concept of relative atomic mass. The numerical expressions of these two properties of an atom are normally identical, since relative atomic weights are always measured in a common gravitational field. To-day, the two terms are often used interchangeably, with some confusion of meaning that could be avoided if 'atomic mass' were reserved for the species of matter now known as nuclides and 'atomic weight' were used only in its traditional connotation, that is, for elements.

The precision and the accuracy of the determination of atomic weights increased steadily during the latter part of the nineteenth century. They reached their height in the early part of the twentieth century in the work of T. W. Richards and G. P. Baxter and their associates at Harvard University, and of O. Honigschmid and his many collaborators at Munich. Paradoxically, the remarkable precision of the measured atomic weight of lead from different sources proved beyond question that the atomic weight of an element is not necessarily constant. The consequence of this discovery was the realization that the substances known as elements are not necessarily composed of identical atoms, but that some elements may be composed of a mixture of atoms which are essentially identical in chemical properties but not identical in mass. Such species of matter were given the name 'isotopes'.

To-day, we know that most of the ninety-odd elements occurring in Nature exist as mixtures of isotopes. There are about 300 naturally occurring isotopes, and several hundred additional isotopes can be prepared artificially; but the concept that the mass of an atomic particle is constant is now more firmly established than it was before isotopes were discovered. This is so both because of advances in the knowledge of the fundamental nature of matter and because modern methods make possible much more exact measurements of nuclidic masses. However, it should be noted that although the masses of individual nuclides that constitute an element can be measured very accurately, their abundances in an element cannot be determined, as yet, with equal accuracy.

The chemist is no longer unaware of the existence of isotopes or of the fact that the chemical properties of isotopic species are not strictly identical. Nevertheless, in most of his work he deals with the chemical elements as they exist in Nature, whether as a single nuclide or as a complex mixture of isotopes. Therefore, when he is concerned about the proportions in which elements react with one another he needs to know the average mass of the mixture of nuclidic species which constitute the element. This is the quantity that has been called the atomic weight of the element, and the term is still useful provided certain limitations are attached, particularly the limitation that the element be in its natural condition, unmodified in its isotopic composition by human intervention or as the result of the naturally occurring phenomena of radioactivity. In contrast, the atomic physicist is usually concerned with individual nuclidic species—the differences in their nuclear characteristics and the nuclear, as opposed to chemical, changes they undergo.

These divergent interests of the chemist and the physicist have been reflected in the use of different bases of reference for denominating nuclidic masses and atomic weights. Early in this century chemists differed in their ideas about the best way to express atomic weights. Some believed it was best to base the scale of atomic weights on unity as the atomic weight of hydrogen, the lightest element. Prout's hypothesis, that all the elements are composed of hydrogen and thus that their atomic weights should be multiples of the atomic weight of hydrogen, undoubtedly had a strong influence on this choice of scale. However, oxygen was more useful than hydrogen as an operational reference species, because it forms stable and manageable compounds with more elements. This consideration led to an international agreement, early in the twentieth century, to base the scale of atomic weights on oxygen as the reference species, with an assigned integral atomic weight of 16. There was a transition period during which text and reference books continued to publish two tables of atomic weights, one based on H = 1 and the other on O = 16. The oxygen scale had been firmly established when Aston, about 1920, built the first mass spectrograph and began to measure atomic masses.

The later discovery that natural oxygen contains small proportions of isotopes of nominal masses 17 and 18 made it evident that Aston's mass ratios were not based on natural oxygen, as he had supposed, but on the dominant isotope of oxygen. However, Aston continued to use the integral number 16 as the relative mass of this isotope and thereby established a new scale of nuclidic masses, which came to be known as the physical scale. This was done with the approval of the International Commission on Atomic Weights, of which Aston himself was a member. At that time it was scarcely possible to foresee the ultimate effects of the decision. The concentrations of ^{17}O and ^{18}O in natural oxygen were not known and would not be determined accurately for some years. Furthermore, it does not appear to have been foreseen that mass spectroscopy would provide a valuable technique for the determination of atomic weights. Thus, when refinements of the technique proved it to be a reliable method for determining atomic weights, it was necessary always to convert data measured and reported on the 'physical' scale to the established scale by a factor based on the isotopic composition of natural oxygen. This composition proved to vary slightly with the source of the oxygen; but from 1940 the International Commission stabilized the conversion factor at 1·000275 and thus established a conventional, although never formally defined, relation between the physical scale of atomic (nuclidic) masses and the chemical scale of atomic weights.

Scientists were thus confronted with a situation similar to that existing when the hydrogen and oxygen scales were in simultaneous use. However, the difficulties were now much more serious because, in the meantime, there had been accumulated an enormous volume of data on physico-chemical properties of systems the composition of which was expressed in gram-equivalents or gram moles, on the chemical scale. Some molar quantities, such as Avogadro's number, the Faraday constant, and the universal gas constant were expressed sometimes on one scale and sometimes on the other, without identifying the scale. Not infrequently thermodynamic quantities began to appear which were derived partly from data on one scale and partly from data on the other, without stating the distinction. Some of the data were accurate enough so that the difference between the two scales could not be neglected without impairing the usefulness of the derived quantities.

In view of these circumstances and the prospect that confusion would increase with further improvement in the accuracy of physico-chemical measurements, the International Commission on Atomic Weights began in 1955 to study the possibility of unifying the scales or of adopting a new scale. Scales based on ^{1}H = 1 and ^{4}H = 4 had been suggested earlier, but neither of these appeared satisfactory. The simplest procedure would have been to abandon either the chemical or the physical scale in favour of the other but neither alternative was acceptable. Chemists recognized the logical superiority of a scale based on a reference species the atomic weight of which was invariant but were reluctant to face the problems resulting from introducing a systematic change of nearly 3 parts in 10,000 into many recorded molar data. From this point of view a new scale, to be acceptable, should not result in more than very small numerical changes in quantities based on the existing chemical scale. Physicists, on the other hand, were understandably opposed to exchanging a single nuclide for a mixture of nuclides as a reference species, even if the composition of the mixture were to be fixed by definition. Furthermore, they considered it essential to use an integral number as the assigned relative mass of the reference nuclide and thus to provide a simple basis for the definition of the 'absolute mass unit'. For example, the absolute mass unit based on the ^{16}O scale is 1/16 of the mass, expressed in grams, of an atom of ^{16}O. By preference the integral number taken as the assigned relative atomic mass of the reference nuclide should be identical with its 'mass number'. (The 'mass number' of a nuclide is the sum of the nucleons, protons and neutrons, contained in its nucleus.) It is desirable that the mass number be identical (by definition) with the atomic mass of the reference nuclide, even though no other nuclide will have an exact integer for its atomic mass.

If the chemist's desire to avoid more than a minimal change in the values for atomic weights and the quantities based on atomic weights was to be satisfied, the choice of a new reference species was limited to a small number of nuclides. Use of the following nuclides would result in the indicated changes from values expressed on the chemical scale: ^{12}C, −43 parts per million; ^{15}N, −30 p.p.m.; ^{17}O, +9 p.p.m.; ^{18}O, +4 p.p.m.; ^{19}F, +41 p.p.m. By contrast, the adoption of ^{16}O (the existing physical scale) would cause a change of −275 p.p.m. Of the foregoing, only ^{12}C was fully acceptable to physicists as a possible replacement for ^{16}O. As the principal isotope of carbon this nuclide has long served as an operational reference species in mass spectroscopy. In the form of singly, doubly, or triply charged hydrocarbon ions ^{12}C is capable of supplying an almost unlimited number of masses for the doublet technique of determining mass differences by mass spectroscopy. ^{12}C appeared also to be satisfactory as a basis of reference in the derivation of nuclidic masses from 'Q values'—the net energy changes involved in nuclear reactions. However, the change from ^{16}O to ^{12}C as the reference species would result in changes of about 300 parts per million in values for nuclidic masses.

Various objections can be made to the other nuclides listed already. ^{15}N, ^{17}O and ^{18}O are minor isotopes not readily available for direct experimental

Table 1. INTERNATIONAL ATOMIC WEIGHTS—1961

	Symbol	Atomic No.	Atomic weight
Actinium	Ac	89	
Aluminium	Al	13	26·9815
Americium	Am	95	
Antimony	Sb	51	121·75
Argon	Ar	18	39·948
Arsenic	As	33	74·9216
Astatine	At	85	
Barium	Ba	56	137·34
Berkelium	Bk	97	
Beryllium	Be	4	9·0122
Bismuth	Bi	83	208·980
Boron	B	5	10·811*
Bromine	Br	35	79·909†
Cadmium	Cd	48	112·40
Cæsium	Cs	55	132·905
Calcium	Ca	20	40·08
Californium	Cf	98	
Carbon	C	6	12·01115*
Cerium	Ce	58	140·12
Chlorine	Cl	17	35·453†
Chromium	Cr	24	51·996†
Cobalt	Co	27	58·9332
Copper	Cu	29	63·54
Curium	Cm	96	
Dysprosium	Dy	66	162·50
Einsteinium	Es	99	
Erbium	Er	68	167·26
Europium	Eu	63	151·96
Fermium	Fm	100	
Fluorine	F	9	18·9984
Francium	Fr	87	
Gadolinium	Gd	64	157·25
Gallium	Ga	31	69·72
Germanium	Ge	32	72·59
Gold	Au	79	196·967
Hafnium	Hf	72	178·49
Helium	He	2	4·0026
Holmium	Ho	67	164·930
Hydrogen	H	1	1·00797*
Indium	In	49	114·82
Iodine	I	53	126·9044
Iridium	Ir	77	192·2
Iron	Fe	26	55·847†
Krypton	Kr	36	83·80
Lanthanum	La	57	138·91
Lead	Pb	82	207·19
Lithium	Li	3	6·939
Lutetium	Lu	71	174·97
Magnesium	Mg	12	24·312
Manganese	Mn	25	54·9380
Mendelevium	Md	101	
Mercury	Hg	80	200·59
Molybdenum	Mo	42	95·94
Neodymium	Nd	60	144·24
Neon	Ne	10	20·183
Neptunium	Np	93	
Nickel	Ni	28	58·71
Niobium	Nb	41	92·906
Nitrogen	N	7	14·0067
Nobelium	No	102	
Osmium	Os	76	190·2
Oxygen	O	8	15·9994*
Palladium	Pd	46	106·4
Phosphorus	P	15	30·9738
Platinum	Pt	78	195·09
Plutonium	Pu	94	
Polonium	Po	84	
Potassium	K	19	39·102
Praseodymium	Pr	59	140·907
Promethium	Pm	61	
Protactinium	Pa	91	
Radium	Ra	88	
Radon	Rn	86	
Rhenium	Re	75	186·2
Rhodium	Rh	45	102·905
Rubidium	Rb	37	85·47
Ruthenium	Ru	44	101·07
Samarium	Sm	62	150·35
Scandium	Sc	21	44·956
Selenium	Se	34	78·96
Silicon	Si	14	28·086*
Silver	Ag	47	107·870†
Sodium	Na	11	22·9898
Strontium	Sr	38	87·62
Sulphur	S	16	32·064*
Tantalum	Ta	73	180·948
Technetium	Tc	43	
Tellurium	Te	52	127·60
Terbium	Tb	65	158·924
Thallium	Tl	81	204·37
Thorium	Th	90	232·038
Thulium	Tm	69	168·934
Tin	Sn	50	118·69
Titanium	Ti	22	47·90
Tungsten	W	74	183·85
Uranium	U	92	238·03
Vanadium	V	23	50·942
Xenon	Xe	54	131·30
Ytterbium	Yb	70	173·04
Yttrium	Y	39	88·905
Zinc	Zn	30	65·37
Zirconium	Zr	40	91·22

comparisons. They were unacceptable to physicists for other reasons also. The element fluorine, which is composed solely of ^{19}F, was somewhat attractive to chemists because of its possible use as an operational standard for chemical determinations of atomic weights. It would not be suitable, however, as an operational standard for mass spectroscopy. Thus, the only possible basis for achieving a unified scale appeared to rest on the acceptance of ^{12}C as the reference species. Fortunately, this choice proved to be acceptable to both chemists and physicists.

In 1959 the International Commission on Atomic Weights recommended the adoption of ^{12}C as the reference species, with assigned relative mass of 12, provided physicists would also accept it in replacement of ^{16}O. During the ensuing year the proposal was thoroughly examined in Europe and North America. Under the persuasive leadership of Prof. J. Mattauch, the proposal generally came to be viewed with favour among physicists, and in September 1960 it was formally adopted by the General Conference of the International Union of Pure and Applied Physics. In 1961 the International Union of of Pure and Applied Chemistry confirmed the recommendation that had been made provisionally in 1959.

The adoption of the new unified scale of nuclidic masses and atomic weights by the two international scientific bodies most directly concerned with a matter of this kind does not necessarily ensure that the scale will be universally used. Scientists are not obliged to adopt standards or practices recommended by scientific organizations, whether national or international. However, chemists have long looked to the International Union of Chemistry and its Commission on Atomic Weights for authoritative judgments on matters relating to atomic weights. They are therefore very likely to accept without delay the "1961 Table of Atomic Weights", which is based on the new scale. Physicists have not had until recently a similar body to recommend 'best values' for nuclidic masses, but a Commission on Nuclidic Masses was established by the Union of Physics in 1960. Concurrently, a "Table of Nuclidic Masses" has been published[1]. This "Table" gives the results of a thorough analysis of all available sources of information. Data are tabulated on the basis of both the ^{12}C and ^{16}O scales, thus permitting ready comparisons. It is to be hoped that henceforth new physical data will be published in terms of the ^{12}C scale and preferably on this basis exclusively. It would be unfortunate indeed if the new unified scale should fail to displace, within a reasonable time, both of the scales which have been used heretofore.

In connexion with its recommendation to adopt the ^{12}C scale, the Commission on Atomic Weights sponsored a thorough and complete review of the experimental evidence from which atomic weights can be derived. The "1961 Table of Relative Atomic Weights" (Table 1) lists the recommended values for atomic weights that resulted from this review.

Those familiar with earlier "International Tables of Atomic Weights" will find this one different in a number of respects. There are changes in the values for many elements, resulting in part from the system-

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* The atomic weight varies because of natural variations in the isotopic composition of the element. The observed ranges are boron, ±0·003; carbon, ±0·00005; hydrogen, ±0·00001; oxygen, ±0·0001, silicon, ±0·001; sulphur, ±0·003.

† The atomic weight is believed to have an experimental uncertainty of the following magnitude: bromine, ±0·002; chlorine, ±0·001; chromium, ±0·001; iron, ±0·003, silver, ±0·003. For other elements the last digit given is believed to be reliable to ±0·5.

Table 2. THE RADIOACTIVE ELEMENTS—1961

Atomic No.	Name	Symbol	Isotope	Half-life	Mode of disintegration
43	Technetium	Tc	97	$2·6\times10^6$ years	Electron capture
61	Promethium	Pm	147	2·6 years	β-
84	Polonium	Po	210	138 days	α
85	Astatine	At	210	8·3 hours	α
86	Radon	Rn	222	3·8 days	α
87	Francium	Fr	223	22 minutes	β-
88	Radium	Ra	226	1,622 years	α
89	Actinium	Ac	227	21 years	β-, α
90	Thorium	Th	232	$1·4\times10^{10}$ years	α
91	Protactinium	Pa	231	$3·4\times10^4$ years	α
92	Uranium	U	238	$4·5\times10^9$ years	α
93	Neptunium	Np	237	$2·1\times10^6$ years	α
94	Plutonium	Pu	242	$3·8\times10^5$ years	α
95	Americium	Am	243	$7·8\times10^3$ years	α
96	Curium	Cm	247	$>4\times10^7$ years	α
97	Berkelium	Bk	247	c. 10^4 years	α
98	Californium	Cf	249	360 years	α
99	Einsteinium	Es	254	c. 320 days	α
100	Fermium	Fm	253	3 days	α
101	Mendelevium	Md	256	c. 1·5 hours	Electron capture
102	Nobelium	No		c. 10 minutes	α

atic conversion to the new scale, and in part from recalculation of the experimental work from which atomic weights are derived. Many values are given to a higher degree of precision than before. For several elements the precision is sufficient to require stating the range over which the atomic weight varies because of natural variations in isotopic abundances.

It will be noted that atomic weights are not given for radioactive elements other than thorium and uranium. Because the others are likely to be of uncertain isotopic composition, due to the circumstances attending their separation from natural materials or their artificial production, the assignment to them of atomic weights is difficult and of little value. Instead, the radioactive elements are listed separately in Table 2, which indicates the half-lives and modes of disintegration of selected isotopes. The review on which the "1961 Table" is based is the first comprehensive review since 1925 and the third in the history of the International Commission, the first having been made in 1909. The complete report of the current review will be published later this year in *Pure and Applied Chemistry*, the official journal of the International Union of Pure and Applied Chemistry.

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THE UNIVERSITY OF LONDON 1961 BEERENBERG EXPEDITION

By FRANK J. FITCH
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THE 1961 University of London Beerenberg Expedition to North Jan Mayen was planned as a continuation of previous British expeditions to this remote Norwegian island in the Greenland Sea[1–11]. The principal objective of the Expedition was to make a geological study and map of the great oceanic basalt volcano, Beerenberg (71° 05′ N., 8° 10′ W.), which dominates the northern half of Jan Mayen. The situation of Beerenberg on the arctic extension of the seismically and volcanically active Mid-Atlantic Ridge makes it especially interesting to geologists and geophysicists. Combined with the geological mapping was to have been a complete glaciological survey of the whole Beerenberg icefield and glacier system. Detailed investigations of Sørbreen, the large southern glacier of Beerenberg, had been made on previous expeditions[12–16], and it was intended that these should be repeated and extended. Among other objectives were certain mountaineering projects around the summit crater of Beerenberg essential to the scientific programme, the making of various rock and plant collections, general observations on the flora and fauna and the production of a colour ciné film. The expedition was financially supported by NATO, the Department of Scientific and Industrial Research, the Royal Society, the Royal Geographical Society, the Mount Everest Foundation, the University of London and by numerous commercial firms. Assistance and encouragement of every kind was obtained from the Norwegians, both on the island and in Norway.

A ten-man advance party sailed from Inverness in a chartered Norwegian sealer, the 133-ton *Signalhorn* of Aalesund, on June 10, 1961. The members of this advance party were F. J. Fitch (leader and scientific director, Birkbeck College), P. Smith (deputy leader and glaciological team director, Imperial College of Science and Technology), T. R. W. Hawkins (geologist, H.M. Geological Survey), D. Thomas (climber, H.M. Geological Survey), B. Roberts (geologist, Birkbeck College), C. M. Smith (glaciologist, Imperial College), J. D. Booth (glaciologist, Imperial College), J. R. Fraser (glaciologist, Imperial College), J. F. Cole (photographer, General Post Office Research Station) and P. J. D. Guile (technician, Imperial College). The geological party was landed north of the Beerenberg on June 15. By June 24 stores depots had been laid down at various points around the coast of Nord Jan, a base camp erected at Krossbukta and advanced camps and depots established high on the mountain to the north and south. The scientific work was well under way. The glaciological team under Peter Smith had already re-surveyed the snout area of Sørbreen and inserted four lines of stakes across the glacier for flow and ablation measurements, while in the north the geological mapping was progressing rapidly.

The early success of the expedition was destroyed, however, by a tragic accident on June 25, in which five men lost their lives and the leader was rendered unfit for several weeks. Fitch, P. Smith, Booth, Cole, Fraser and C. M. Smith were travelling north along the coast, to join the geologists for a combined attack on the Beerenberg crater, when their motor-boat was capsized in a sudden mountain squall. The five men who died were killed by the intense cold of the water before they could reach the beach only 100 yards away. As a result of a sea-search carried out by the Norwegian frigate *Garm*, the body of C. M. Smith was recovered and brought to the United Kingdom. The body of J. F. Cole was discovered later by a member of the crew of *Signalhorn* and buried on Jan Mayen.

After a period of uncertainty, it was decided that every effort should be made to complete the scientific programme of the expedition, especially the projects begun by the young men whose lives had been lost. A second geological party, J. Banfield (deputy leader and geologist, Neville Cross College, Durham), Miss R. J. Conran (geologist and naturalist, British Museum (Natural History)), Miss B. B. Fox (geologist, Overseas Geological Surveys), J. D. Slade (geologist, Birkbeck College), C. H. Talbot (geologist, Imperial College) and A. W. Wells (geologist, Birkbeck College), relieved the first party on July 14, and Fitch returned with a second glaciological party, D. J. J. Kinsman (glaciologist and geologist, Imperial College), J. W. Sheard (glaciologist and botanist, Imperial College), D. C. Birch (climber, Ilkley) and D. Thomas (climber, H.M. Geological Survey) on August 7. Before the expedition left Jan Mayen much of the original programme was completed. Only the parts which would have involved dangerous climbing on the east coast and in the summit crater had to be abandoned. Beerenberg was climbed on August 14 by Kinsman and Birch, and *Signalhorn* returned the remaining members to Inverness on August 20. Before leaving the island a memorial plaque to the lost men was erected near the head of J. F. Cole's grave at Krossbukta.

Most of the geological mapping programme was completed. 1 : 10,000 field slips cover all but the summit crater rim, the highest interglacial ridges and a short interglacial section of the east coast between Willebreen and Austbukta. Reasonably accurate interpretation of the geology of the area not covered by the large-scale mapping is possible on the basis of reconnaissance observations and rock collecting, and with the aid of extensive ground and aerial photography. The upper part of the mountain is, in fact, a large single lava cone of a distinctive basalt type, sitting on the crest of the main basalt dome of the Beerenberg. Its extent, even where hidden by the icefield, is indicated by a very clearly defined change of slope. The 1 : 10,000 mapping was based on a provisional stratigraphy proposed by Fitch and Banfield in 1959[11]. In an area of recent volcanics such as Jan Mayen, stratigraphical division is most obviously made between rocks of successive eruption cycles. Where there is a petrographic difference between rocks of successive cycles, geological mapping of these divisions can proceed in the usual way, but when, as in Nord-Jan, the petrographic difference between rocks of successive cycles is often not obvious in the field, alternative mapping methods must be used. Fitch and Banfield found that geomorphological evidence, based on a study of the denudation chronology, could be used to supplement the more usual geological criteria, and their mapping technique was successfully adopted by the expedition. As mapping proceeded it was found possible to extend the original stratigraphy, and to erect a series of rock-stratigraphic units to supersede it (Table 1). The age of the submerged volcanic basement of Jan Mayen is unknown, but for a variety of reasons it seems reasonable to assume that it is Tertiary. The growth of the Beerenberg in Nord-Jan began in the Pleistocene. Its rocks can be seen to rest on the old basement in places, and fragments of the older volcanics appear in many of the recent explosion breccias. In its structure and development Beerenberg closely resembles the Hawaiian volcanoes. It straddles a major multiple rift zone that runs north-east–south-west, following the local trend of the Mid-Atlantic Ridge. A subsidiary rift zone radiating towards the south-east from the summit gives it the typical Hawaiian tribrachial structure. A system of minor radial fissures is recognizable in places, and towards the north-east, confluence of fissures suggests the closure of the main volcanic structure in that direction. The overall structural pattern, the prevalence of fissure eruptions, open volcanic rifts, extensive dykes and normal cross-faults, combined with what evidence there is of the submarine topography of the Norwegian and Greenland Seas, strongly supports the view of Heezen and Ewing[17] that Jan Mayen is a volcano built on the flanking mountains of a major sub-oceanic rift valley structure.

Table 1

	Rock-stratigraphic units · North Jan Mayen	
Jan Mayen volcanic series		Smithbreen formation
	Nordkapp volcanic group	Kokslетta formation Tromsøryggen formation
		Sentralkrateret formation
	Kapp Muyen volcanic group	Nordvestkapp formation Havhestberget formation Storfjellet formation Kapp Fishburn Tillite Krossbukta formation
	Hidden volcanic group	
	Submerged volcanic group	

An extensive tillite horizon, the Kapp Fishburn Tillite, is thought to represent the deposits of the last Pleistocene ice-cap to form on Jan Mayen, its disappearance being correlated with the retreat of the Scandinavian ice from the Fennoscandian moraines. If this correlation is correct, the major part of the present Beerenberg is built of the products of eruptions occurring over the last 12,000 years. Certain features of the Havhestberget Formation suggest eruption during a period of cold climate that might be referred to the Younger Dryas Maxima. The ankaramitic basalt dome was completed by Nordvestkapp Formation volcanism. The building of the Sentralkrateret summit cone on top of the earlier dome, an event comparable to the late alkali–basalt volcanism of, say, Mauna Kea in Hawaii, most probably occurred during the Post-Pleistocene 'climatic optimum'. Evidence for this conclusion is principally geomorphological, and includes the smaller size of the summit cirques of this time, and the history of development of major sea-cliffs, now preserved in a fossil state. More recent volcanism, included in the Tromsøryggen and Kokslетta formations, has been essentially fissure eruption, both explosive and effusive, from the fissures of the main north-east–south-west rift zone. Fossil cliffs and a raised beach platform were formed in the interval between these two eruption cycles: they represent the maximum advance of the Post-Pleistocene sea some 3,500–4,000 years ago.

The present ice-field and glacier system is almost entirely the product of the 'little ice age' of the past 350 years. The breaching of the Beerenberg crater rim and the formation of the great glacial valleys of the north coast occurred during this period. The moraines of the present glaciation and those of an earlier advance, probably that known elsewhere to have occurred between 0 and 500 B.C., rest unconformably on the volcanics of the Kokslетta formation. The extensive coastal platforms and lava fields of this formation are therefore probably between 2,500 and 3,500 years old. Although no important volcanism has occurred since the Kokslетta eruptions, minor activity has been reported, and there is no valid reason for supposing that eruptions will not occur again on Jan Mayen, as they have on Tristan da Cunha. Moraines of an earlier glacier advance are found on top of the Tromsøryggen volcanics. The fossil sea-cliffs, 3,500–4,000 years old, post-date these moraines, so they must represent an ice advance about 4,000 years ago, at the close of the Post-Pleistocene warmer period.

The late Peter Smith and a team of glaciologists from Imperial College recorded a present-day minor re-advance of Sørbreen in 1959, and it was in order to extend these observations that a full glaciological survey of the Beerenberg ice was planned for 1961. Although certain of the high-level projects had to be abandoned as a result of the loss of the first glaciological team, it was possible to complete detailed observations on Sørbreen and to make a general survey of the other glaciers and of the summit rime domes.

Re-determination of the snout position of Sørbreen revealed a further advance of 124 metres between 1959 and 1961, making a total re-advance of 271 m. since 1949. The glacier has now almost regained its 1938 position. Surface flow measurements at the 130-, 310- and 910-m. stake lines across Sørbreen, made after an interval of between six and seven weeks, produced values ranging from 4 to 40 cm./day, the highest values being recorded from the 900-m. line and the lowest from the snout region. Ablation figures ranged from 1 to 5 cm./day. Observations on the other glaciers clearly demonstrate that the re-advance of Sørbreen is not an isolated feature, and that the whole Beerenberg ice system is in an active state. Spectacular advances can be seen in numerous places, accompanied by thickening of snout areas and the development of large seracs in the lower parts of glaciers.

It is apparent from a comparison of the Jan Mayen evidence with the evidence from other North Atlantic areas that the present Beerenberg ice-field expanded rapidly some 350 years ago. The main glaciers radiating from the mountain made a series of advances culminating about 1750, when the largest group of modern lateral and terminal moraines were produced. Since that time the glaciers have been in oscillatory retreat, each intervening re-advance reaching less far than its predecessor. Re-advances are thought to have occurred in 1810–20, 1850–70 and 1915–20, each producing its own distinctive moraines. The present-day re-advance began around 1954, and is probably near to its climax. Analysis of the climatic data for Jan Mayen and the surrounding areas reveals a remarkable correlation between glacier fluctuations and the weather patterns in the North Atlantic. There is nothing in the evidence from Jan Mayen to suggest that the current advance, or the present lowering of world temperatures, is more than a temporary regression in the course of the climatic amelioration that has been noticeable since the turn of the century. It seems likely that this long-term amelioration will continue until the climate of the North Atlantic region recovers its milder, pre-1600 A.D. character.

Extensive rock collections were made by the expedition for future petrographic and petrochemical investigation. Specimens were collected by a new technique for palæomagnetic study. A collection of 250 lichens was made for the British Museum (Natural History). Considerable photography was undertaken by the expedition. This included a colour ciné film and scientific record photography in black-and-white and colour. A novel camera-head, designed by Peter Smith, and constructed by P. J. D. Guile, made panoramic record photography possible in locations where only an ice-axe was available for support. It was thoroughly tested in the field and proved very satisfactory and useful. Although certain of the more adventurous parts of the mountaineering project had to be abandoned, such as the plan to enter and camp in the summit crater, considerable experience was gained during traverses of the larger glaciers and the ice-field, and on the climb of King Haakon VII Topp (7,470 ft.) made by Birch and Kinsman on August 14.

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NATIONAL RESEARCH ORGANIZATIONS IN SOUTH-EAST ASIA

THE natural sciences programme of Unesco covers a wide range of activities, among the less-publicized of which are the science co-operation offices. These offices are facilitating co-operation in scientific work in the developing countries in a very practical way and are showing results which are out of proportion to the all-too-meagre resources allotted to them. A recent example of the important work of the South-East Asia Science Co-operation Office was the regional meeting of representatives of national research organizations called by that Office in December 1961 to discuss problems of the organization of scientific research in the area. It was held at Hong Kong in association with the Golden Jubilee Celebrations of the University of Hong Kong, and arose from a similar meeting held two years earlier at Bandung (*Nature*, 186, 859; 1960). Representatives of Australia, the Republic of China, Hong Kong, India, Indonesia, Japan, Korea, the Federation of Malaya, New Zealand, the Philippines, Singapore and Thailand were present. The meeting was inaugurated with an address by the Hon. Sir Sik Nin Chan, who stressed the importance of science and technology in assisting the industrial development of South-East Asia and the need for close collaboration if this was to be achieved. Dr. (now Sir) Lindsay Ride, vice-chancellor of the University of Hong Kong, was elected chairman of the meeting.

The general theme of the meeting, which had a rather wider aspect than that adopted at Bandung, was concerned with the development of national research policies in the South-East Asia region. The report, *Current Trends in Scientific Research* (the Auger Report), prepared by Unesco as a result of the resolution of the General Assembly of the United Nations on November 14, 1958, that a survey should be made of the main trends of inquiry in the field of natural sciences and the dissemination and application for peaceful ends of such scientific knowledge, was used as a background document.

The stage of scientific development, as represented by the national research organization, varied considerably in the countries of the area. Some already had large and effective national organizations which had made important contributions to their national development. Other research bodies were in a much earlier stage but had been successfully launched, while in other countries again national organizations had not gone far beyond the planning phase. Detailed accounts were presented of the progress that had been made in the two years which had elapsed since the Bandung meeting. These all reflected an increasing appreciation by Governments of the importance of scientific research, and the meeting considered some of the factors which were limiting expansion.

The great variation in the relative expenditures by different Governments was discussed. It was, of course, appreciated that the optimum effort would vary from country to country according to need, and also that the amount of money spent was not necessarily an accurate measure of the amount of research. It was felt, however, that it would be valuable to compare the expenditures of countries of the region as percentages of gross national products or of national budgets and the Unesco Office was asked to obtain and analyse figures for the countries of the area. (The meeting did not feel it would be proper for it to recommend a minimum figure for Government expenditure on research, though one delegate was prepared to go on record that this should be 1 per cent of the national budget.)

The question of extension—making use of research results obtained—should be a vital feature of a national research policy. Although in agricultural research the problems were now fairly generally understood, this was not the case in the industrial field. The translating of results of research to industrial practice was not an activity in which the research scientist was skilled, and various means of doing this (for example, the establishment of development authorities) were discussed. In many countries of the region, however, particularly those just beginning to build up industrial activity, the important need was to provide day-to-day assistance in solving smaller technical problems arising in the course of production—the application of existing basic knowledge and 'know-how'. In New Zealand this had been solved by the establishment of industrial development laboratories, and this principle was being followed by at least one other country in the area.

Although the meeting was primarily concerned with national research organization, it discussed the role of research in universities since the national organizations are dependent on the universities to provide them with trained personnel. It was vital for universities to undertake active research programmes so that students could be trained in methods of research. In some countries it was, of course, the policy to carry out much of the basic research in the universities or other institutes attached to them. In other countries the national research organization played a major part in assisting university research—this was particularly useful where the high cost of research facilities was a problem. In any event, the universities must occupy a position of high status in the national research scheme and there should be the closest collaboration between them and the national research organizations. Although it was emphasized that the responsibility for postgraduate training must be in the university system, it was felt that the recognition by the universities of outstanding scientists in the national research organizations as honorary university officers was most desirable.

Another problem which came under vigorous discussion was that of the promotion of science. In order to secure adequate support for scientific work it is essential that there should be an awareness throughout the community of its aims and objectives and the practical effect it can have in raising living standards and promoting national welfare. This was particularly important in countries in the South-East Asia region and it was agreed that two groups should be considered: Government and general public. If the ministerial head of the national research organization were a senior minister or his equivalent, science could make itself felt much more effectively. In India the fact that the Prime Minister himself was the minister concerned with the national research body had had a tremendous effect on its development. Every possible step should be taken by national research organizations to emphasize the value of investment in scientific research. Use should be made of all the mass media—the Press, films, radio, television, etc., for developing an awareness of science and its results. In particular, the Press reached a great proportion of the public, and provision of informative, well-written material was essential. Writing for this medium was a specialized task, and the number of competent scientific journalists available was very limited. The value of science museums in increasing public awareness of science was also stressed. It was important that young people should be interested at an early age not only to increase their understanding but also to interest them in the attractions of a career in science. The increasing demand for scientific manpower was making this more important, and in discussing this the meeting emphasized two important points. The first of these was the need in most countries for improving science education at the school-level. It would not be possible to attract the brightest children to a scientific career unless the subject was made interesting and exciting from the beginning. This required inspired teaching which would catch the imagination of young people and give them an idea of the excitement of the subject and its practical relevance in improving living standards and social conditions. In many countries the schoolteacher did not enjoy sufficient status to attract men and women of adequate intellectual calibre to the profession. The other point which was emphasized was the need for adequate pay and status for the scientist. In some countries of the area he did not enjoy the same standing as the administrative Civil servant and was obliged to undertake additional employment to ensure an adequate income. In these circumstances, the best minds would never be attracted to a research career, and it was desirable that countries should adopt a scientific policy which would materially improve the status of scientists.

The meeting emphasized the importance of scientific documentation centres, particularly in developing countries. Such centres had now been established, with the assistance of Unesco, in India, Pakistan and the Philippines, while one was being set up in Thailand and another planned in Indonesia. It was suggested that it would be impossible for each centre to build up a completely comprehensive service and the Unesco South-East Asia Science Co-operation Office at Djakarta was asked to examine the possibility of close liaison and co-ordination between the various centres. The Office was also asked to study the possibility of establishing regional scientific journals in the area. Although there was little difficulty in placing scientific papers in international journals in basic subjects like physics and chemistry, it was often hard to provide for publication of papers of specialized regional interest, for example, zoology, parasitology, botany, phytochemistry. Because of cost and limited output of papers, it was difficult for smaller countries to establish their own journals to cater for this type of material. Regional journals in such subjects would overcome the difficulties and ensure that the work could be properly recorded and made more readily available by libraries.

The meeting also examined a number of other aspects of co-operation, for example, technical assistance, regional collaboration in research and training, the exchange of scientists, regional scientific meetings, etc. The formal outcome of the meeting was a series of recommendations to the Director-General of Unesco that the South-East Science Co-operation Office should undertake a number of specific activities designed to strengthen the development of science in the countries of the region. The meeting also emphasized the importance of the work being carried out by the Office, which thoroughly justified the provision of additional funds to enable a substantial expansion of its activities.

The South-East Asia region embraces countries the stage of development of which varies over an extremely wide range, and the conference—more effective perhaps because of its small size and intimacy—should make a contribution towards achieving the purpose of the recommendations of the Auger Report: the better utilization of science for improving living standards. This is a goal which is common to the countries of the region, as is evidenced by the establishment or planning of national research organizations by all the Governments concerned. The desire for constructive international co-operation, demonstrated convincingly at this meeting as well as at Bandung, should have important practical results in helping the growth and work of these organizations, particularly in the less-developed countries. It should also have as a secondary, though no less important, consequence, a political dividend in the building up of international trust and confidence. This may be small in relation to the magnitude of international tensions, but it is nevertheless an important aspect of international scientific gatherings, particularly those like that described where there is a common interest in working together for constructive ends.

GUY B. GRESFORD

OBITUARIES

Prof. A. H. Compton

ARTHUR HOLLY COMPTON died on March 15 in Berkeley, California, as a result of cerebral hæmorrhage suffered two weeks earlier. He was sixty-nine.

He had retired last year as distinguished service professor of natural philosophy in Washington University (St. Louis), where he had served as chancellor from 1945 until 1953. He was planning to be active in retirement as professor-at-large between Washington University, the University of California (Berkeley) and the College of Wooster (Ohio), and had gone to Berkeley to deliver a lecture series on "Man, Science and Society".

Compton received his B.Sc. from the College of Wooster, where his father, Elias Compton, was professor of philosophy, and received his Ph.D. in 1916 at Princeton University. The next year he was an instructor at the University of Minnesota, and then was for two years a research engineer for the Westinghouse Lamp Company in Pittsburgh. The year 1919–20 was spent at Cambridge (England) as a National Research Fellow, after which he was appointed Wayman Crow professor and head of the Department of Physics at Washington University, where he remained until 1923. During 1923–45 he served as professor of physics and dean of physical sciences at the University of Chicago, until he returned to Washington University as chancellor in 1945. He was president of the American Physical Society in 1934, of the American Association of Scientific Workers in 1939–40 and of the American Association for the Advancement of Science in 1942. In 1927 he shared the Nobel Prize in physics with C. T. R. Wilson.

His career was marked by an extraordinary range of great accomplishments in physics, in higher education, in war-time scientific research, and in efforts to improve human and international relations.

While a student at Princeton he devised a beautiful demonstration of the Earth's rotation, which ought to be known to all teachers of physics. A toroidal glass tube is filled with water, and mounted so that it can be 'flipped' through 180° about a diameter as axis. Before flipping, the water is at rest relative to the tube, which is turning, relative to the fixed stars, in its own plane with the component of the Earth's rotational angular velocity normal to that plane. After flipping, the water drifts relative to the tube, at a rate proportional to that angular velocity component. By measuring the drifts produced by three successive flips about each of three mutually perpendicular axes, one finds the Earth's angular velocity as a vector; that is, one can infer which local direction is north, what is the observer's latitude, and what is the absolute value of the length of the day.

His first major discovery was the detailed measurement and interpretation of the wave-length change occurring when X-rays are scattered, especially by materials of low atomic number. This is now generally known as the Compton effect. He showed that the loosely bound electrons in the material scatter the X-rays in accordance with the principles of conservation of momentum and energy, as if they consist of a stream of photons, each having momentum of $h\nu/c$, as well as energy, $h\nu$. The energy aspect goes back to Planck and Einstein; but the Compton effect afforded the first clear demonstration that the X-ray photons also carry quantized amounts of momentum.

The next few years were marked by the development of coincidence methods by Compton and A. W. Simon in Chicago, and independently by W. Bothe and H. Geiger in Germany. These experiments showed that individual scattered X-ray photons and recoil electrons appear at the same instant in time, contrary to some views that were then being developed by Bohr, Kramers and Slater in an attempt to reconcile quantum views with the continuous waves of electromagnetic theory.

Compton also discovered the phenomenon of total reflexion of X-rays, and their complete polarization (with C. F. Hagenow), and first obtained (with R. L. Doan) X-ray spectra from ruled gratings. This latter work had an important consequence in leading to a distinct improvement in our knowledge of the electronic charge. By measuring an X-ray wave-length with a ruled grating of known grating space, and then using a crystal to diffract the same rays, one can determine the absolute value of the grating space of the crystal. Combining this with the measured crystal density, it is possible to obtain the Avogadro number, and combining this with the Faraday, the electronic charge is obtained. The outcome was that the Millikan oil drop value had to be revised from $4{\cdot}774$ to $4{\cdot}803 \times 10^{-10}$ c.g.s. E.S.U., it being finally recognized that systematic errors had been made in measuring the viscosity of air, a quantity which enters into the oil drop method.

From about 1930, Compton directed his attention mainly to the study of cosmic rays. In the next ten years he was in charge of a major programme involving a world-wide study of the geographic variations of their intensity. This resulted in full confirmation of some observations made in 1927 by J. Clay, indicating a latitude effect on the intensity. The world survey, in the service of which Compton made many long voyages, showed correlation of the intensity of cosmic rays with geomagnetic, rather than geographic, latitude. This opened the way for extensive subsequent studies of the interaction of the Earth's magnetic field with the incoming isotropic stream of primary charged particles.

In 1941 he was appointed chairman of the National Academy of Sciences Committee to Evaluate Use of Atomic Energy in War. In the autumn of 1941 this Committee worked with those responsible for studying this same problem in Great Britain, and also with the S-1 committee headed by Dr. L. J. Briggs, then director of the National Bureau of Standards. This led to recommendations to the United States Government for the setting up of a major effort starting in January 1942. Compton assumed the active direction of the group, which was known by the 'cover-name' of Metallurgical Laboratory of the University of Chicago, which concentrated on development of controlled uranium fission reactors for the production of plutonium. Within the year the first controlled uranium fission reactor was operating at Chicago, this specific project being largely due to the efforts of E. Fermi, L. Szilard, E. P. Wigner, and a host of co-workers. More experimental reactors were designed and built at Oak Ridge, Tennessee, and at the newly established Argonne Laboratory in the suburbs of Chicago. Here was carried out the work which led to the large plutonium-producing reactors built at Hanford, Washington, which produced the plutonium for the atom bomb that destroyed Nagasaki in August 1945.

Throughout the War, Compton also played an important part in the general planning of the atom bomb project, including the setting up of the laboratory at Los Alamos, New Mexico, and in reaching the military–political decisions about the use of the bombs in Japan. He has given a personal account of these matters in his book, *Atomic Quest* (Oxford University Press, 1956).

Compton became chancellor of Washington University in 1945, bringing with him a close associate from the days of his work on cosmic rays, Joyce Stearns, as dean of faculties. He turned with great vigour to the task of re-making a University which had greatly suffered through the depression years and the War years. In eight years filled with hard work he set it along a path toward greatness along which it continues to move. Then in 1953 he asked to be relieved from administrative duties, so that he could return to teaching, and devote himself entirely to problems of the social impact of science and technology, and to ways of promoting world brotherhood and the relieving of international tensions. He was planning to continue active work on such problems into the retirement which ended soon after it began.

He is survived by his wife, formerly Betty Charity McCloskey, and two sons, Arthur Allen, now in the American Foreign Service stationed at Manila in the Philippines, and John Joseph, a professor of philosophy at Vanderbilt University (Nashville, Tennessee), and by a brother, Wilson Compton, now retired, a former president of Washington State University (Pullman, Washington). Another brother, Karl Taylor Compton, former president of the Massachusetts Institute of Technology, died in 1954.

E. U. CONDON

Prof. A. E. Douglass

DR. ANDREW ELLICOTT DOUGLASS, the noted American astronomer and founder of tree-ring-dating research, died on March 20, aged ninety-four.

Dr. Douglass was born in Windsor, Vermont, on July 5, 1867. At the time of his death, he held four titles in the University of Arizona, where he had been a member of the faculty since 1906: professor of astronomy, professor of dendrochronology, and director emeritus of both the University's Steward Observatory and Laboratory of Tree-Ring Research. His professorships had been on a part-time basis in recent years.

Dr. Richard A. Harvill, president of the University of Arizona, writes: "Dr. Douglass was a brilliant scientist who served the University of Arizona with great distinction for fifty-five years. He was highly creative and extremely versatile in the range of his interests and contributions. Throughout the world, when the University of Arizona was mentioned scientific leaders immediately thought of Dr. Douglass. But he was more than a great scholar. He was a man of most admirable personal qualities. He exemplified qualities of simplicity and of humanity that are traits of a truly great man".

Dr. Douglass went to Arizona in 1894 to assist in selecting a site for the Lowell Observatory. After inspecting many locations, he recommended Flagstaff, and the Observatory that later became famous for planetary research was established there on his recommendation. He served as its first assistant observer until 1901.

In that year he became interested in scientific interpretations of relationships between climatic changes and the annual growth of trees, as shown in their growth-rings. His subsequent investigations led to development of a scientific technique to which he gave the name 'dendrochronology'. The technique is used to date fragments of wood by correlation of annual growth-rings of the trees which produced them.

Prof. Douglass joined the University of Arizona faculty in 1906 as head of the Department of Physics. In 1918 he was made director of the University's Steward Observatory and served in that capacity until 1938, when he became director emeritus. The astronomer-turned-dendrochronologist laid the foundations of tree-ring analysis so firmly that he was able to offer a course in the University—the first of its kind—in 1929. By 1935 his research had advanced the technique to a point that made it possible for the University to establish its Laboratory of Tree-Ring Research under his direction.

Prof. Douglass's studies of tree rings became so widely known that they obscured to some extent his earlier prominence as an astronomer. After graduation from Trinity College in Connecticut with a B.A. degree in 1889, he served five years with Harvard Observatory. During that period he was a member of the Harvard–Boyden expedition to South America to select a southern hemisphere astronomical observatory site. He helped in choosing the Arequipa, Peru, site and later served as a member of the Harvard solar eclipse expedition to Chile.

Both the Harvard experience and an early developed interest in the planet Mars served Prof. Douglass well in the selection of the site for the Lowell Observatory. Despite many other activities, he continued his long-term studies of the red planet through the years. In 1924, using the new Steward Observatory 36-in. telescope, he found that melting of the south polar cap on Mars was followed by distinct 'greening' of the planet's dark markings. The University of Arizona instrument showed the green markings so clearly in the 1920's that many 'public night' observatory visitors saw the colour change. Also in 1924 Prof. Douglass first pointed out that Mars may support a low form of organic life—a contention that earned him world-wide scientific attention.

A man of broad capabilities, Prof. Douglass ran for political office in 1902, was elected, and served two terms as probate judge of Arizona's Coconino County. During that period he also laid out the Navajo–Coconino county boundary, taught a year at Arizona State College, Flagstaff, and pursued the early tree-ring studies which he later developed at the University of Arizona. He married Ida Whittington of Los Angeles on August 3, 1905.

Soon after joining the faculty of the University of Arizona in 1906, Prof. Douglass realized the opportunities for astronomical research at the University if a large observational instrument could be obtained. His years of effort on this project culminated in 1916 when Mrs. Lavinia Steward gave the University sufficient funds to establish the Observatory bearing her husband's name. The 36-in. telescope mirror, first of its size made in the United States, was completed and mounted in 1922 under the direction of Prof. Douglass.

During his long and distinguished career, Prof. Douglass served the University of Arizona not only as teacher and scientist but also in two major administrative positions. He was acting president of the University from December 1910 until May 1911, and dean of the College of Liberal Arts during 1915–18.

In the years since Dr. Douglass established the Laboratory of Tree-Ring Research, dendrochronology has made significant contributions to several areas of science. One of his most widely recognized successes came in 1929 when he completed an Arizona climatic sequence extending back to A.D. 700. The achievement made it possible to date more than 30 prehistoric Indian ruins through examination and correlation of cross-sections of beams used in constructing the early Indian dwellings.

Prof. Douglass is survived by his wife, Ida Douglass, and a nephew, Andrew W. Douglass, both of Tucson, Arizona.

NEWS and VIEWS

Royal Society Visiting Professorship :
Prof. M. Szwarc

PROF. M. SZWARC, research professor in the State University College of Forestry at Syracuse University, New York, has been appointed Royal Society visiting professor for the academic year 1962–63. He is expected to take appointment on August 1, 1963, and to work in the Department of Inorganic and Physical Chemistry in the University of Liverpool on ionic and anionic and stereospecific polymerization. Prof. Szwarc has achieved distinction in many fields of physical chemistry and his name is associated with the discovery of the valuable 'toluene carrier gas techniques' for measuring bond dissociation energies and the elucidation of kinetics mechanism. He has also worked on 'living polymers' and electron transfer processes in non-aqueous solutions and is connected with many of the pioneering developments in anionic polymerizations.

Smithsonian Institution Langley Gold Medal :
Dr. H. L. Dryden

DR. H. L. DRYDEN, deputy administrator of the National Aeronautics and Space Administration, has been awarded the Langley Gold Medal of the Smithsonian Institution. The Langley Medal is founded in memory of Samuel Pierpont Langley, pioneer in aviation and secretary of the Smithsonian Institution during 1887–1906. It has previously been awarded only nine times in the fifty-four years since its establishment in 1908, the first being given to the Wright brothers in 1909. Dr. Dryden was selected "in recognition of his important applications of experimental science to the problems of flight and for his wise and courageous administration of much of America's research and technical developments that now make possible the conquest of air and space".

American Geophysical Union Awards

The William Bowie Medal, the principal award of the American Geophysical Union, has been presented to Prof. Sydney Chapman, for "outstanding contributions to the field of astro-geophysics in the course of five decades".

The John Adam Fleming Award for original research in geomagnetism, atmospheric electricity and aeronomy has been presented to Dr. Lloyd V. Berkner, formerly president of the Union. Dr. Berkner is president of the Graduate Research Center of the Southwest in Dallas, and treasurer of the U.S. National Academy of Sciences and chairman of the Academy's Space Science Board.

The James B. Macelwane Award for "outstanding contributions to the geophysical sciences by a scientist under the age of thirty-five" has been presented to Dr. James N. Brune, of Lamont Geological Observatory.

The Commonwealth and International Library of Science, Technology and Engineering

On May 23, the Pergamon Press, Oxford, headed by Robert Maxwell, is launching the new Commonwealth and International Library of Science, Technology and Engineering. The chairman of the Library is Sir Robert Robinson and the executive editor Mr. Cyril W. Wood. The Library, with a target of 1,000 volumes by 1967, contains about fifty subject divisions covering the different branches of science, technology and engineering. Further, in an attempt to broaden the outlook of the scientist, there are included such divisions as liberal studies and the history of science. The editors of the separate divisions have drawn up lists of titles designed to give a continuity of study within each division, and, in those subjects such as mathematics, physics, chemistry and biology, which are taught in schools, this continuity stretches over the age-range of 12+ to that of the honours graduate, thus covering the needs of secondary modern and grammar schools, colleges of technology and universities. Although the Library consists essentially of text-books, some smaller number of monographs on specialist subjects for the postgraduate student will be included. To achieve this continuity of study and to avoid too heterogeneous a collection, all the books of the Library are to be freshly written and are not reprints of existing publications. Furthermore, very close liaison between editors of related divisions is being maintained.

The books will be small, of an average length of 150 pages, and will be published in a common format in two styles—a soft-cover edition within the price-range of 7*s*. 6*d*.–17*s*. 6*d*. (1.25–2.75 dollars) and a more expensive one bound in a hard cover for libraries. Certain important advantages can be claimed for this type of book. The relatively small size makes it possible to select authors who are specialists in the particular section of the subject to be covered. Moreover, a series of small books on a subject can be more easily kept up to date than can one large volume in the same field. Because of the low price, the student may be encouraged to build up a library for himself during his course of study. This last-named objective is an especially worthy one, since too many students, for varying reasons, are now buying either new or second-hand books and discarding or selling them as soon as they have finished the courses which such books cover.

British Iron and Steel Research Association

A Process Physics Section has been set up in the Physics Department of the British Iron and Steel Research Association in place of the previous Metal Physics Section. The new Section is headed by Dr. R. V. Williams, who recently joined the Association from the Thermo-nuclear Section of the Associated Electrical Industries, Ltd., Research Laboratories at Aldermaston. The object of this Section is to study current research in certain new fields such as magneto-hydrodynamics and plasma physics, and to institute a programme of work on projects that show promise of being important to future steelmaking and processing practice. It will also include work on new materials research with special reference to fibrous reinforcement. Work is starting on the following: (1) electric resistance heating of flames using the ionic conductivity in a flame or hot gas as a means of flame or gas temperature boosting; (2) use of electromagnetic effects for control of liquid steel streams; (3) use of steel fibre reinforcement in non-ferrous materials.

New Australian Radio Heliograph

The Commonwealth Scientific and Industrial Research Organization is to build a giant camera for taking detailed radio pictures of the Sun. Construction of the radio heliograph is to be made possible by a gift to the Organization of 550,000 dollars by the Ford Foundation. It will consist of 100 saucer-shaped aerials, each measuring 42 ft. across. These will be arranged in the form of a circle two miles wide. Special receivers and computers will combine the waves received by the 100 individual dishes to give a motion picture of the Sun. The exact location of the new instrument has not yet been decided. It will probably be built at Parkes, N.S.W., not far from the Organization's 210-ft. radio telescope, which was completed last year. Mr. J. P. Wild, leader of the Organization's group studying the Sun, has designed the radio heliograph to give a detailed picture of the radio flares which accompany the explosions near sunspots which can be seen through optical telescopes. All in all, the Division of Radiophysics of the Commonwealth Scientific and Industrial Research Organization has been granted 1,333,000 dollars from American sources (361,000 and 250,000 dollars respectively from the Rockefeller Foundation and the Carnegie Institution towards the cost of the 210-ft. radio telescope, and 172,000 dollars from the National Aeronautics and Space Administration for research on radio antenna systems of advanced design).

Capital Expenditure on Education in Britain

In reply to a question in the House of Commons on May 1, the Economic Secretary to the Treasury, Mr. A. Barber, said that during 1956–57 capital expenditure by public authorities on education in Great Britain was £117 million and present expenditure £546 million, the total representing 3·6 per cent of the Gross National Product. For 1957–58 the corresponding figures were £133 million, £613 million and 3·8; for 1958–59, £134 million and £670 million and 4·0; for 1959–60, £137 million, £742 million and 4·1; and for 1960–61, £133 million, £812 million and 4·2.

Educational Research in Scotland

On May 2, the Under-Secretary of State for Scotland said that Exchequer assistance for educational research in Scotland was normally channelled through the Scottish Council for Research in Education. Work in hand included continued research into intelligence and intelligence tests, the teaching of arithmetic and writing, and the influence of nursery schools on a child's subsequent educational performance. An important project had been initiated, in co-operation with the University of Edinburgh, bearing on educational requirements for success in higher education, and the Scottish Education Department intended to assist directly the University of Aberdeen in research into programmed teaching.

University Development in Scotland

In replying for the Government in a debate on a fifth university for Scotland in the House of Commons on April 19, the Financial Secretary to the Treasury, Sir Edward Boyle, said that the Government's decision that no further universities in England, Wales or Scotland would be nominated before the Robbins Committee reported next year remained firm. He pointed out, however, that pressure on university places was still less severe in Scotland than in England. During the past five years the number of students increased by 20 per cent to more than 18,000, of whom a quarter came from outside Scotland, and Sir Edward anticipated that with the increased Exchequer grants recently announced the Scottish universities and the Royal College of Science and Technology would be able to provide for a further substantial expansion of student members. In addition to these places the central institutions and colleges of further education accommodated 4,000 students in advanced courses, broadly comparable with first degree courses, and this number was planned to rise to 6,000–7,000 by 1970, while the seven teacher training centres, with about 5,500 students, would accommodate at least 6,500 students by 1970. Scotland's needs had been fully recognized, claimed Sir Edward, and the amount allocated to Scotland had risen since last June from £9·3 million to £12·5 million as a result of continuous review by the University Grants Committee.

The University Museum, Oxford

The seventy-third annual report of the delegates of the University Museum, Oxford, for the year ending July 31, 1961, records that the new microbiology building and the extension to the Pharmacology Department came into use during the year. The additional storey on the Lindemann building of the Clarendon Laboratory was completed. In the Museum the first-floor rooms on the north-west side of the court were modified for the purposes of the Scientific Collections Committee by the insertion of an additional floor.

Dinosaurs

The Trustees of the British Museum (Natural History) are supplying a popular demand by the publication of a small pamphlet on dinosaurs. They are fortunate in that it was written by Dr. W. E. Swinton before he left England for an important museum post in Toronto. A brief historical sketch of the group is followed by a general description of dinosaurs and details of the various orders. A final chapter discusses the problem of their extinction. There is a useful note on restorations and a full glossary of the technical terms used in the text. The hand-book is well illustrated, including a coloured plate which accounts for the rather high price. (*Dinosaurs*. Pp. xi + 44 (11 plates). London: British Museum (Natural History), 1962. 5*s*.)

Archæology in Saudi Arabia

Two so-called neolithic collections of flint implements from the southern corner of Saudi Arabia where it borders on the Yemen are described by Philip Smith of the University of Toronto and George Maranjian of the Arabian American Oil Co. in the February issue of *Man*. Some of the tools found are illustrated. Arrow-heads, both leaf-shaped and tongued with barbs, occur, as well as scrapers, grinding stones, etc. No pottery has been found. There is no means of dating these finds and the animal bones recovered are too fragmentary to be identified, but it is thought sheep and goats may occur. Carbon dating for a somewhat similar site east of Jiladah, which was examined by Henry Field, is given as approximately 3,000 B.C. One of these collections has been given to the Peabody Museum at Harvard, the other is on loan to the same museum. Henry Field and F. E. Zeuner have both directed attention to flint tools from this region, but these latest finds are of considerable interest.

Science Abstracts: Subject Index (Section A)

Science Abstracts is published in two separate sections—Section *A* (Physics) and Section *B* (Electrical Engineering). Five-year cumulative author- and subject-indexes for the years 1950–54 have been in preparation since October 1955. The author-index containing approximately 100,000 entries, arranged alphabetically, was published in the autumn of 1956, and the subject-index for Section *B* in April 1959. The new subject-index for Section *A* is now available. It contains more than 100,000 entries arranged under nearly 1,200 different subject headings, and provides comprehensive bibliographies of all subjects covered by the Section. The price is 35*s*. (4.90 dollars) and it can be obtained, together with copies of any of the previously issued cumulative indexes, from the Institution of Electrical Engineers, Savoy Place, London, W.C.2.

Conferences, Meetings and Training Courses in Atomic Energy

The list of *Conferences, Meetings and Training Courses in Atomic Energy* published every two months by the International Atomic Energy Agency is divided into three parts. The first part covers fully all meetings which will be held during the next twelve months, and the second covers meetings during the subsequent twelve months, although in many cases specific information is lacking. This second part is issued in order to assist the planning of future meetings and to prevent duplication. The third part gives specialized training courses at different levels, and some of the sources from which the information has been derived are listed in the annex. The Agency intends this list to be as comprehensive as possible and therefore publishes the fact that it welcomes suggestions and additions giving the fullest information available. The names and addresses of more than forty sales agents for the International Atomic Energy Agency are given at the end of the list. Copies are available from the International Atomic Energy Agency, Kaerntnerring, Vienna 1.

Low-Temperature Physics at Karlsruhe

To mark the installation of the two hundredth Collins helium cryostat manufactured by the A. D. Little Company, a conference on "Low-temperature Physics" was held at the Physikalischen Institut der Technischen Hochschule, Karlsruhe, during March 27–30. It was in the nature of an informal gathering, and it was agreed that no papers presented should be published. In consequence, there was considerable freedom to discuss preliminary results or to indicate the development of apparatus which had not necessarily achieved its final form. Altogether, some 110 people attended, representing fourteen countries, and a useful interchange of information and opinion took place, not only in the two and a half days of formal sessions, but also during the social events of each evening. Visits were arranged to the low-temperature laboratories at the Technischen Hochschule and the reactor establishment near Karlsruhe. The participants enjoyed a stimulating conference with a greater opportunity for informal discussion than is sometimes possible at larger international events. Hospitality was provided by the A. D. Little Company and the conference arrangements were in the hands of Prof. W. Buckel.

Solar Flares and Cosmic Rays

In recent years there has been increasing interest in the observation of cosmic-ray primaries at ground-level following solar flares, and ten such cases have been observed since 1942. M. A. Ellison, S. M. P. McKenna and J. H. Reid have now given a summary of the characteristics of these ten flares, a review of their geophysical effects, and a detailed bibliography (Dublin Institute for Advanced Studies: Observatory of the School of Cosmic Physics. *Dunsink Observatory Publications*, 1, No. 3: *Cosmic Ray Flares*. Pp. 51–88. Dublin: Dublin Institute for Advanced Studies, 1961). The flares observed on the disk were notable for the flash of radiation in Hα occurring in a time of 2 or 3 min., for their large area of order 2,000 millionths of the solar hemisphere, for the twin bright filaments crossing the sunspot group, and for the obscuration of the umbræ with the highest field strengths. The flares occurred near to maximum development of the sunspot groups. All the flares produced sudden ionospheric disturbances and strong radio continuum emission. Some of these flares were among the greatest ever recorded, others were not optically of great importance. The disk co-ordinates of these flares showed remarkable asymmetries in contrast to normal intense flares: there was a strong preponderance to the west limb. All except one occurred north of the solar equator. The implication is that easier and more direct paths to the Earth exist from the north-westerly quadrant of the solar disk, due possibly to the solar rotation and space motion. The authors conclude that the arrival of high-energy particles capable of producing cosmic-ray effects at ground-level is mainly determined by factors other than flare importance, such as the variable magnetic fields in interplanetary space, the disk position of the flare and the magnetic field conditions in the sunspot region.

Joint Health Physics Committee

The Joint Health Physics Committee (the formation of which was noted in *Nature*, **193**, 1136; 1962) is sponsoring a series of symposia on different aspects of health physics during the next two years, the first of which is to be held at the Atomic Energy Research Establishment, Harwell, on June 5 under the title "Provision of Health Physics Services: Problems and Organization". Full details are being sent direct to the members of the collaborating organizations, and if accommodation permits other people will be welcome at the symposium. Further information can be obtained from Mr. W. A. Langmead, Radiological Protection Division, Authority Health and Safety Branch, U.K. Atomic Energy Authority, Harwell, Didcot, Berks.

Paramagnetic Resonance

The first international conference on "Paramagnetic Resonance" will be held in the Hebrew University of Jerusalem during July 16–20. Subjects to be discussed will include: paramagnetic resonance of transition elements—the iron group, the rare earth group, the uranium group, the palladium and platinum group; double resonance ENDOR experiments; theoretical advances in energy-level calculations; resonance spectra of point defects and radiation centres in single crystals; spectra of ion pairs; relaxation phenomena and line shape—correlation with paramagnetic relaxation experiments; correlation of paramagnetic resonance spectra with optical spectra; advances in experimental techniques; influence of electric fields and pressure on paramagnetic resonance spectra. Further information can be obtained from Prof. W. Low, First International Conference on Paramagnetic Resonance, the Hebrew University of Jerusalem, Jerusalem.

Congress of the History of Science

The tenth International Congress of the History of Science will be held at Cornell University, Ithaca, New York, during August 26–31, 1962, and at the American Philosophical Society, Philadelphia, Pennsylvania, during August 31–September 2, 1962. The following subjects will be covered: (I) general problems in the history of science; methods, philosophy and historiography of science; (II) history of technology and applied science; (III) science in antiquity; (IV) science in the Middle Ages and the Renaissance; (V) mathematics and the exact sciences after 1600—(1) history of mathematics, (2) history of physics and astronomy, (3) history of chemistry (including pharmacy); (VI) biological and earth sciences after 1600—(1) natural history and biology (including medical biology), (2) geography, exploration, geology and oceanography; (VII) sciences of man (psychology, anthropology, sociology, linguistics). Further information concerning presentation of papers and attendance can be obtained from the secretary, Dr. C. Doris Hellman, Tenth International Congress on the History of Science, Cornell University, Ithaca, N.Y.

The Association of Consulting Scientists

The following officers and members of Council of the Association of Consulting Scientists have recently been elected: *Chairman*, Dr. M. Barent (Barent and Johnson); *Vice-Chairman*, Mr. F. G. Sarel Whitfield (Avebury Research Laboratories, Ltd.); *Hon. Treasurer*, Dr. G. W. Ferguson (Parry and Ferguson); *Hon. Secretary*, Mr. W. H. Stevens (Park House, Hawthorne Road, Bromley, Kent); *Members of Council*, Dr. H. H. Chambers (Sondes Place Research Laboratories, Ltd.), Dr. G. P. L. Miles (G. P. L. Miles), Dr. R. F. Milton (R. F. Milton), Mr. H. Pritchard (H. Pritchard), Mr. A. N. Worden (Huntingdon Research Centre).

The Royal Institution, London

At the annual meeting of the Royal Institution on May 1, the following officers were elected: *President*, Lord Brabazon of Tara; *Treasurer*, Mr. W. E. Schall; *Secretary*, Brigadier H. E. Hopthrow; *Managers*, Dr. A. Clow, Sir Harold Roxbee Cox, Mr. E. R. Davies, Dr. T. F. Gaskell, Prof. A. G. Gaydon, Prof. Alex. Haddow, Miss E. Henrietta Jebens, Mr. L. B. W. Jolley, Dame Kathleen Lonsdale, Dr. H. Lowery, Mr. Percy H. Schwarzschild, Mr. W. R. Stevens, Mr. Cyril Sweett, Wing-Commander H. F. Tiarks and Prof. W. D. Wright; *Visitors*, Mr. A. D. R. Caroe, Mr. Norman Clarke, Miss F. M. Eastwood, Mr. A. H. Ewen, Mr. G. J. Gollin, Mr. F. Greenaway, Mr. C. R. Hopper, Mr. S. Rees Jones, Mr. W. C. Lister, Miss E. Whittall, Mr. F. Poperwell, Dr. A. A. Moss, Dr. H. R. Rishworth, Miss D. Sidebottom and Mr. H. Stanesby.

National Research Council of Canada

The following have been appointed members of the National Research Council of Canada for a three-year term: Prof. F. A. Forward, head of the Department of Mining and Metallurgy, University of British Columbia; Dr. F. K. Hare, dean of the Faculty of Arts and Science, McGill University; Dr. G. A. Krotkov, head of the Department of Biology, Queen's University at Kingston; and Mr. W. F. McLean, president of Canada Packers, Ltd., Dr. F. R. Hayes, head of the Department of Biology and director of the Oceanographic Institute, Dalhousie University, has been re-appointed to the Council for a further three-year term. The new appointments fill vacancies caused by the retirement from the Council of Dr. I. McTaggart Cowan, University of British Columbia; Dr. A. D. Misener, director of the Ontario Research Foundation; Dr. B. W. Sargent, Queen's University at Kingston; and Dr. D. L. Thomson, McGill University. Dr. R. J. Uffen, principal of University College, University of Western Ontario, has been appointed to four of the Council's executive committees.

The Night Sky in June

New moon occurs on June 2d. 13h. 27m. U.T. and full moon on June 18d. 02h. 03m. The following conjunctions with the Moon take place: June 5d. 03h., Venus 4° N.; June 21d. 07h., Saturn 0·5° S.; June 23d. 12h., Jupiter 2° N.; June 28d. 10h., Mars 5° N.; June 29d. 18h., Aldebaran 1° S.; June 30d. 06h., Mercury 0·8° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Pollux on June 11d. 19h., Venus being 5° S. Mercury is too close to the Sun for observation. Venus is an evening star, visible in the west after sunset. It sets at 22h. 40m., 22h. 40m. and 22h. 25m. on June 1, 15 and 30, respectively; its stellar magnitude is −3·4. Its distance decreases during the month from 127 to 109 million miles and the visible portion of the apparent disk decreases from 0·847 to 0·757. Mars is still too close to the Sun for easy observation, rising about two hours before the Sun on June 15. Jupiter rises at 0h. 55m., 0h. 00m. and 23h. 00m. at the beginning, middle and end of the month, respectively. It is in Aquarius, its stellar magnitude is −2·1 and its distance from the Earth in the middle of the month is 440 million miles. Saturn is in Capricornus, rising at 23h. 50m., 22h. 55m. and 21h. 50m. on June 1, 15 and 30, respectively. Its stellar magnitude is +0·7 and its distance from the Earth on June 15 is 860 million miles. There are no occultations brighter than magnitude 6 visible at Greenwich. The summer solstice occurs on June 21d. 21h.

Announcements

Prof. Léon Van Hove, leader of the Theory Division at CERN, has been awarded the Dannie Heineman Prize for mathematical physics by the American Physical Society and American Institute of Physics for his contributions to statistical mechanics and to field theory, as examples of outstanding publication in the field of mathematical physics.

Mr. B. S. Shenstone, a director and chief engineer of British European Airways, has been elected president of the Royal Aeronautical Society for 1962–63. Born in Canada, Mr. Shenstone graduated at the University of Toronto. He is also vice-chairman of the Society's Man-Powered Aircraft Group Committee.

Mr. Richard Hindle Fowler has been appointed director of the Institute of Applied Science of Victoria, Melbourne. Mr. Fowler, who has been assistant director of the Institute since 1956, succeeds Dr. C. M. Focken, who has retired.

The annual meeting and summer conference of the Society for Applied Bacteriology will be held in the School of Agriculture, University of Nottingham, during July 17–19. Included in the meeting will be a symposium on "*Staphylococci* and *Micrococci*". Further information can be obtained from Dr. Ella M. Barnes, Low Temperature Research Station, Downing Street, Cambridge.

A two-day summer meeting of the Royal Meteorological Society will be held in the University College of North Wales, Bangor, during July 5–6. The programme will include two symposia on "The General Circulation of the Atmosphere" and on "The Electrification of Thunderstorms". Further information can be obtained from the Assistant Secretary, Royal Meteorological Society, 49 Cromwell Road, London, S.W.7.

The 1962 National Bureau of Standards course in "Radio Propagation" will be held during July 16–August 3, 1962. The course, administered by the National Bureau of Standards, Boulder, Colorado, Laboratories, is open to scientific and engineering personnel in other Government agencies, industry, or universities. Further information can be obtained from the National Bureau of Standards Office of Technical Information, U.S. Department of Commerce, Washington 25, D.C.

An international conference on "The Physics of Semiconductors", arranged by the Institute of Physics and the Physical Society on behalf of the International Union of Pure and Applied Physics and the British National Committee for Physics, will be held in the University of Exeter during July 16–20. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Erratum. In the communication entitled "Electron-Diffraction Studies of Fatty-Acid Monolayers", by B. R. Banerjee, B. Ostrofsky and H. E. Ries, jun. (*Nature*, 193, 873; 1962), lines 14–17 in the second column of p. 874 should read: ". . . to the orientation indicated by the spots. Fig. 4, after collapse at 42 dynes per cm., shows no evidence for orientation; the sharp and continuous diffraction lines suggest random orientation and thicker films".

BRITISH POST OFFICE AND TELECOMMUNICATIONS SERVICES

IN a debate on the Post Office in the House of Commons on March 28, the Postmaster-General, Mr. R. Bevins, said that out of capital expenditure of £132 million during 1962–63, Britain will spend about £122 million on telecommunications, or 11 per cent more than in the present year, partly in modernizing the system as new techniques developed, by automatization through the development of subscribers' trunk dialling, by new international cables and, in the long term, by satellites, and partly in reducing the waiting list. Inland trunk traffic is growing at the rate of about 12 per cent a year, and by the end of next year there will be about 37,000 trunk circuits as against 24,000 three years ago. It is planned to make subscriber trunk dialling available to about 90 per cent of subscribers by 1970, and it is estimated that savings to subscribers during 1962–63 on the same number of calls as with the old tariff will be £7 million. The waiting list has been reduced in six years from 250,000 to 53,000. Later this year Britain will be opening the first electronic exchange in Europe to carry public telephone calls. Present techniques, said Mr. Bevins, limited to about 100 the number of telephone systems which can be carried by long-distance submarine cables, but a cable in the design stage might increase this to about 250. Traffic demands in ten or twenty years time are likely to justify the high cost of communication satellites, which can probably offer 1,000 simultaneous telephone calls and perhaps a television channel. He hoped that the Commonwealth will play its part in this, and he had welcomed representatives from all Commonwealth countries who had come to consider Commonwealth policy. Britain is also co-operating with the Americas in test transmissions through satellites, but we may be many years away from a full, universally operated system.

In the debate, Mr. P. Holland asked for some information on the experiments in automation being conducted at Luton and Norwich, but this point was not covered in a full reply on the debate by the Assistant Postmaster-General, Miss M. Pike, who, however, instanced the new telephone exchange at Altrincham and the head post office at Hitchin as illustrating the possibilities of introducing new ideas and techniques into post office buildings. Both buildings were designed by a special research team, a joint enterprise with the Ministry of Works. A large-scale experiment covering parcel post for the whole of East Anglia is planned to start this year, and the Post Office is experimenting with the sorting and segregation of mail as well as with the sorting of parcels. Demand for telex has doubled in the past four years, and 1,600 new lines during 1962–63 will bring the total to more than 10,000. About 20,000 subscribers are expected by 1970, and to cope with this growth 21 new telex exchanges will be required; work on six will be started during 1962–63. Referring to the Post Office's increasing concern with the transmission of data and the use of telecommunications networks, Miss Pike stressed the need for ensuring a background of submarine communications throughout the Commonwealth. Despite its huge effort in developing satellite communications, the American Telephone and Telegraph Company has decided not to change its large investment in plans in submarine cables between now and 1965–66.

PREPARATION OF SCIENTIFIC REPORTS AND BOOKS

THE theme of this year's conference of the Scottish Branch of Aslib (Association of Special Libraries and Information Bureaux) was the writing, editing, publishing, binding and storage of the technical report. The meeting was of a historic nature as it was the first two-day residential course which the Scottish Branch has held. To enable as many as possible to attend, it was held in Perth, once the capital of Scotland and a point easily reached from all parts of the country. The meeting was accorded a civic reception from the Lord Provost of Perth, Mr. John T. Young, and the magistrates.

The first speaker was Dr. Archie Clow, producer-in-charge, Science Unit of the British Broadcasting Corporation, who spoke on "Communication in Science". Dr. Clow said that his experience over the past ten years was that interest in science in the public as a whole is very high. Dr. Clow spoke on the need for careful writing and editing of a broadcast science talk pointing out that the speaker had to do everything possible to maintain the flow of thought. The listener could not stop the speaker and ask for explanations or, as in reading, turn back the pages. In one aspect broadcasting did not differ from other forms of communication and that was intelligibility in the sense of what the listener could recall an hour or a week later. He agreed that there was a great need to present the hard dry bones of scientific investigations with imagination.

The second paper was "The Editorial Function", which was given by Mr. G. N. J. Beck, assistant editor of *Endeavour*, who is principally concerned with the production of the foreign-language editions of that journal. He spoke on the choice of the subject of articles and their commissioning from suitable authors. He outlined how the flow of material was kept going between the editorial offices and the printers of the foreign issues. Pointing out that they used the services of translators belonging to the countries covered, he indicated the occasional difficulties that arose in translation of an idiomatic phrase comprehensible to the original author but of which a literal translation would mean nothing. He also emphasized the need for clear and intelligent writing and proof reading.

Mr. D. Ainslie Thin, director of Messrs. Oliver and Boyd, Ltd., followed with a talk on "Publishing Scientific and Technical Material". Mr. Thin dealt with scientific material from the publishers' point of view. He spoke of author's royalties and the need for modern attractive book jackets. He also referred to the vexed subject of indexes, which was later a matter for discussion from the floor. It was a question of whether or not it was better for the author or a trained indexer to do this work. Mr. Thin went into detail on the various stages in the publication of a book, the necessity for a clear layout, illustrations to match the text, type faces to be used and so on. It was suggested to him that it would be useful if publishers were to print the bibliographical detail on the flyleaf and to indicate the font-type used. The first of these suggestions he thought to be a useful one, but doubted if it would be practical to elaborate on the type as in some books several fonts were used. Mr. Thin also went into the question of the age of books and stocks. One suggestion from the floor was that when a publisher saw his stock of a particular book running low he should consider the advisability of making a microfilm of it. He again spoke of the difficulty met in the publication of a translated version of a book. It was sometimes difficult to get the translator and the author to agree on the final version to be issued.

At the end of the first day there was some time given for discussion. In addition to the items already mentioned the question of the use of microfilm was also raised. It apparently is still not quite acceptable to the average scientist, who prefers paper in his hands. There was also some discussion on the need and methods for getting information over to the working man at the factory bench. Dr. Clow had found there was interest expressed in the broadcasts, but it was essential that the correct speaker was employed to present the matter in an interesting way.

The second day of the conference opened with a talk by Mr. David Moncur, manager, Dunn and Wilson's, Falkirk. Mr. Moncur, who illustrated his talk with a series of transparencies, went into elaborate detail of the process of binding books and periodicals in Britain. He spoke of a recent visit to the United States where the process of binding had been streamlined but had poor effect. One library he spoke of had all its books bound in one colour, which was extremely monotonous to the eye. In Britain much is done to vary the colour. Mr. Moncur also spoke of the new trends in binding by the use of plastic sleeves and front boards.

The final paper of the day and of the conference was given by Dr. D. J. Urquhart, director of the National Lending Library at Boston Spa. He dealt with the availability and translation of foreign publications. He spoke on the building up of the National Lending Library and its endeavour to provide a 48-hr. service to borrowers. He pointed out that the person who worked in a sizeable research organization and perhaps had access to big libraries was better off than he who worked in a small industry remote from technical college and university libraries. He thought that the public libraries had a great responsibility to increase their guides to scientific literature, supplying the basic reference tools and using the resources available to them through Boston Spa. Reference was also made to the Russian translations held at Boston Spa and how they were available to borrowers.

THE EAST AFRICAN VIRUS RESEARCH INSTITUTE

IN his annual report*, the acting director of the East African Virus Research Institute, Dr. J. D. Gillett, explains that the middle of Africa is the home of many different virus diseases, particularly those which are transmitted to man by mosquitoes. It is not enough merely to study virus, vector and vertebrate host, including man, as separate entities. These, their predators and parasites, their associated plants and, indeed, the whole community of living things must be considered, together with the physical environment, as a single interacting system. It is only thus that one can begin to understand the nature of the complex factors which play a part in the maintenance of virus populations, and of the variations in these factors which lead to the infection of man from wild animals and bird hosts, or to the sudden occurrence of human epidemics. It is only by such a broad approach that explanations will be found for 'epidemic years'. While the paucity of technical facilities for virus research in Africa is well known, the richness in natural facilities is unsurpassed anywhere.

The approach to virus research in Africa must be mainly ecological. If, however, it is to avoid the danger of becoming merely descriptive, field observation must be supported by laboratory experiments whenever possible. Laboratory studies, if properly planned, can produce short-cut answers and suggest valuable clues to complex problems of behaviour. It would be difficult to find a better place for this complementary approach to virus studies. New viruses are being discovered every year. Ten years ago these were mostly being recovered from wild populations of mosquitoes, and their importance to man was largely unknown. With the development of new techniques, however, emphasis has swung more and more to the human patients themselves as a source of new viruses, and now the Institution is in a position to find the cause of many fevers which previously would have been accepted as pyrexias of unknown origin. Many new viruses and virus strains are being discovered. Most of the fevers associated with these newly discovered viruses are relatively mild, or their unpleasant symptoms pass off without permanent after-effects. Apart from individual discomfort, their main importance would appear to be the economic loss to the country concerned that must accompany wholesale absence from work.

The most dramatic of these new virus infections has been the huge epidemic of o'nyong-nyong. This appears to have begun in Uganda in 1959, moved steadily forward in a south-easterly direction for about a year and then, coming up against natural ecological barriers to spread farther in that direction, halted before breaking out in separate foci many hundreds of miles apart. The line of the initial advance depended on almost continuous permanent concentrations of the main vector, the malaria mosquito,

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* East Africa High Commission. East African Virus Research Institute Report, July 1960–June 1961. Pp. ii+56. (Entebbe: East African Institute for Medical Research.) Sh. 3.

Anopheles funestus, and its limits and extent could be fairly accurately forecast by a study of endemic malaria conditions. Its present distribution in isolated pockets appears to have resulted from lines of human communication, such as steamers on Lake Victoria and railways from the lake ports. The appearance of the disease in epidemic form, however, has finally depended on the appearance of the main vector in high concentration, and of course, on a non-immune susceptible human population.

O'nyong-nyong virus is very closely related to chikungunya virus, which was isolated for the first time in 1953 during an epidemic in Tanganyika; the symptoms of infection in man are very similar indeed, and resemble those of dengue. Furthermore, infection from either virus produces hæmagglutination, inhibiting antibodies to both viruses. It is interesting to note, however, that, so far, o'nyong-nyong virus has in every instance been associated with the two main malaria mosquitoes, *A. funestus* and *A. gambiae*, whereas chikungunya outbreaks can appear in the absence of these species. Moreover, both o'nyong-nyong and chikungunya are occurring in contemporaneous, but spatially separated, epidemics. If these two agents are merely variants, does the insect host play a part in bringing about selection for one or the other ? Are these extra-human cycles in other vertebrates, and, if so, are they the same for each virus ?

Dr. Gillet indicates that the most important development of the year has arisen from the observation that the distribution of a malignant lymphoma in African children coincides with that of certain arthropod-borne diseases such as yellow fever. Thus a possibility exists that this revolting fatal disease is arthropod-borne, or at least associated with arthropod-borne disease. This is at present no more than a slender clue, but one which is being followed up vigorously. Work on this important subject is likely to proceed if the close collaboration of a team of cancer experts can be secured. In the meantime, the investigators have been screening several sera from cases and relatives against a range of viruses, in an attempt to demonstrate antibodies that would indicate previous implication of an arthropod-borne virus. So far, these have given largely negative results, except for Bunyamwera virus, where antibodies have been demonstrated in a suggestively high proportion. Further information is urgently needed on the general distribution of Bunymwera antibodies in the human population before any conclusions can be made.

THE REGIONAL RESEARCH LABORATORY, HYDERABAD

THE annual report for 1960–61 of the Regional Research Laboratory, Hyderabad, follows the broad pattern of previous years*, but the presentation is addressed more particularly to the general reader. Lists of publications during the year and of patent applications filed and accepted are appended, as well as of staff and of members of the Executive Council and its sub-committees.

Work in the Division of Oils and Fats included hydroxylation and descriptive studies on castor oil, ricinoleins as surfactant bases, the processing of cotton-seed and tallow-like products from cotton-seed oil, the hydrogenolysis of fatty acids, fat-splitting and distillation of fatty acids, and fundamental studies on autoxidation. In the Division of Surface Coatings and Pigments, besides fundamental studies on dehydrated castor oil, the styrenation of drying oils, the preparation of long oil alkyds from drying-oil fatty acids, of emulsion paint vehicles from alkyds, and of varnishes from cashew nut-shell liquid and drying oils, the use of styrene–cardanol co-polymers in surface coatings and exposure tests for composite aluminium, red lead and other conventional primers were all investigated.

In organic chemistry some twenty-one *N*-(alkylaminoalkyl)-1:2:3:4-tetrahydroquinolines were synthesized for pharmacological examination, a number of *N*-dialkylaminoalkyl-β-phenylethylamines were prepared, as well as terpineol and terpin hydrate from 3-carene and pimenes. In entomology, the mechanisms of synergism of sesamin, 'Sesoxane', piperonyl butoxide and other insecticides were examined, as well as the mechanism of physiological action at different temperatures of toxaphene and pyrethrum, and the effect of X-rays on the biology and biochemistry of the spermatozoa of insects and mammals. The mechanism of biosynthesis of itaconic acid was further examined, as well as the uptake of phosphorus-32 and of labelled glucose by *Escherichia coli* in the logarithmic phase of growth, the nucleic acid and protein metabolism of liver cells in suspension. Electrophoretic studies of proteins before and after treatment with acid and studies of the metabolism of proteins and nucleic acid in bovine spermatozoa also continued.

Other work was concerned with the utilization of Indian cotton linters, the production of hand-made paper of high bursting strength, the recovery and fractionation of tar acids from tar oils and ammoniacal liquor, the chemical examination of tar acids from low-temperature tar, distillation characteristics of low-temperature tars and pilot-scale distillation of these tars, as well as low- and medium-pressure hydrogenation of low temperature tars and oils. Electrode binder and electrode coke were prepared from these tars, and spectrophotometric studies made of tar acids obtained by low-temperature carbonization. Further work was carried out on the production and development of active carbons and on bleaching earths for vegetable and mineral oils. A molybdenum cobalt oxide on alumina catalyst was prepared for the hydrogenation of tar oil as well as one on silica alumina and a nickel oxide on alumina catalyst. Other work was concerned with the hydrolysis of titanium salts, the preparation and properties of butyl titanate, white cement, the synthesis of silicon carbide, active carbons for water treatment, the treatment of boiler feed water, vapour-phase esterification, and design and development studies on benzyl chloride, phenylacetic acid and phenylacetamide.

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* India: Council of Scientific and Industrial Research. Regional Research Laboratory, Hyderabad. Annual Report, 1960–61. Pp xii+118. (Hyderabad: Regional Research Laboratory.)

HIGH-ENERGY RESEARCH AT CERN

THE monthly, *CERN Courier*, which was temporarily suspended during 1961, made a welcome comeback at the beginning of 1962. The January issue (2, No. 1; 1962) contains, in addition to a message from Prof. V. F. Weisskopf, the director-general of the European Organization for Nuclear Research (CERN) at Meyrin near Geneva, a comprehensive article by J. Baarli, the head of the health physics division, on radiation safety at CERN. Some 800 people out of a total personnel of 1,440 are subject to permanent radiation control. They are those who work either directly with radiation or in areas where a radiation dose-rate exceeding normal background has been measured. They carry γ-film badges and in the case of about 150, neutron film badges in addition. The records for 1960 show that the maximum radiation exposure for more than 700 of the 800 controlled personnel was less than 1 rem and only one person had an exposure exceeding 5 rems. During 1961 there was a somewhat greater exposure, especially of those groups who carried out maintenance work and repair on the synchro-cyclotron accelerator, but it was still below the recommended maximum permissible radiation exposure. Other articles in the January issue include reports on the 1961 international conference on theoretical aspects of very high-energy phenomena held at CERN during June 5–9, 1961, and on the twelfth Solvay Physics Meeting held at the Free University of Brussels during October 9–13, 1961.

The first part of an article based on a paper prepared by the director-general for the twentieth session of the CERN council meeting on December 19, 1961, entitled "CERN and High-Energy Physics" is the main feature of the February issue of the *Courier*. Prof. Weisskopf, after outlining the present state of high-energy physics, considers the activities of the different high-energy research centres, particularly those contributions which have opened new avenues of research or revealed decisive results.

The main aim of high-energy physics is the study of the properties of elementary particles, and large high-energy installations are required because the smaller the object, the higher is the energy needed to penetrate into its structure. Studies to date have not yet given satisfactory answers as to the structure of the particles, but they have revealed an unexpected wealth of phenomena—nucleon structure, strange particles, weak interactions, and anti-matter—which represent a new aspect of nature, very different from experiences at lower energies.

The work with the synchro-cyclotron at CERN has been directed mainly towards weak-interaction physics, and the first important contribution was the discovery of the electron decay of the pion in 1958. Following the addition of a muon channel, the first measurement of the anomalous magnetic moment of the muon was made, and at present measurements of muon capture by hydrogen are being carried out. The proton synchrotron, which came into use in 1960, has been used for the study of peripheral collisions, and a number of new phenomena relevant to the understanding of the pion-cloud structure has been demonstrated. Experiments planned for the immediate future include the neutrino experiment, in which a neutrino beam of high energy is obtained when fast pions produced in a target decay in flight, and runs with the 81-cm. bubble chamber in a separated anti-proton beam of more than 3 GeV., in order to obtain pictures of annihilations of fast anti-protons and their by-products.

S. WEINTROUB

DAYTIME SKY RADIANCE FROM FORTY TO EIGHTY THOUSAND FEET

By GORDON A. NEWKIRK and JOHN A. EDDY*

Department of Astro-Geophysics, High Altitude
Observatory, Boulder, Colorado

THE possibility of flying large astronomical equipment on balloons to altitudes of 80,000–100,000 ft. has led to increased interest in the radiance of the sky at these altitudes. The daytime sky radiance is of interest to the instrument designer, who is concerned with the contrast between the target (for example, a planet, artificial satellite, the solar corona, etc.) and the sky. It is of equal interest to the astronomer, who can learn about the sedimentation of micrometeorites through the upper atmosphere by means of the scattering of sunlight from meteoric debris, and to the meteorologist.

As part of a programme to investigate the feasibility of coronal observations from balloons, the High Altitude Observatory has flown an externally occulting coronagraph up to heights of 82,000 ft. to measure the angular and the wave-length distribution of the daylight sky. The coronagraph was a modification of the instrument designed by Evans[1] and used by one of us[2] for ground-based observations of the solar aureole. The balloon-borne instrument was pointed at the centre of the solar disk to within an accuracy of ± 1 min. of arc by the guiding gimbals developed and used in Project Stratoscope[3–6].

The sky radiances were recorded in three ways: (1) photographically, at scattering angles $\varphi = 9 \cdot 6°$, $20 \cdot 7°$, $31 \cdot 4°$, $40 \cdot 6°$, and $57 \cdot 8°$ at $\lambda = 0 \cdot 44\mu$ with electric vector vibrating perpendicularly to the scattering plane; (2) photographically, at scattering

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* Present address: National Bureau of Standards, Boulder, Colorado.

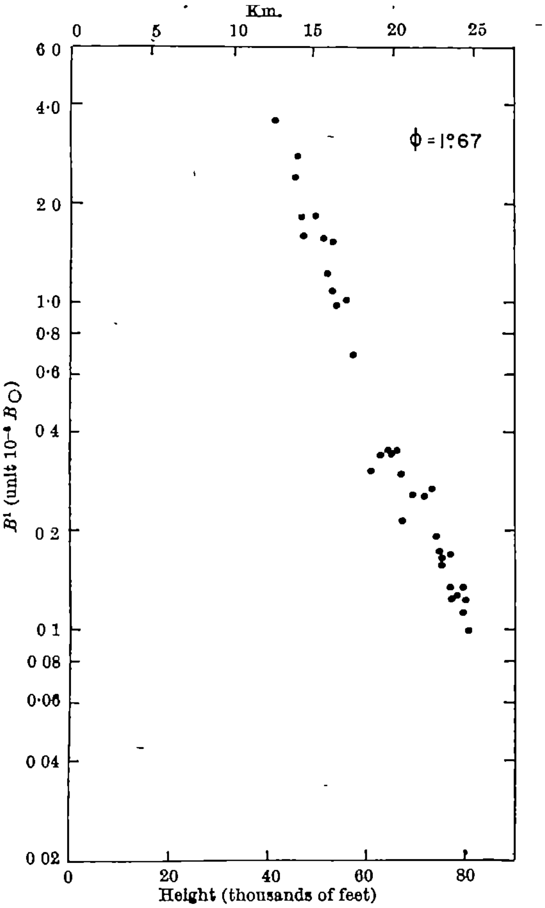


Fig. 1. Daytime sky radiances with electric vector perpendicular to the scattering plane at scattering angles φ at $\lambda = 0{\cdot}44\mu$. Measured photographically on October 3, 1960

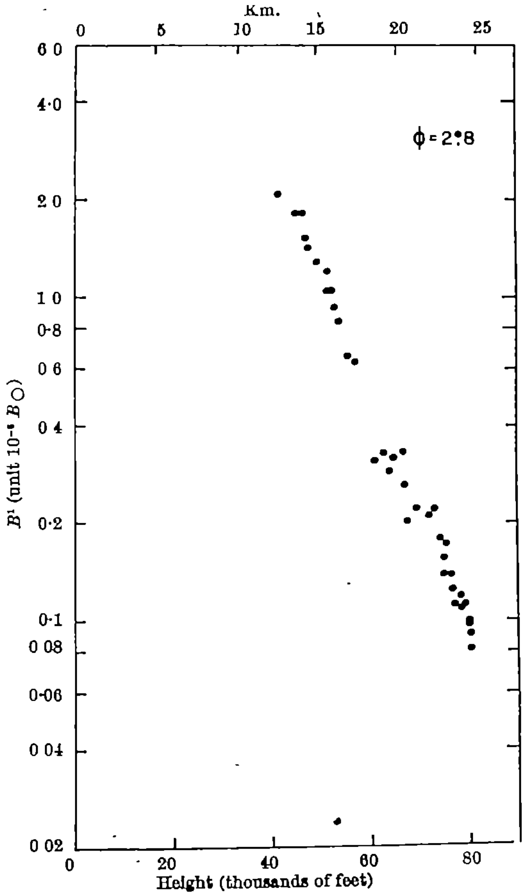


Fig. 2. See legend to Fig. 1

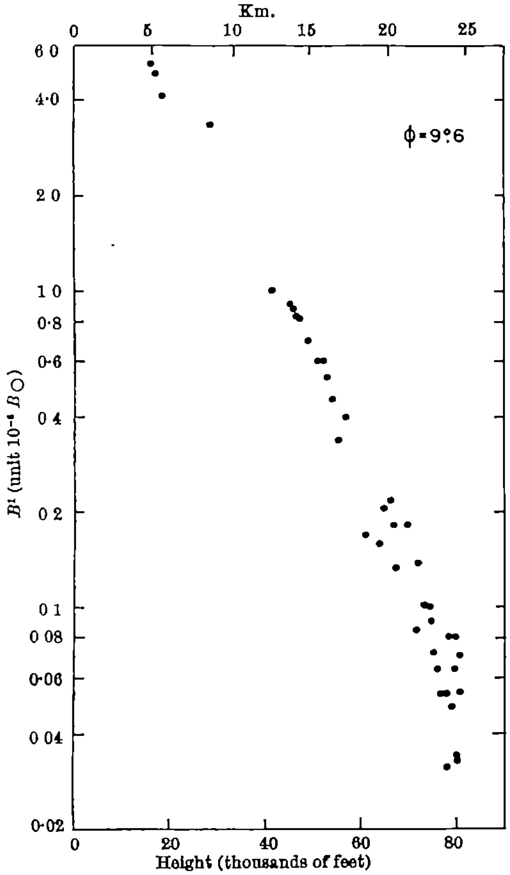
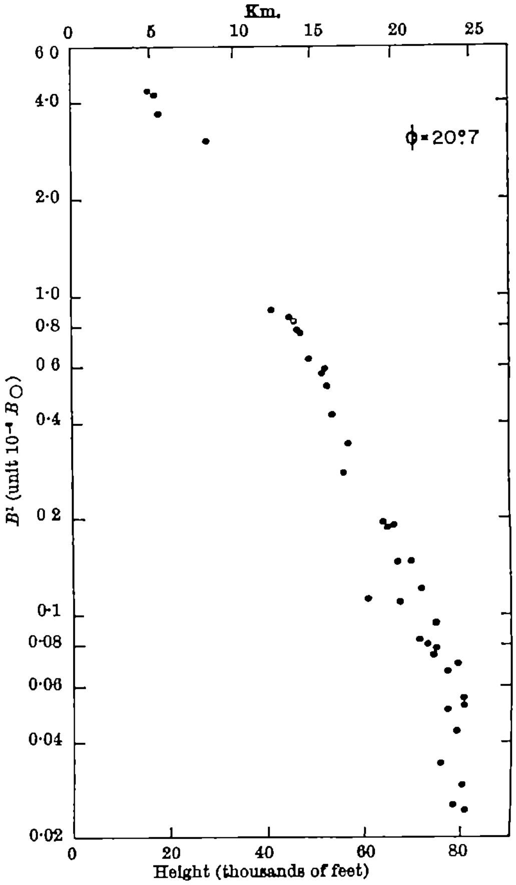


Fig. 3. See legend to Fig. 1



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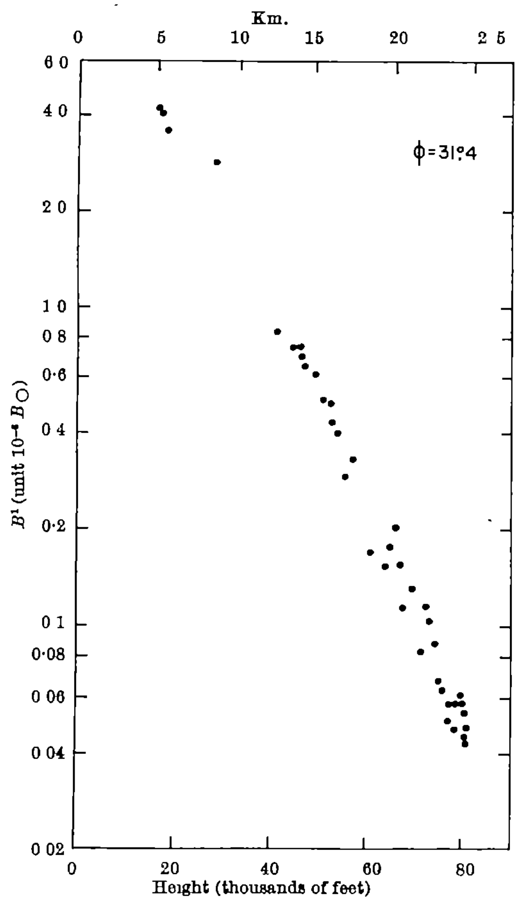

Fig. 5. See legend to Fig. 1

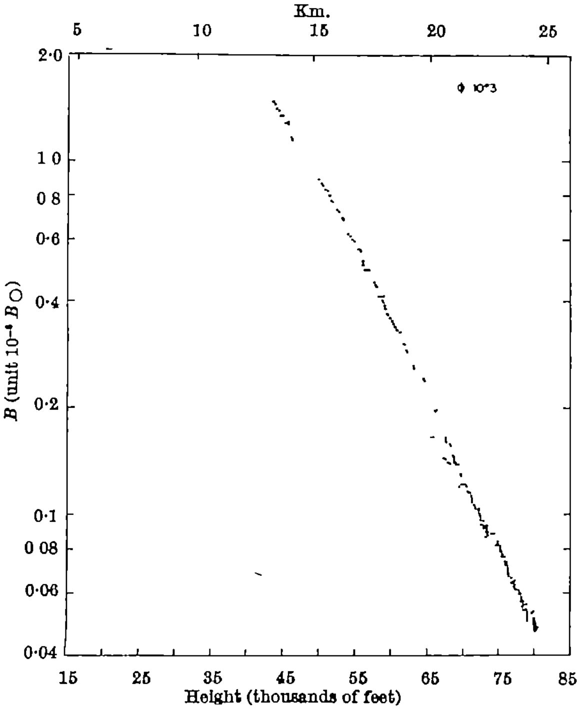

Fig. 6. Total daytime sky radiance at $\lambda=0\cdot52\mu$ at a scattering angle of 10·3° from the solar centre. Measured photoelectrically on October 3, 1960

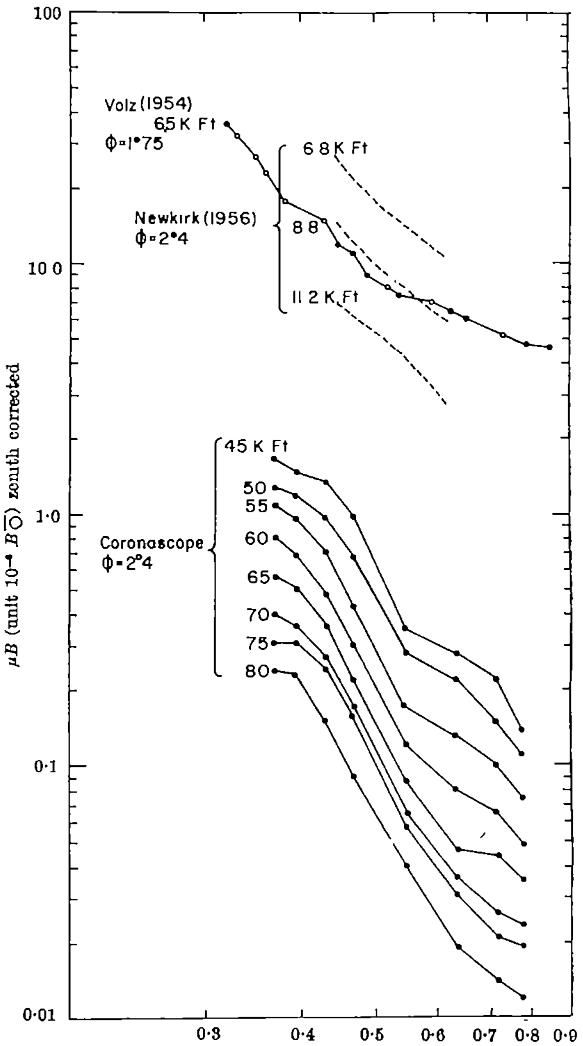

Fig. 7. Spectral energy distribution of the total daytime sky at a scattering angle of 2·4° as measured on October 3, 1960, and corrected to the zenith by multiplication by $\mu=\cos Z_0$. The results are compared with earlier observations at low altitude (refs 2 and 8)

angles from 1·67° to 2·8° with a low-dispersion spectrograph covering λλ 0·37–0·79μ; (3) photoelectrically, at a scattering angle 10·3° at λ = 0·52μ.

The spectrograph and the photoelectric photometer recorded total radiance. Standardization was provided by a sunlit, calibrated, opal glass and step wedge combination that was photographed simultaneously with the spectrograph and the 9·6°–57·8° fields. The photoelectric photometer was standardized frequently in flight with a calibrated opal illuminated by sunlight.

In view of the potential usefulness of these results, we present some of the measurements made on our more successful flight, that of October 3, 1960, without interpretation (Figs. 1–7). All values are given in units of the radiance of the mean solar disk at the height of observation. Two points should be noted. First, during the ascent of the equipment from 53,000 to 80,000 ft. and the descent from 80,000 to 40,000 ft., the zenith distance of the Sun was, of course, changing. Secondly, the observations are made in neither the vertical nor the horizontal circle through the Sun, but in an instrumental plane in-

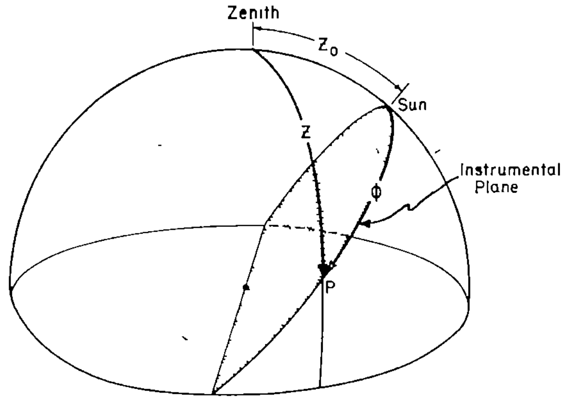

Fig. 8. Relation between the zenith angles and the scattering angle φ on the celestial sphere

clined to the horizontal and including the Sun. The relation between the solar zenith angle Z_0, the scattering angle φ and the zenith angle Z of the point P in the sky is displayed in Fig. 8. Table 1 gives a brief résumé of the quantities $\mu_0 = \cos Z_0$ and $\mu = \cos Z$ for 10,000-ft. intervals during the flight.

Table 1. TABLE OF COSINES OF ZENITH DISTANCE AT SCATTERING ANGLES φ, OCTOBER 3, 1960

Height (10^3 ft.)	Central standard time	$\varphi = 0$	$\varphi = 2\cdot8°$	$\varphi = 9\ 6°$	$\varphi = 20\ 7°$	$\varphi = 31\ 4°$
60	09 · 40	0 539	0·533	0 532	0 505	0 497
70	10 · 00	0 572	0 572	0 565	0 587	0 525
80	10 : 30	0 615	0 615	0·607	0 576	0 560
80	11 : 50	0 662	0 662	0 653	0 620	0 598
70	14 · 36	0 481	0 480	0 474	0 451	0 450
60	15 10	0 404	0 404	0 399	0 379	0 386
50	15 44	0 316	0 315	0 311	0 296	0 312
40	16 01	0 267	0 267	0 263	0 250	0 271

The scatter in the photographic measurements is sufficient to mask the small difference between the observations made during ascent and descent. The photoelectric observations (Fig. 6) are more accurate; the ascent branch, beginning at 66,000 ft., can be distinguished lying slightly below the descent branch. The difference is a result of the difference in zenith distance between the two branches.

It is of interest to compare these observations with the sky radiance expected from a pure, molecular sky at, say, 80,000 ft. Within the first two degrees of the Sun, the sky is approximately twice the Rayleigh value. At a scattering angle of 10° the observed radiance exceeds the Rayleigh value by only about 10 per cent. At 20° and beyond the two are nearly indistinguishable.

The only other observations which describe even roughly the quantities reported here are those published by Stakutis[7]. A detailed comparison is impossible because the wave-length at which the latter observations are made is not specified. However, his minimum radiance in the neighbourhood of the Sun is 10^{-5} at 98,000-ft. altitude. Our observation at $\lambda = 0{\cdot}52\mu$ at 80,000 ft. gives a radiance 5×10^{-8}, a factor of 200 smaller.

This work was supported by the Office of Naval Research under contract *Nonr* 393(05).

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GEOMAGNETIC MICROPULSATIONS WITH PERIODS 0·3-3 SEC. ('PEARLS')

By PROF. J. A. JACOBS and E. J. JOLLEY

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GEOMAGNETIC micropulsations of nearly sinusoidal shape and with periods ranging from about 0·3 to 3 sec. have been called 'pearls'. The wave-trains usually exhibit beats—the envelope of a sequence of oscillations resembling a necklace of oval pearls. It is because of this resemblance that Troitskaya[1] has named this phenomenon pearls. The pulsations may occur as separate bursts lasting 1–2 min. and as a continuous series of pulsations lasting for an hour or more. Fig. 1 shows an example of pearls recorded at Abbotsford, British Columbia. The curves show pearls in their pure form, longer-period pulsations which were also present having been filtered out. The appearance of pearls is not very common. Benioff[2] observed that over the four-year period 1955—59 the number of occurrences in Southern California was about 30 a year. This number may only be representative of low-middle latitudes and may be increased by a factor of two or three in the polar regions[3].

During the course of a combined field operation in the summer of 1960, pearls were recorded once at Abbotsford during two weeks of continuous operation. The onset of pearls at Suffield, Alberta, some 450 miles to the east, was not simultaneous, but occurred 38 min. earlier. At Austin, Texas, they were not observed at all. Records were obtained from Benioff for Isabella (California), Ruth (Nevada), Huancayo (Peru), Reykjavik (Iceland) and Uppsala (Sweden). (The latitudes and longitudes of these stations are given in Table 1.) It appears that pearls were observed on this occasion progressively earlier at eastern stations. Fig. 2 shows a plot of the time of occurrence of pearl activity against (geographic) longitude. The graph is practically linear, the onset

Table 1

Station	Geographic Latitude	Geographic Longitude	Geomagnetic Latitude	Geomagnetic Longitude
Resolute Bay (Canada)	N.74°41′	W.94°55′	N 82.9°	289.3°
Reykjavik (Iceland)	N 64°11′	W.21°42′	N.70.2°	71 0°
Uppsala (Sweden)	N 59°58′	E.17°39′	N 58.7°	104 5°
Suffield (Canada)	N 50°15′	W.111°10′	N 57.3°	306.4°
Abbotsford (Canada)	N.49°03′	W.122°17′	N.54.6°	293 6°
Victoria (Canada)	N.48°25′	W.123°22′	N.54 1°	292.6°
Memambetsu (Japan)	N.43°55′	E.144°12′	N.34.1°	208.3°
Ruth (U S.A.)	N.39°16′	W 114°59′	N.46.4°	305.3°
Isabella (U.S.A.)	N 35°40′	W.118°28′	N.42.6°	303.1°
Huancayo (Peru)	S.12°03′	W.75°20′	S.0.6°	353.8°

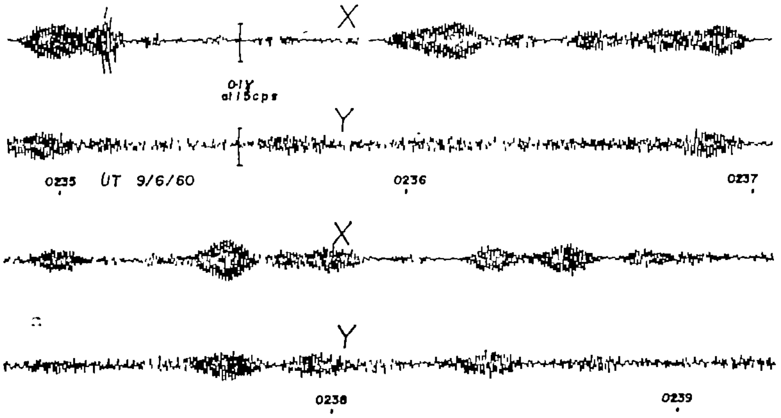

Fig. 1. Records of X and Y, Abbotsford, June 9, 1960, filtered.

of these pulsations moving westward at an approximate rate of 14°/hr. An even better linear fit is obtained if the time of onset is plotted against geomagnetic longitude.

To investigate further the L.M.T. or U.T. dependence of the onset of pearls, the records at Victoria, British Columbia, were examined for all cases of pearl activity from October 1960 until February 1961. The corresponding records for the times of occurrence were obtained from Benioff for Isabella, Ruth, Huancayo, Reykjavik, Resolute Bay and Uppsala. There is not sufficient difference in longitude between Isabella, Ruth and Victoria satisfactorily to resolve the question of local time dependence. In most cases pearl activity at these three stations was reported about the same time. At Resolute Bay it was impossible to recognize any pearl activity. However, the field is usually quite disturbed at that latitude and the sensitivity of the recording equipment was thus reduced so that many examples of pearls may have been missed. For the same reason, little pearl activity could be identified on the records from Reykjavik. At Huancayo the record is invariably quiet with an occasional burst of pearl (?) activity lasting for not more than 1 min. Such bursts have periods of about 2 sec., show some damping in amplitude and little or no correlation with records from other stations. The records from Uppsala, on the other hand, often showed pearls about 9 hr. earlier than their onset on the west coast of North America—in agreement with the linear trend of Fig. 2.

The magnetic observatory at Memambetsu (Japan) began rapid run recordings of Earth currents in December 1960 and the records for the first three months of operation were compared with the data from Victoria and Isabella. Only one out of eight occurrences of pearls at Memambetsu was also observed at Victoria and Isabella, although on this occasion the results were extremely interesting. Pearls were observed at Isabella about 6–7 hr. before their onset at Memambetsu and than again at Isabella some 17–18 hr. after their appearance at Memambetsu. The linear trend of Fig. 2 was thus extended right round the world on this occasion and is shown in Fig. 3, which also shows the double occurrence at Uppsala on consecutive days. The gradient of the line in Fig. 3 is 15°/hr.

The most obvious explanation of these results is that the onset of pearls depends entirely on local time. It is also possible that the graph expresses the azimuthal drift of charged particles trapped in the geomagnetic field. For a proton travelling at $1 \cdot 5 \times 10^6$ m./sec. (the velocity V of the solar wind under disturbed conditions), the azimuthal drift is about 17°/hr. The day in question, however, was not particularly disturbed, and a value of V for quiet solar conditions would reduce this figure by a factor of 10. Moreover, if pearls are due to the drift of protons trapped in the Earth's magnetic field, one would expect to observe the phenomenon with the frequency of the mirror oscillations, which is about 50 times too large for that of pearls, even under disturbed conditions.

The comparative rarity of pearls indicates that the ionosphere is an opaque shield for waves of this frequency most of the time. It is possible, of course, that pearls are more frequent but that their amplitude is in general below the threshold of the detecting equipment. In middle latitudes pearls are recorded mainly at sunrise and sunset and to a lesser extent during the night, and usually under geomagnetic quiet conditions. This tendency may be different in equatorial and polar regions. Troitskaya[3] has found that pearls are more frequent in polar regions and may occur at any time.

The results shown in Figs. 2 and 3 and the consistent time difference of occurrence between Uppsala and Victoria would indicate that, in these latitudes at any rate, pearls are L.M.T. dependent. The conditions which make the ionosphere transparent to

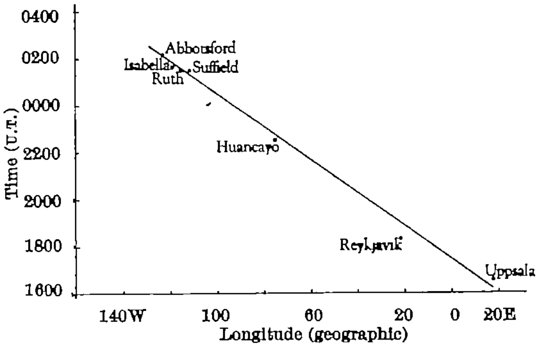

Fig. 2 Onset of pearl activity, June 8–9, 1960.

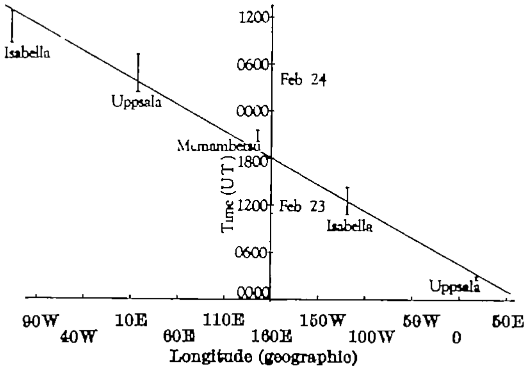

Fig. 3. Pearl activity, February 23–24, 1961

waves of pearl frequency are not clearly understood. In particular, the duration of suitable conditions is of interest—it is only very rarely that they exist for more than one rotation of the Earth, although pearls often occur on consecutive days. From the limited amount of data so far analysed, pearls were often observed at Victoria some 9 hr. after their appearance at Uppsala (longitude of separation 141°), but only once were they observed at Memambetsu after their appearance at Victoria (longitude of separation 92°). There were, however, many cases of their appearance at Memambetsu and their non-appearance at Victoria (longitude of separation 268° if their onset moves westwards). There is only 4° difference between the geomagnetic latitudes of Victoria and Uppsala, whereas Memambetsu is 20° to the south of Victoria and may thus reflect different conditions at low-middle latitudes.

No relationship was found between the occurrence of pearls and other ionospheric phenomena. Pearls usually occur about sunrise or sunset—the time of rapid change of f_0F_2. However, these changes in f_0F_2 take place daily, although more pronounced in winter, whereas pearls are comparatively rare. If the source is solar radiation, then a seasonal effect should be observed and their duration should be determined by solar conditions. There should also be a correlation with other phenomena in the ionosphere, which as yet has not been found. Moreover, any electromagnetic radiation effects would be propagated to the Earth in a few minutes, yet pearls are almost never observed during the daytime. If the source is corpuscular, either by direct injection or from the Van Allen radiation belts, no seasonal effects should be observed. Further analysis covering a longer period is desirable—particularly any results from conjugate points.

This work was supported by the Office of Naval Research under Contract *Nonr* 3116(00).

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RADIOACTIVE AIR-BORNE DUSTS IN JAPAN

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AFTER the re-opening of the nuclear test explosions by U.S.S.R., during September 1961, fresh nuclear debris was found in ground-level air for the first time on September 12 at our Laboratory. Since then, the concentration of artificial radioactivity in the atmosphere has risen and has kept changing up and down by the successive explosions depending on meteorological conditions. On November 7, 8 and 9, abnormally high concentrations were observed, which ranged from 70 to 125 $\mu\mu$c./m.^{3}. These were considered to be due to the large-scale test explosion of 50 megaton hydrogen bomb carried out on October 30.

Towards the end of September it was generally recognized in Japan that the measured values of radioactive concentration in rain-water differed sometimes appreciably from each other, even though they were obtained at nearby laboratories at the same time. In order to investigate this, we took the autoradiographs of the air-borne dusts collected on large filter papers. It was concluded that such a situation was caused by the presence of a small number of highly radioactive particles in fall-out[1-3,6]. Air-borne dusts were collected on a circular filter paper 18·5 cm. in diameter at the

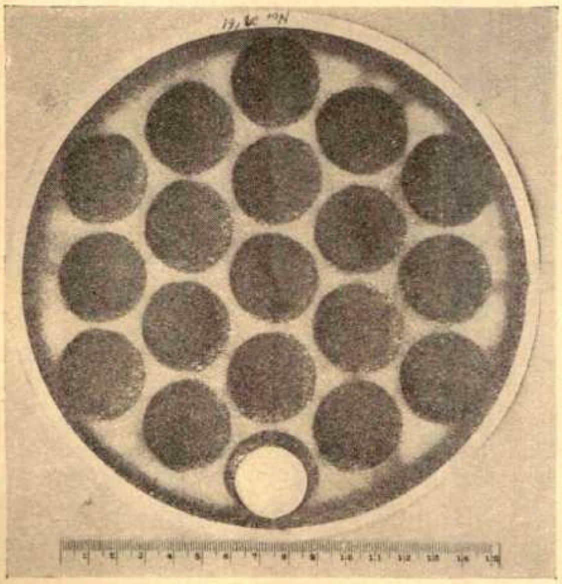

Fig. 1

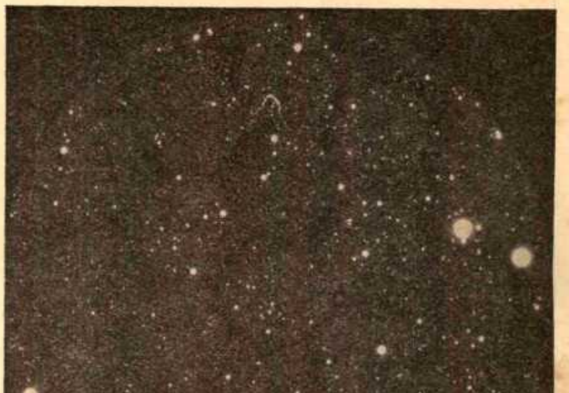

Fig. 2

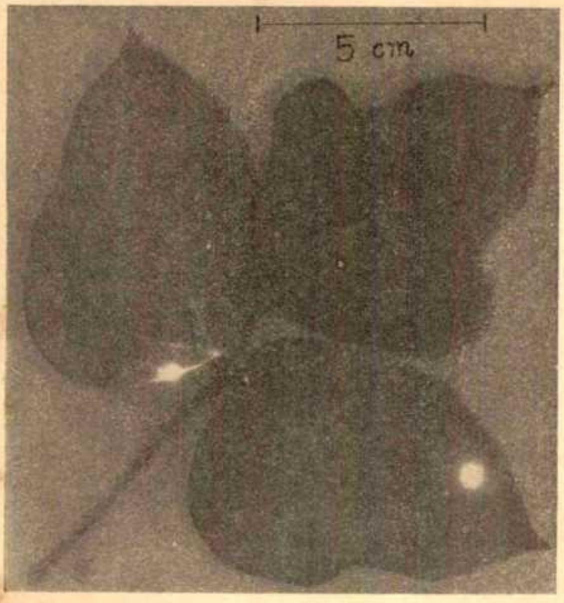

Fig. 3

suction rate of 400 l./min. Fig. 1 shows a filter paper dust sample on which the dusts have been collecting for 24 hr. A small circular area of 25 mm. in diameter was cut off from the filter paper and used as the sample for measuring the activity by a Geiger–Müller counter. Fig. 2 shows the autoradiograph of the dust specimen collected on November 7 by sampling 627 $m.^3$ of air. This was autoradiographed on an X-ray film (Fuji X-ray Film No. 200) for 16 hr. on November 14, one week after the sampling. The activity of the highly radioactive particle which produced the largest spot on this autoradiograph was about 20 mμc. just after sampling.

In order to examine how these highly radioactive particles became attached to the surface of vegetation, autoradiographs of the leaves of wild arrowroot were taken on November 13. About 12 sample leaves were collected at random in the yard of our Laboratory. Fig. 3 is the autoradiograph of one of them. The contour of the leaf was disclosed by irradiation of weak light after autoradiographic exposure in order to find out where the radioactive particles were located on the leaf. On careful inspection of the autoradiograph, it was seen that the blackening took place along veins in the vicinity of a large spot. It was therefore concluded that up-take of the radioactive substances took place through the leaf surface[5].

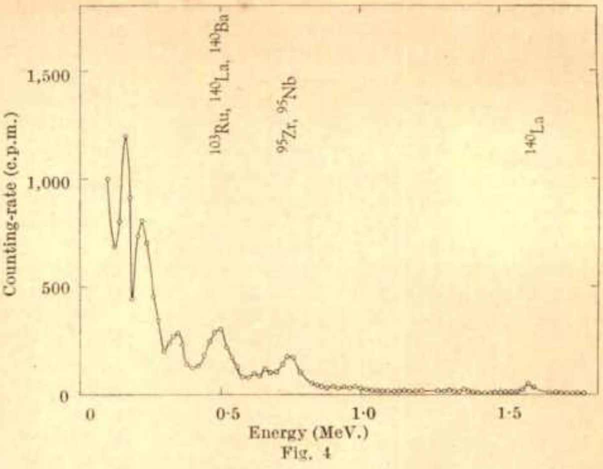

Fig. 4

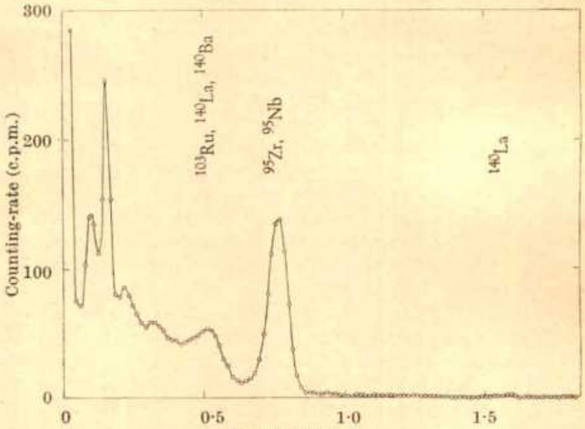

Fig. 5

A γ-ray spectrum of the dust sample collected on November 8 was taken on November 15 and is shown in Fig. 4. A highly radioactive particle was found on the surface of a polyethylene bottle which was left outdoors. Fig. 5 shows the γ-ray spectrum of this particle. The difference of distribution curves seen in Figs. 4 and 5 is considered to come from the so-called chemical fractionation effect[2,4].

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ROCK MAGNETISM AND LOW-ANGLE FAULTING

THE recent article by Norris and Black[1] greatly extends the scope of rock magnetism studies by dealing with the Lewis thrust-sheet, a famous North American example of low-angle faulting with large displacement. From the point of view of palæomagnetism, this type of structure has always been considered unsuitable, because of the desire to select rocks with a remanent magnetization that could be presumed to have suffered no alteration since the rock was formed. The well-known deductions about polar wandering, continental drift, rotation of continents, etc., are wholly dependent on whether this concept of fossil magnetism is valid. Its validity is generally assumed to hold good, even for tilted formations, so long as the rock has not been subjected to metamorphic or gross tectonic effects.

The Lewis thrust-sheet is one of many large sheets situated on the eastern side of the Rocky Mountains.

Advancing on a 200-mile front, the Lewis sheet appears to have been displaced by up to 40 miles or more[2], with the remarkable stratigraphic result that rocks of Pre-Cambrian age are now widely superimposed on Cretaceous sediments. Can it be assumed that such a large-scale dislocation would occur without disturbing the direction of remanent magnetization in the transported rocks ?

This question can scarcely be answered on general grounds while the mode of formation of thrust-sheets is itself in doubt. The earlier picture of thrust-sheets as rigid plates pushed from behind is now recognized as mechanically unsound. Gravity gliding has been suggested as a possible alternative[3], but this requires a co-extensive basal surface of highly regular dip, and suitably lubricated. There is also the problem of how the sheet came to be formed in the first place. Here it should be noted that a low-angle fault does not necessarily coincide with any particular stratigraphic horizon. The Lewis fault, for example, has cut across pre-existing folds[2].

Many thrust-sheets (the Lewis among them) have retained a coherence that is hard to reconcile with the concept of rock as a rigid, brittle substance. Nevin[4] remarks: "the fact that sheets of rock have been displaced a distance much greater than their thickness, without complete shattering, is astonishing". A high degree of coherence in the Lewis sheet is confirmed by the remanence observations of Norris and Black, who have collected 59 samples from three different localities and obtained remarkably consistent directions of magnetization.

It would not be difficult to imagine ice-sheets similar in form to these great sheets of rock. Indeed, the characteristics of thrust-sheets are very much what one might expect if the rocks involved could be regarded as having significant plastic properties. A variety of other geological phenomena likewise suggest that on the geological time-scale all near-surface rocks behave plastically[5]. On this hypothesis thrust-sheets are seen as flow-sheets, generated perhaps by extrusion from the base of young mountains which by this means will tend to subside under gravity towards a state of plastic equilibrium.

Since plastic flow involves shear distortion it might be expected to alter the direction of any vector embedded in the rock, including the remanence vector. The plastic subsidence of tall objects has been briefly described by Orowan[6]; but no detailed analysis is available. In a well-developed thrust-sheet, however, the predominant effects are likely to be those that characterize flow in sheet-like bodies, and this has been extensively studied by glaciologists. In the present context the important feature of such flow is that the speed of movement is greatest at the top of the sheet and diminishes steadily downwards. This has been verified by direct observations on glaciers and also accounted for theoretically[7]. If the remanence vector rotates accordingly, the direction in which the sheet has flowed can be ascertained from the change in the direction of magnetization.

This relationship has been invoked recently in an attempt to reinterpret observations of remanence in western Europe as evidence of continental expansion[8]. Adopting a minimum of assumptions, it is postulated that throughout geological history the geomagnetic field has always approximated to that of a geocentric dipole aligned with the present geographical axis, and that discrepancies indicated by rock magnetism are due to plastic flow in the rock since it became magnetized, rather than to continental drift, etc.

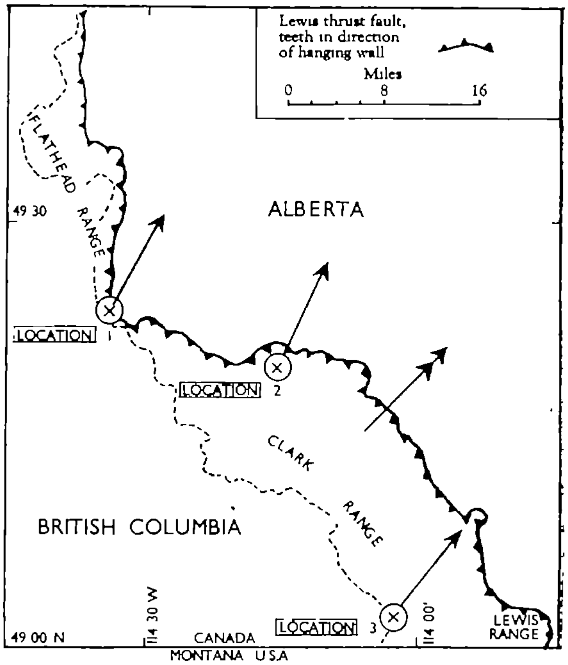

Fig. 1. Direction of movement of Lewis thrust-sheet, Rocky Mountains, Canada. Double arrow is based on geological evidence, single arrows on remanent magnetization. (Map details after Norris and Black (ref. 1).)

Thus the original inclination I_0 of the remanence vector is given by:

$$\tan I_0 = 2 \tan \lambda$$

where λ is the geographical latitude of the sampling locality. The azimuth θ in which the flow has occurred can be represented by:

$$\theta = \tan^{-1}\{\sin D/(\cos D - \tan I/\tan I_0)\}$$

where the remanence vector in its final position has inclination I and declination D. Since the direction of movement of the Lewis thrust-sheet is approximately known, whatever the mechanism might be, the above formula for θ provides some check on whether the plastic flow mechanism is plausible.

The Pre-Cambrian sandstone studied by Norris and Black was sampled at three sites near latitude 49° N. ($\lambda = 49°$), one of them situated near the middle of the Clark Range salient and one near each end. The number of samples and the mean values of declination and inclination measured for each site are listed in Table 1. The computed azimuths of flow (θ) are also given in Table 1, and are shown by means of arrows in Fig. 1, which has been prepared from the map of Norris and Black[1].

Table 1

Location	No. of samples	D	I	θ
1	27	218 5	39 5	29
2	24	213 0	49·0	26
3	8	230 0	38 0	38

All angles in degrees.

On geological evidence the direction of displacement of the Clark Range salient, according to Price[2], is north-eastwards. This direction (shown in Fig. 1 as a double arrow) is close to the directions of flow that have been inferred from the remanence data. It should be mentioned that the equation for θ has essentially only one meaningful solution[8]; there is no ambiguity here such as characterizes the determination of pole position in palæomagnetic studies.

It appears that the hypothesis that low-angle faulting is brought about by plastic flow in the overlying sheet[5], and that the direction of remanent magnetization is altered in the flow process, gains in plausibility from the example of the Lewis thrust-sheet. Further investigation may therefore be warranted along the following lines. Observations of magnetic remanence in other thrust-sheets for which the direction of movement is known would provide further opportunities to test the flow hypothesis. Secondly, the Lewis thrust-sheet might be examined for evidence of plastic deformation. Stress differences as small as 10^6 dynes/cm.^{2}, operating through geological time, may be capable of producing such deformation, the microscopic effects of which may thus be entirely different from those of the large stress differences usually contemplated in geology. The angle of "magnetic shear" indicated by the results of Norris and Black is about 55°; the angle of mechanical shear may be much smaller[8].

Norris and Black have interpreted their remanence observations in the standard palæomagnetic fashion. They mention that their calculated pole positions agree well with other results, as yet unpublished, for the late Pre-Cambrian of North America. In view of the entirely different interpretation suggested above, these further results will be awaited with interest.

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WE are interested in Dr. Evison's application of our basic palæomagnetic data[1] to the mechanics of emplacement of the Lewis thrust-plate in the south-eastern Cordillera of Canada. He questions whether the ancient remanence vectors could have been effectively preserved in the plate in view of the possibility that it may have undergone plastic flow.

It is our view that the rocks of the Lewis thrust-plate did not undergo significant plastic flow and that the remanence vectors maintained a common Pre-Cambrian pole position before, during and after the movement history of the plate.

That there has been deformation beyond the elastic limit within the plate is without question, but the geology of the plate offers little to support the contention that it reached its present position as a flow-sheet. As will be mentioned briefly, geological mapping confirms that displacement of the Lewis thrust-sheet was accomplished through relative motion on a shear surface or zone. Deformation within the sheet was limited almost exclusively to thrust faulting, folding and interbed slip.

The Lewis thrust is made up of a series of glide surfaces or zones parallel or sub-parallel to the layering with interconnecting shoulders or steeper portions where the thrust cuts through the stratigraphic succession either along or across the strike. It either selectively parallels specific zones[3] or, with local exception, cuts up section in the direction of relative displacement of the thrust plate. From surface and well data in the area studied it is known to parallel a zone a few hundred feet below the top of the Pre-Cambrian Waterton Formation for an area in excess of 500 square miles. Only locally does it truncate folds[3].

Except in the immediate vicinity of the thrust surface, movement within the Lewis thrust-plate consisted primarily of displacement on significantly few fault splays and of development of broad, open folds[3]. Of secondary importance was differential forward movement within the mass accomplished by interbed slip as well as by shear deformation in incompetent units.

The fact that this differential shearing motion was limited to bedding surfaces and to incompetent units is demonstrated by the remarkable preservation of even the most delicate primary structures, of both organic and sedimentary origin in the competent rocks making up the plate[4,5]. So obvious is this phenomenon that early geologists mistook these Pre-Cambrian rocks to be as young as Cambrian to Triassic[6].

The competent rocks from which our oriented samples were collected showed no signs of fracture cleavage or flow. Oolites and stromatolites from competent Pre-Cambrian rocks slightly lower in the plate do not appear to be deformed in such a manner as might be anticipated if shearing deformation were pervasive through the rock. We have observed moreover that pre-Laramide joints are statistically perpendicular to bedding, deviations being to one side or other, and showing no indication whatsoever that blocks outlined by joints have been deformed by simple shear.

It is unlikely therefore that the mechanism of transport of the Lewis thrust-plate could have been one of pervasive plastic flow as outlined by Dr. Evison. Macroscopically, movement was along well-defined fault surfaces. Microscopically, any plastic flow that may have occurred was so insignificant that the primary fabric of the rock was left unaltered except for local shear zones.

The logical deduction that the remanence vectors in these Pre-Cambrian rocks have not been significantly altered by plastic flow at the scale of the 1-in. cubes studied by us is confirmed moreover by a comparison of our pole positions from rocks in the plate with pole positions for undisturbed rocks of comparable age in North America[7].

It is therefore concluded with reasonable certainty that the orientation of the remanence vectors has not been significantly altered as a consequence of the tectonic history of the Lewis thrust-plate and that the plate was transported as a coherent but flexible mass and not as an extruded flow sheet.

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FIVE-DIMENSIONAL COSMOLOGY AND THE HYPOTHESIS OF NEGATIVELY CHARGED STARS

PROF. V. A. BAILEY[1] has shown that many astronomical phenomena can be explained by the hypothesis that a star, such as the Sun, carries a net negative charge. He suggests (hypothesis H_2) that such a charge could arise if the universe were five dimensional, and that the laws of conservation hold exactly, not in the four-dimensional continuum of space-time, but in the more fundamental five-dimensional continuum. Accordingly, an investigation was made into a statement of the conservation laws for the five-dimensional model used in the unified field theory of Kaluza and Klein[2,3].

The first fundamental form for the five-dimensional continuum is taken as:

$$d\sigma^2 = \gamma_{\mu\nu}\, d\xi^\mu\, d\xi^\nu. \qquad (\mu, \nu = 1, 2, 3, 4, 5)$$

A unit vector field A^μ is postulated, by means of which a four-dimensional Riemannian space:

$$ds^2 = g_{ab}\, dx^a\, dx^b. \qquad (a, b = 1, 2, 3, 4)$$

is constructed. A special system of co-ordinates, ξ^μ, is chosen for which $\xi^a = x^a$, and the $\gamma_{\mu\nu}$ are taken to be independent of ξ^5. This latter, the A-cylindrical condition, prevents difficulties with the unfamiliar ξ^5 co-ordinate in the physical equations. As yet, it will not be assumed that γ_{55} is constant. A skew-symmetrical tensor, f_{ab}, also is introduced by means of this material.

The geodesic equations in five dimensions:

$$\frac{\delta}{\delta\sigma}\left(\frac{d\xi^\mu}{d\sigma}\right) = 0$$

where δ denotes intrinsic differentiation, give the set of four equations:

$$\frac{\delta}{\delta s}\left(m_0 \frac{dx^a}{ds}\right) + \frac{e}{c^2} f^a{}_b \frac{dx^b}{ds} + \frac{1}{2} \frac{e^2}{m_0 c^4} g^{ab}\left(\frac{1}{\gamma_{55}}\right)_{,\,b} = 0 \qquad (1)$$

together with:

$$\frac{de}{ds} = 0 \qquad (2)$$

where m_0 and e are scalars in the four-dimensional space. The $,\,b$ denotes ordinary partial differentiation with respect to x^b.

If γ_{55} is constant, (1) takes exactly the form of the equations governing the motion of a charged particle in ordinary general relativity, and (2) expresses the conservation of e.

The natural five-dimensional generalization of the equations of motion and conservation for a continuous medium is:

$$J^{\mu\nu}{}_{;\,\nu} = 0$$

where $J^{\mu\nu}$ has the form:

$$\rho \frac{d\xi^\mu}{d\sigma} \frac{d\xi^\nu}{d\sigma} + \tilde{\omega}\gamma^{\mu\nu}$$

and ; denotes covariant differentiation in five dimensions. This leads to the four equations:

$$\left(r \frac{dx^a}{ds} \frac{dx^b}{ds} + p g^{ab}\right)_{:\,b} - \varepsilon_1 f^a{}_b \frac{dx^b}{ds} - g^{ab}\,(\sqrt{\gamma_{55}})_{,b} \left(\frac{\varepsilon_1{}^2}{r\gamma_{55}{}^{3/2}} + \frac{p}{\sqrt{\gamma_{55}}}\right) = 0 \qquad (3)$$

together with:

$$\left(\varepsilon_1 \frac{dx^b}{ds}\right)_{:\,b} = 0 \qquad (4)$$

where : denotes covariant differentiation in the four-dimensional space, and r, ε_1 and p are scalars in this space related to ρ and $\tilde{\omega}$. When γ_{55} is constant, (3) resembles the ordinary relativistic hydrodynamical equations governing the motion of a charged medium in an electromagnetic and a gravitational field. (4) corresponds to the equation of conservation of charge.

In (1) and (2), the 'charge' e is related to the 'mass' m_0 by:

$$e = m_0 c^2 \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds}$$

Corresponding to this, we should expect the 'charge density' ε to be related to the 'mass density' r_0 by:

$$\varepsilon = r_0 c^2 \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds} \qquad (5)$$

Now, in ordinary four-dimensional relativity, the energy-momentum density tensor in terms of the rest density r_0 and pressure p_0 is:

$$T^{ab} = -(r_0 c^2 + p_0) \frac{dx^a}{ds} \frac{dx^b}{ds} + p_0 g^{ab}$$

Referring to (3), it is seen that the scalar r introduced there is given in terms of density r_0 by:

$$r = -(r_0 c^2 + p_0)$$

while:

$$p = p_0$$

But the ε_1 in (3) and (4) is found to be given by:

$$\varepsilon_1 = -r \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds}$$

So, in terms of ordinary density, this is:

$$\varepsilon_1 = (r_0 c^2 + p_0) \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds}$$

Comparing this with (5):

$$\varepsilon_1/\varepsilon = 1 + \frac{p_0}{r_0 c^2}$$

ε_1 plays the part of charge density of the medium, regarded as continuous. (4) expresses the conservation of this charge. ε is the charge density of the medium, regarded as a distribution of discrete, unconnected particles, each having a constant charge.

These cannot both be the true charge. It is natural to assume that ε, the one depending on the discrete particles, is the true charge, because of the fundamental nature of such charged particles as the electron. The equation (4) then shows that this true charge is not conserved in a moving medium in which the pressure and density are variable. In terms of true charge density, (3) and (4) are, for the case where γ_{55} is constant:

$$\left\{-(r_0 c^2 + p_0) \frac{dx^a}{ds} \frac{dx^b}{ds} + p_0 g^{ab}\right\}_{:\,b} - \varepsilon\left(1 + \frac{p_0}{r_0 c^2}\right) f^a{}_b \frac{dx^b}{ds} = 0$$

$$\left\{\varepsilon\left(1 + \frac{p_0}{r_0 c^2}\right)\frac{dx^b}{ds}\right\}_{:\,b} = 0$$

where r_0 is the actual rest density, p_0 the pressure, and ε the charge density. From a macroscopic point of view, if a given portion of the material is transported from place to place, the charge it contains varies as $1\Big/\left(1 + \frac{p_0}{r_0 c^2}\right)$.

Thus, it appears that conservation in five dimensions implies that there is not conservation in the four-dimensional space, in general.

It might be expected that gravitational forces would tend to cause a settling of the heavier, positively charged, particles towards the centre of the Sun against the effects of the electric forces and thermal motions. Since it is likely that $1\Big/\left(1 + \frac{p_0}{r_0 c^2}\right)$ will decrease towards the region of greater pressure, this will cause a net negative charge on the Sun, as required by Bailey's hypothesis, and apparently confirmed by the interplanetary magnetic measurements recorded by the solar satellite *Pioneer V* (ref. 4).

As well as giving equations which closely resemble those of ordinary relativity, choosing γ_{55} constant is satisfactory also from a theoretical point of view, since the A-cylindrical condition can then be expressed simply in a manner covariant for all the corresponding systems. However, suppose now that γ_{55} is not constant, and consider the case of a particle momentarily at rest with respect to a geodesic Lorentz co-ordinate system. The time component of equation (1) is:

$$\frac{\mathrm{d}m_0}{\mathrm{d}t} = -\tfrac{1}{2}\frac{e^2}{m_0 c^4}\frac{\partial}{\partial t}\left(\frac{1}{\gamma_{55}}\right)$$

Thus it appears that the γ_{55} term can destroy the conservation of rest mass. The use of co-ordinate systems which are not special could introduce additional terms having the same effect. Bailey[5] has suggested that expanding models of the universe which depend on the continual creation of matter might be consistent with the postulate of conservation in the five-dimensional space. The above result indicates that this may be so, but whether the expanding universe of constant density described by Hoyle[6] can be obtained as a by-product of the five-dimensional theory depends on the discovery of suitable field equations.

Mathematical appendix. An outline of the derivation of the equations (1)–(4) is given here. The description of the theory follows that of Bergmann[3] to a large extent.

In the space of the five-dimensional co-ordinate system, ξ^μ, four parameters, $x^s \equiv x^s(\xi^\mu)$, are chosen. Greek suffixes range from 1 to 5, and Latin suffixes from 1 to 4. Let:

$$\gamma^{a}_{\alpha} = x^{a}{}_{,\alpha} \equiv \partial x^{a}/\partial \xi^{\alpha} \qquad \text{(i)}$$

Then the field A^α is defined by:

$$\begin{cases} \gamma_{\alpha} A^{\alpha} = 0 & \text{(ii)} \\ A^{\alpha} A_{\alpha} = 1 & \text{(iii)} \end{cases}$$

The reciprocal γ^{α}_{a} are defined by:

$$\begin{cases} \gamma^{a}_{\alpha}\gamma^{\alpha}_{b} = \delta^{a}_{b} & \text{(iv)} \\ \gamma^{\alpha}_{a} A_{\alpha} = 0 & \text{(v)} \end{cases}$$

The fields g_{ab} and φ_{ab} are then taken to be:

$$g_{ab} = \gamma^{\alpha}_{a}\gamma^{\beta}_{b}\gamma_{\alpha\beta} \qquad \text{(vi)}$$

$$\varphi_{ab} = \gamma^{\alpha}_{a}\gamma^{\beta}_{b} A_{\alpha\beta} \qquad \text{(vii)}$$

where:

$$A_{\alpha\beta} = A_{\alpha,\beta} - A_{\beta,\alpha} \qquad \text{(viii)}$$

The equations (ii)–(v) lead to:

$$\gamma^{\alpha}_{a}\gamma^{a}_{\beta} = \delta^{\alpha}_{\beta} - A^{\alpha} A_{\beta}$$

giving, with (vi):

$$\gamma_{\mu\nu} = g_{ab}\gamma^{a}_{\mu}\gamma^{b}_{\nu} + A_{\mu} A_{\nu} \qquad \text{(ix)}$$

and:

$$\gamma^{\mu\nu} = g^{ab}\gamma^{\mu}_{a}\gamma^{\nu}_{b} + A^{\mu} A^{\nu} \qquad \text{(x)}$$

where:

$$g^{as}g_{bs} = \delta^{a}_{b}$$

For the special system in which:

$$\xi^{s} = x^{s} \qquad \text{(xi)}$$

(i) gives:

$$\gamma^{a}_{\alpha} \equiv \begin{pmatrix} \gamma^{a}_{b} \\ \gamma^{a}_{5} \end{pmatrix} = \begin{pmatrix} \delta^{a}_{b} \\ 0 \end{pmatrix}$$

and so, from (ii) and (iii):

$$A^{\alpha} = (0,0,0,0,\ 1/\sqrt{\gamma_{55}})$$

Hence.

$$A_{\alpha} = \gamma_{5\alpha}/\sqrt{\gamma_{55}} \qquad \text{(xii)}$$

Writing this as:

$$A_{\alpha} = \sqrt{\gamma_{55}}\,(\varphi_{a},\ 1) \qquad \text{(xiii)}$$

(iv) and (v) are solved to give:

$$\gamma^{\alpha}_{a} \equiv \left(\gamma^{b}_{a},\ \gamma^{5}_{a}\right) = \left(\delta^{b}_{a},\ -\varphi_{a}\right)$$

Thus, from (ix):

$$\gamma_{\alpha\beta} = \begin{pmatrix} g_{ab} + \gamma_{55}\varphi_a\varphi_b, & \gamma_{55}\varphi_a \\ \gamma_{55}\varphi_b, & \gamma_{55} \end{pmatrix} \qquad \text{(xiv)}$$

and (x):

$$\gamma^{\alpha\beta} = \begin{pmatrix} g^{ab}, & -g^{as}\varphi_s \\ -g^{bs}\varphi_s, & \gamma_{55}{}^{-1} + g^{rs}\varphi_r\varphi_s \end{pmatrix}$$

The A-cylindrical condition:

$$\gamma_{\alpha\beta,5} = 0 \qquad \text{(xv)}$$

gives, from (xii):

$$A_{\alpha,5} = 0$$

from (xiii):

$$\varphi_{a,5} = 0$$

and from (xiv):

$$g_{ab,5} = 0$$

Since the field equations must be a generalization of those of ordinary relativity, the tensor $J^{\alpha\beta}$ must depend on $\gamma^{\alpha\beta}$, and so, even though the field equations might not be determined as yet, it is possible to say, from (xv), that:

$$J^{\alpha\beta}{}_{,5} = 0$$

From (vii) and (viii):

$$\varphi_{ab} = \sqrt{\gamma_{55}}(\varphi_{a,b} - \varphi_{b,a})$$

By (xi), the , a denotes partial differentiation with respect to x^a as well as ξ^a for those functions which, through the A-cylindrical condition, are independent of ξ^5. The array:

$$f_{ab} = \varphi_{ab}/\sqrt{\gamma_{55}}$$

therefore is derivable from a four-vector potential in the same way as the electromagnetic field tensor in ordinary relativity, and so satisfies part of Maxwell's equations.

Using the above formulæ, the components of the five-dimensional Christoffel symbols can be evaluated in terms of those in the four-dimensional space. It is found that:

$$\left\{ \genfrac{}{}{0pt}{}{s}{\alpha\beta} \right\}_{(5)} = \gamma^a_\alpha \gamma^b_\beta \left\{ \genfrac{}{}{0pt}{}{s}{ab} \right\}_{(4)} + \tfrac{1}{2} g^{sr} \gamma^\rho_r \left(A_\alpha A_{\rho\beta} + A_\beta A_{\rho\alpha}\right)$$

$$A_\mu \left\{ \genfrac{}{}{0pt}{}{\mu}{\alpha\beta} \right\}_{(5)} = \tfrac{1}{2}(A^\rho A_\alpha A_{\rho,\beta} + A^\rho A_\beta A_{\rho,\alpha} + A_{\alpha,\beta} + A_{\beta,\alpha})$$

$$\left\{ \genfrac{}{}{0pt}{}{\sigma}{\alpha\sigma} \right\}_{(5)} = \gamma^a_\alpha \left\{ \genfrac{}{}{0pt}{}{s}{as} \right\}_{(4)} + A^\sigma A_{\sigma,\alpha}$$

With these, operating on the geodesic equations.

$$\frac{\delta}{\delta\sigma}\left(\frac{d\xi^\mu}{d\sigma}\right) \equiv \frac{d^2\xi^\mu}{d\sigma^2} + \left\{ \genfrac{}{}{0pt}{}{\mu}{\alpha\beta} \right\}_{(5)} \frac{d\xi^\alpha}{d\sigma} \frac{d\xi^\beta}{d\sigma} = 0$$

with γ^a_μ gives (1), and operating with A_μ gives (2). In (1) and (2):

$$m_0 = \alpha \frac{ds}{d\sigma}, \alpha \text{ constant,}$$

and:

$$e = m_0 c^2 \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds}$$

Operating on the equations of conservation:

$$J^{\mu\nu}{}_{;\nu} \equiv \left(\rho \frac{d\xi^\mu}{d\sigma} \frac{d\xi^\nu}{d\sigma} + \tilde{\omega}\gamma^{\mu\nu} \right)_{;\nu} = 0$$

with γ^a_μ gives (3), and operating with A_μ gives (4). In (3) and (4):

$$r = \sqrt{\gamma_{55}}\, \rho \left(\frac{ds}{d\sigma}\right)^2$$

$$p = \sqrt{\gamma_{55}}\, \tilde{\omega}$$

$$\varepsilon_1 = - r \sqrt{\gamma_{55}}\, A_\mu \frac{d\xi^\mu}{ds}$$

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The suggestion that a five-dimensional model (U_5) of the Universe may be useful in cosmology[1] led quite naturally to the view that the laws of conservation of momentum, energy and electric charge are strictly true in such a universe U_5 and only approximately true in our space–time universe U_4.

This view led later to the conclusion that stars like the Sun carry large negative charges[2] and in this way provided a unified theory of several different astrophysical phenomena and also led to successful predictions concerning the interplanetary magnetic field measurements of the solar satellite *Pioneer V* [3].

These facts show that Dr. Taylor's quantitative discussion of five-dimensional cosmology is of distinct importance, for it not only confirms the view stated above but also is remarkable for its derivation of the empirically correct sign of the Sun's net charge[3].

It is to be hoped that in due course the correct field equations will be derived from the five-dimensional model. It is true that Kaluza has given an associated variational procedure[4] by means of which he derived Maxwell's electromagnetic field equations and Einstein's gravitational field equations; but this particular procedure probably yields only a first approximation for weak fields, for Einstein has pointed out that the existence of stable charged particles like electrons requires that the correct electromagnetic field equations be non-linear in the field variables. Guides to finding a correct procedure may perhaps exist in current non-linear field equations such as those due to Born, Infeld and Dirac[5].

It may be noted that Dr. Taylor's conclusion that the charge density ε varies inversely as the quantity $1 + p_0/r_0c^2$ leads also to the conclusion that the rate of apparent creation of net charge in interstellar space is small compared with the rate near and inside stars. This and the negative sign for the charge are conclusions which disagree with the hypotheses of Lyttleton and Bondi[6].

Lastly, it may be pointed out that additional direct observations of interplanetary magnetic fields, made by means of a pair of differently moving space vehicles in a relatively small region, would offer additional evidence on the hypothesis that the Sun carries a large negative charge and so perhaps offer evidence concerning the five-dimensional model of the Universe.

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GROWTH OF ALLOPREGNANE SINGLE CRYSTALS

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PART of our programme of determining the molecular structures of steroids by X-ray crystallographic methods is the growth of good single crystals. For our purposes a good single crystal is one having the following characteristics: (1) a unique lattice orientation; (2) absence of strain; (3) absence of impurities; (4) a reasonably small number of vacancies and dislocations; (5) an ideally imperfect nature; (6) a diameter within the 0·2–1·0 mm. range; (7) well-defined external faces.

Recently, we have examined the crystallization properties of a group of allopregnanes. Considering the usual factors affecting the growth of single crystals from solution (degree of supersaturation, saturation velocity, temperature of crystallization, nature of the solvent, presence of impurities; presence of seeds, and agitation of the solution during crystallization), we have empirically recognized certain relationships between allopregnane polycrystalline aggregates and the conditions of crystallization. These relationships are of proved utility in establishing procedures which have resulted in the growth of good single crystals of the allopregnanes.

Good allopregnane crystals usually crystallize from solution only within a narrow range of conditions. Since these ideal conditions are different for each solvent–solute system, finding these conditions for any given system is sometimes difficult, and is always based on empirical techniques. Our observations of more than 300 crystallizations and crystallization products suggest that some features of allopregnane crystalline precipitates are often related to determinable and controllable conditions present during the process of crystallization. These features, therefore, may indicate the paths to be followed to obtain ideal conditions of crystallization, and good single crystals. The purpose of this article is to present our empirical findings on this subject.

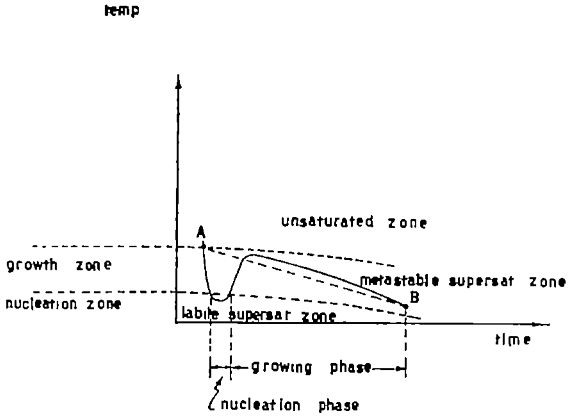

Fig. 1

In general, time–temperature plots of allopregnane saturated solutions prepared at elevated temperatures and cooled are divided into three zones (Fig. 1). These zones include an unsaturated zone, a metastable supersaturated or growth zone, and a labile supersaturated or nucleation zone. We have observed that if saturated allopregnane solutions are allowed to cool normally, that is, in a straight-line path either rapidly or slowly, many nuclei can form and polycrystalline aggregates (either in the form of spherulites or concretions) can result. Good single crystals have been obtained in many instances by altering the cooling path as indicated in Fig. 1. In this alteration, the cooling path is forced to dip briefly into the zone of nucleation, resulting in the production of a relatively small number of nuclei. The temperature is then raised to a point just below the upper boundary of the growth zone. Next, the solution is forced to cool along the indicated path, which usually yields good single crystals.

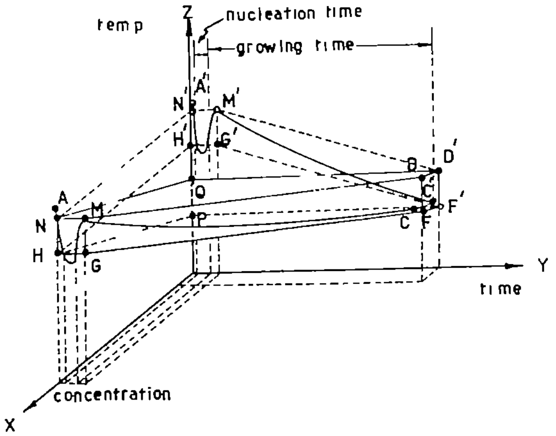

Fig. 2

Fig. 2 illustrates in more detail the process described above, namely, solute-phase crystallization across a large metastable supersaturated zone. Curve *AC* represents the general path which the solution must follow in order to yield single crystals as a product. Curve *A'C'* is the orthogonal projection of curve *AC* on the *yz*-plane (time and temperature). The surface *NMDFGH* is a cross-section of that portion of the metastable supersaturated zone within which the concentration, temperature, and time vary. The surface *N'M'D'F'G'H'* is the orthogonal projection on the *yz*-plane of the cross-section of the metastable supersaturated zone. Lengths *NN'* and *CC'* represent respectively initial and final concentrations of the solution. In three dimensions the metastable supersaturated zone is represented by a 'stratum' dipping in the direction of negative concentration. The surface *PQD'F'* is the cross-section of this zone by the *yz*-plane. In examining the temperature–concentration–time relationships of the cooling paths of any particular allopregnane, the upper and lower limits (lines *MD* amd *GF*, respectively) of the metastable supersaturated zone must be determined experimentally. By initially referring to a temperature time diagram (such as Fig. 1) and determining *M'D'* and *G'F'*, this operation can be facilitated.

When working with allopregnane solutions, which have been prepared at room temperature and allowed to evaporate to dryness, the following relationships appear to be valid:

(1) Concretions are due to a very high degree of supersaturation. Single crystals may be obtained by decreasing the amount of evaporation, decreasing the speed of evaporation, or supplying seeds (which allows crystal growth in the metastable zone).

(2) Allopregnane dendrites have two causes: (*a*) The time of crystallization is not long enough. Good crystals may be obtained by decreasing the speed of evaporation. (*b*) The temperature–concentration–time system has a large metastable supersaturated zone, which prohibits nucleation until the last stage of evaporation. The time remaining before dryness is not enough to yield crystallization. Single crystals may be produced by adding seeds during evaporation, or by inducing early nucleation by scratching, stirring, pouring, etc.

(3) We observed that whiskers result from the formation of a gel (also see Amelinckx[1]), hence single crystals may be formed by preventing the formation of a gel by stirring, adding traces of other solvents, and shortening the evaporation time. Whiskers

growing from single crystals indicate the presence of a 'poison' in the solution. A 'poison' is defined by Sears[2] as a small amount of soluble impurity in a solution which will appreciably alter the normal growth behaviour. This change may involve both the growth-rate of various crystal faces and the final crystal habit. The following techniques have been successfully used to obtain single crystals when impurities are present: (*a*) purification of the allopregnane or the solvent, or both; (*b*) beginning with more solution, halting evaporation as soon as the crystals reach the desired size, and pouring away the remaining solution.

(4) When only spherulites occur, crystallization takes place under conditions which we found impossible to control using the same solvent–solute system. Good crystals can sometimes be obtained by changing the solvent.

(5) When impurities reach a critical concentration in the last stage of evaporation, drusy surfaces result. Single crystals with non-drusy surfaces may be obtained by halting the process of crystallization before the last stage of evaporation is reached.

(6) Glassy-capped crystals result from the formation of a film during evaporation, and may be avoided by stirring or agitating the evaporating solution.

(7) Crystals with serrated edges generally occur when the crystal has emerged from the solution during evaporation. These may be avoided by starting with more solution and stopping the evaporation before dryness.

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ANNUAL RHYTHM OF WOOL GROWTH

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THE influence of climate on the annual rhythm of wool growth has puzzled physiologists for many years. We have discussed the earlier literature elsewhere[1], and since our review Hart[2] has given more details of his work on the effects of day-length. Recently, Morris[3] reported experiments from which he concluded that the seasonal cycle is induced primarily by seasonal changes of day-length because reversing the photoperiodic seasons reversed the rhythm. He was unable to detect an effect of temperature. In our article we report such an influence on the rhythm and data on its persistent nature.

We took two groups of 14 adult ewes, half of them Southdowns and half medium-wool Merinos, which had been maintained indoors for 6 months in the normal indoor temperatures and day-lengths of Prospect, New South Wales (lat. 34° S.), except that the lighting was artificial. One group, called in this article the controls, was exposed to normal indoor temperatures and to exaggerated photoperiodic seasons of normal phase representing a latitude of 60° S. The experimental group was subjected to a régime of reversed and somewhat exaggerated thermal seasons and a constant equatorial day-length beginning September 17, 1958. Wool was gathered every 28 days by clipping two tattooed patches, each 10 × 10 cm. in area, of which one was on the mid-side and the other low on the thigh. The remainder of the fleece was kept at a fairly constant length by shearing to 1 cm. for the Southdowns and 1·5 cm. for the Merinos every 56 days. Three Southdowns and four Merinos in each group had an insulating cover strapped over the mid-side patch, comparable in thickness with the rest of the fleece. None of the thigh patches was covered. The ration fed was nearly constant for each animal and allowed a small increase in weight. One of the Southdowns, in the control group, died during the experiment.

The annual rhythm of the Merinos was very small, but in general gave the same responses to treatment which are shown for the Southdowns in Fig. 1. The rhythm of wool production of the controls was similar to that found by earlier workers[4]. In this group the seasonal variations for the three types of patch (covered mid-side, uncovered mid-side and thigh) were similar.

The covered mid-side patches of the experimental group showed a rhythm which was nearly the same as in the mid-side and thigh patches of the control group, except that the maxima were slightly delayed by about four weeks (Fig. 1*B*). Thus, when the day-length was constant, and the seasonal variations of temperature were reversed, a nearly normal annual rhythm persisted.

The maxima for the uncovered mid-side and the thigh patches were considerably delayed by about 12 weeks (Fig. 1*C*, *D*). The displacement of the minima for the thigh patches was about 5 months, but was variable for the uncovered mid-side, suggesting some topographical difference in the reactions to the experimental climate.

The phase shift in the rhythm of the thigh and uncovered mid-side patches of the experimental group indicates that temperature can influence the expression of the rhythm when there is little or no wool cover. This effect must be local, because a nearly normal rhythm of wool growth occurred beneath the insulating covers, while simultaneously a modified rhythm was shown on the uncovered thigh patches of the same animals. The phase shift appears to be an effect of cold because, as already mentioned, it was only shown to a marked degree on the uncovered patches.

In March and April covered patches in the experimental group produced much less wool than in December and January, despite the higher ambient temperature and, therefore, local temperature of the patch; it seems as though the wool follicles entered a refractory phase following a period of high wool production. In March and April the uncovered

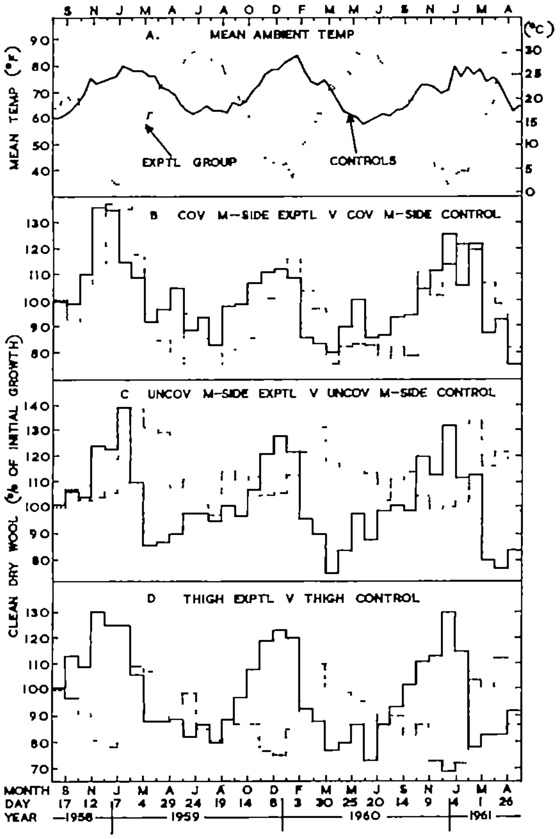

Fig. 1. Clean dry wool gathered from tattooed patches on the Southdown ewes, related to mean ambient temperature. Wool growth is expressed as a percentage of that over the 28 days preceding September 17, 1958, when the treatments started. When their mean ambient temperature exceeded 70° F., the experimental group had a diurnal rhythm of temperature with a minimum of 70° F., there was no diurnal rhythm over the rest of the year The controls had the natural indoor diurnal rhythm ... experimental group; —— control group, *M-side*, mid-side; *cov.* covored.

patches produced much more wool than the covered patches, although the local temperature must have been lower for these than for the covered patches. It would seem that the period of high production, delayed by the cold of December and January, could occur when temperature began to rise. Thus, temperature shifted the phase of the rhythm rather than causing an additive effect. It seemed to act like the various modifiers, which can influence the time of breeding of those species of birds the reproductive cycle of which is primarily controlled by day-length[5]. The fact that it was possible for sheep with covered mid-side patches to exhibit simultaneously two different rhythms raises the intriguing possibility that the rhythmicity may be to some degree controlled within the wool follicles themselves.

Our observation that the annual rhythm can be persistent provides a possible explanation of the long photoperiodic treatment that Morris found necessary to reverse the rhythm. Morris detected no effect of temperature on uncovered patches when he reversed the thermal seasons while maintaining exaggerated photoperiodic seasons of normal phase, but his winter temperatures may have been higher than ours (only mean monthly maxima were recorded). We, indeed, observed little effect of temperature in the warmer microclimate of the covered mid-side patches. It would be expected that wool growth in the fleeced sheep would be similar to that on our covered patches and, therefore, not easily influenced by ambient temperature.

We conclude that the annual rhythm of wool growth can in certain circumstances be modified by temperature. The modified rhythm can occur on one part of the skin, while on other parts (covered mid-side patch of the experimental group) an almost normal rhythm continues; the latter, like many circadian rhythms, can persist for a considerable time in the absence of the climatic stimuli which are presumed to have initiated it, and normally to maintain it. Our observations do not imply that the rhythm, once established, is independent of climate in the fleeced sheep. For the work of Hart and Morris and also our own and Wildman's unpublished observations indicate that the rhythm can be modified by photoperiodic treatment.

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ANATOMY OF HUMAN SKIN TUMOUR CAPILLARIES

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THE vigorous vascular proliferation at the edge of rapidly growing tumours[1], and the rich vasculature in the body of many transplantable experimental neoplasms[2] and spontaneous human tumours[3], have been clearly demonstrated. However, that the presence of large numbers of capillaries in tumour tissue does not necessarily imply an abundant supply of nutrient materials is indicated by tissue oxygen tension measurements[4] which suggest that this rich vascular bed is functionally inefficient. Recent advances in extra-corporeal perfusion techniques, the observation of potentiation of ionizing radiation effects by increased tissue oxygen tension, the suggestion that chronic hypoxia[5] and stromal changes[6] may play an important part in carcinogenesis, and that specific changes develop in cutaneous capillaries during experimental cancer induction[7,8], emphasize the importance to the oncologist of knowing more about the structure and function of tumour blood vessels.

The capillaries supplying the skin and cutaneous appendages can be easily seen in frozen sections prepared by the Gomori technique for demonstrating alkaline phosphatase. This method is superior to the

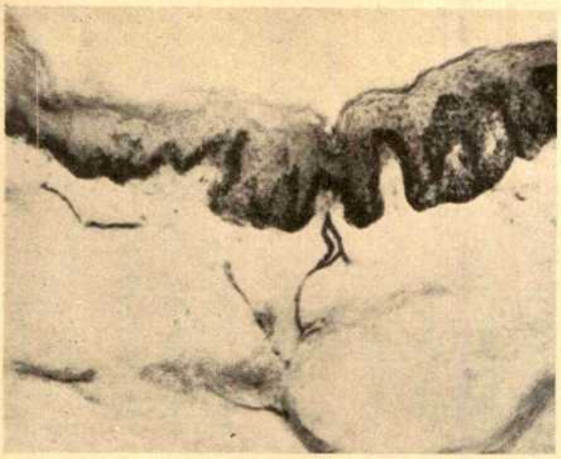

Fig. 1

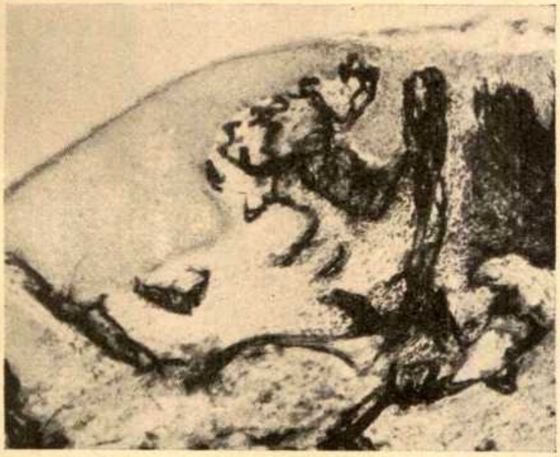

Fig. 2

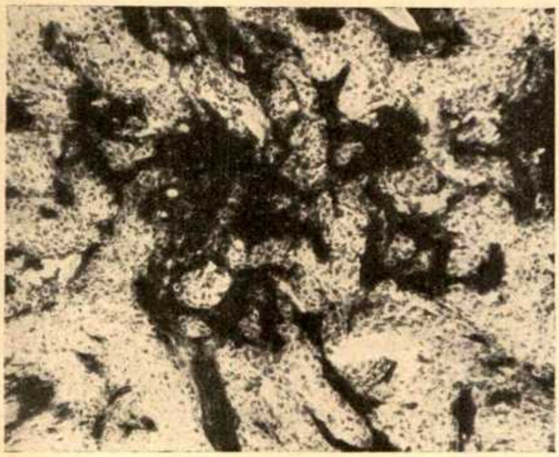

Fig. 3

conventional benzidine and silver impregnation techniques, showing even collapsed or blocked vessels of capillary calibre. Alkaline phosphatase activity appears to be intense only in the end-arterial or capillary endothelium, while the walls of venules and larger arterioles show either little or no enzyme activity.

A variety of cutaneous human pre-malignant and malignant lesions was examined, and a number of benign neoplasms and inflammatory skin diseases was investigated for control purposes. The tissue was fixed for several hours in 10 per cent cold neutral buffered formalin, and frozen sections were cut between 30 and 50μ in thickness, utilizing a Sartorius model 22 freezing microtome. The sections were prepared by the Gomori method for alkaline phosphatase[9].

The normal anatomy of cutaneous capillaries has been described by Ellis *et al.*[10], Kopf[11] and Winkleman[12]. In sections stained for alkaline phosphatase, capillary loops entering the dermal papillæ are seen, with a richer capillary plexus surrounding the cutaneous appendages (Fig. 1).

In sections of malignant skin neoplasms, the capillary structure is strikingly altered. There is a significant increase in the number of visible capillaries which are elongated, dilated and tortuous, showing many anastomoses and cross-linkages. The altered vessels surround and extend beneath the proliferating islands and strands of tumour cells of basal cell carcinomas (Fig. 2). In fifteen such specimens no capillaries were found within the neoplastic epithelium even when the tumour strand-width exceeded 300–350μ. In contrast to this picture, there are large numbers of short, widely dilated anastomosing

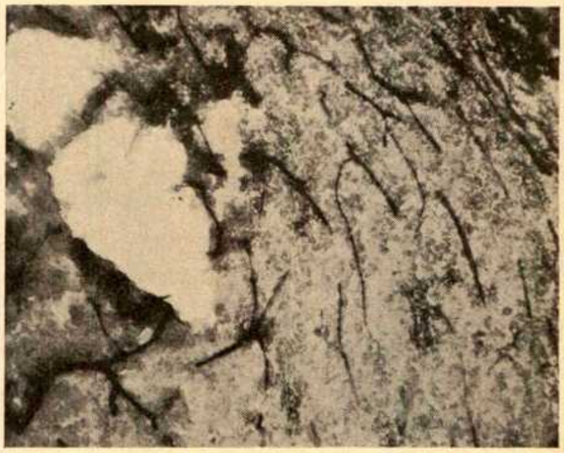

Fig. 4

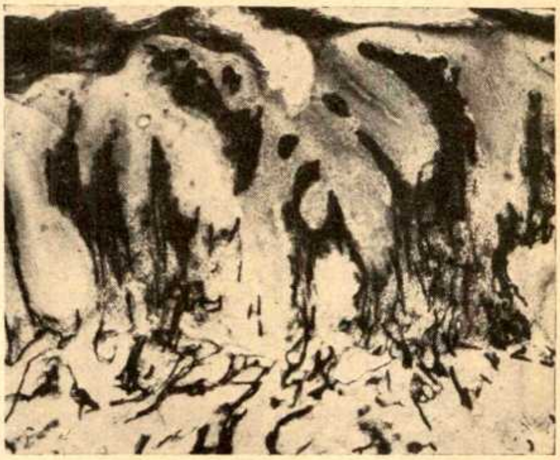

Fig. 5

capillaries in the main body of squamous cell carcinomas (Fig. 3), while in malignant melanoma the capillaries appeared to be extremely long, often arranged in parallel rows, and sometimes branching and anastomosing (Fig. 4). In both the squamous cell epithelioma and malignant melanoma, the vessels seem to be intermingled with the mass of tumour cells, unaccompanied by any significant amount of connective tissue. In the tissue surrounding both tumours there are increased numbers of altered capillaries similar to those seen around the basal cell carcinomas.

No such vessel changes have been noted in warts, nevi, xanthomas, cutaneous lymphomas or inflammatory skin diseases.

Most striking capillary abnormalities underlie the cutaneous pre-malignant lesions of senile keratosis and Bowen's disease. The changes in the vessels consist of endothelial proliferation, elongation, tortuosity, ectasia and shunting. These appear sharply at the point where epithelial hyperplasia begins, and are most evident under areas of major epithelial atypism or anaplasia (Fig. 5).

The changes in vessels found in senile keratosis and Bowen's disease correspond well to the observations of Dreifuss[8] and Kreyberg[7] in mouse skin painted with tar, and the tumour vessels seen in squamous cell carcinomas and melanomas are very similar to the description of the vascular anatomy of Algire's chamber transplanted mouse tumours[2].

Investigations are proceeding to determine whether these striking vascular changes preceded, accompany or follow experimental tumour induction, and whether similar abnormalities occur in neoplasms or other human organ systems.

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HEMICHOLINIUM No. 3 AND MAMMALIAN NEUROMUSCULAR TRANSMISSION

By DR. W. W. HOFMANN, DR. G. A. FEIGEN and G. H. GENTHER

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RECENT reports suggest that at least one of the defects responsible for the weakness of myasthenia gravis is at the level of the motor nerve endings[1,2]. Desmedt has expressed the view that a failure of transmitter synthesis or release by motor nerve terminals could account for the clinical and electromyographic features of the disease[1,3]. As partial evidence he cites experiments with cats where hemicholinium compound number 3 (HC3), an agent capable of inhibiting the synthesis of acetylcholine in autonomic ganglia and in minced brain[4,5], produced a block in neuromuscular transmission. Responses to cholinesterase inhibitors were not mentioned, but the findings were interpreted as showing that HC3 could impair the synthesis or release of transmitter at motor nerve twigs. Others have referred to these experiments as evidence that the neuromuscular block after HC3 may, indeed, stem from prejunctional effects[2,6,7]; but more recent results indicate that the compound can also act directly on the end-plate[7,8], at least in certain preparations.

Dahlback *et al.* examined single end-plates directly in biopsy specimens from myasthenic patients and reported abnormally low frequencies of miniature end-plate potentials[2]. Since the miniature end-plate potential frequency could not be raised with potassium ion but the end-plate sensitivity to acetylcholine remained normal, these findings lend strong support to the concept of a prejunctional defect in the disease. In light of these observations we wished to determine whether HC3 could produce a myasthenic type of neuromuscular block in human subjects and whether, indeed, such a result might be explained on the basis of prejunctional failure.

Four volunteer subjects were examined. All were adult males with no known disease of the neuromuscular apparatus. Myasthenic subjects were not used because their known sensitivity to a number of different classes of compounds might make interpretation of results difficult. Percutaneous, supramaximal ulnar nerve stimulation was carried out in trains of 4–5 pulses at train-intervals of from 1 to 2 sec. while hemicholinium base was being given through the ipsilateral brachial artery in doses of 0·1–0·3 mgm./kilo arm weight. The evoked compound action potentials and isometric ergograph were photographed with moving film from a dual beam oscilloscope before and after drug administration. The frequency of shocks in each train was purposely made high (20–50/sec.) to maximize any effects which might depend on interference with rapid turn-over of transmitter stores. As shown in Fig. 1, HC3 was quite inert in the doses used during the time of acute exposure. The stimulations were not carried on for long periods after the arterial injections, since it appeared unlikely that any delayed type of block would develop while blood inflow was normal. It should be pointed out that, while the total doses used were slightly lower than the LD_{50} for guinea pigs[7], and considerably less than those producing neuromuscular block in the cat[1], the arterial route of administration might be expected to offset these differences to some degree. In all subjects the frequency of shocks was gradually increased after

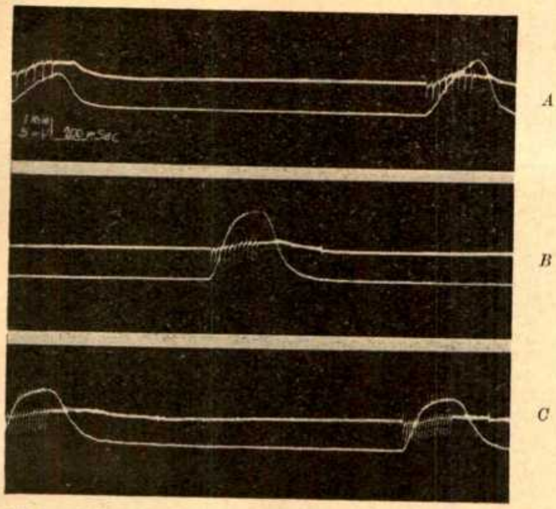


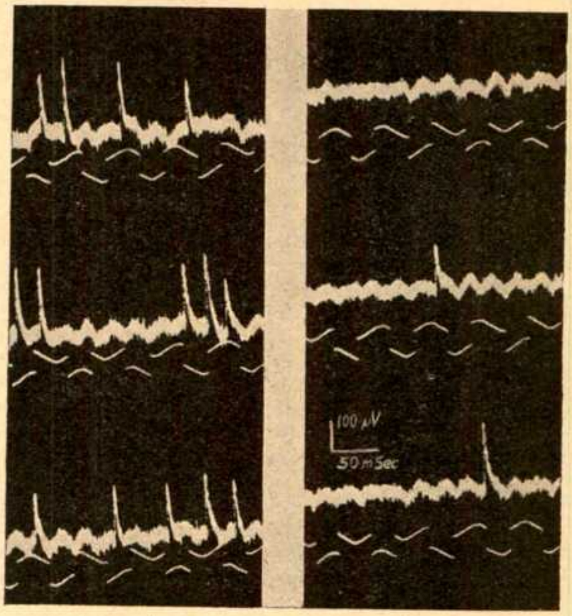
Fig. 1. Action potentials (upper trace) and tetanus tension (lower trace) in human subject in response to supramaximal ulnar nerve stimulation. All records after HC3 injection to show normal responses at high stimulation rates. *A*, 1 min. after arterial injection of 5 ml. HC3 concentration 0·1 mgm./ml., in physiological saline; *B* and *C* consecutive tracings 12 min. after *A*

injection of HC3 to see at what point the muscles would fail to 'follow'. As shown in Fig. 1*C* there was no block at very high rates[9] of stimulation.

More detailed investigations of HC3 were then made on animal preparations. The miniature end-plate potentials of rat and mouse intercostal muscles were recorded by inserting microelectrodes filled with potassium chloride into end-plate regions with a micromanipulator and microscopic visual control. The bathing solution was that described by Liley[10]. The bath temperature was kept at 37 ± 0·1° C. Miniature end-plate potentials were photographed from an oscilloscope fed by a cathode follower circuit output. Electrode resistances varied between 3 and 12 megohms. The HC3 was first given intraperitoneally to some animals prior to study in doses roughly approximating those used by others[7]. In other experiments, the agent was added to the tissue preparation before or during recording from the end-plates of individual cells in doses near those producing neuromuscular block[11]. In the first instance, two mice were killed with doses of 0·15 and 0·3 ml. of HC3, 10^{-4} mgm./ml., respectively. Each animal showed intense irritability and excitement prior to respiratory paralysis, and, even while respiration was so depressed as to cause visible cyanosis, these mice finally had such severe convulsions as to throw them clear of their cages. Miniature end-plate potentials of normal size and frequency[12] were recorded from the muscles of these animals for hours after death (Fig. 2*A*). In other experiments muscle preparations from untreated animals were soaked for 2–4 hr. in a bath of nutrient solution containing HC3, 10^{-4}–10^{-3} mgm./ml. Perfectly normal miniature end-plate potentials were also recorded from these cells. When, however, 1–2 ml. HC3, concentration 10^{-4}–10^{-1} mgm./ml., was added to the bath while microelectrodes were in place there was a rather consistent temporary lowering of miniature end-plate potential frequency without marked change in voltage (Fig. 2*B*). Any drop in amplitude could be restored promptly with neostigmine. Conversely, HC3 given after neostigmine dramatically reduced the high-voltage signals due to cholinesterase inhibition (Fig. 3). In cells from treated animals as well as from tissue soaked in HC3 the frequency of miniature end-plate potentials could be greatly increased by addition of guanidine. Subsequent application of HC3 lowered the amplitude of the miniature end-plate potentials to some degree and definitely decreased the frequency.

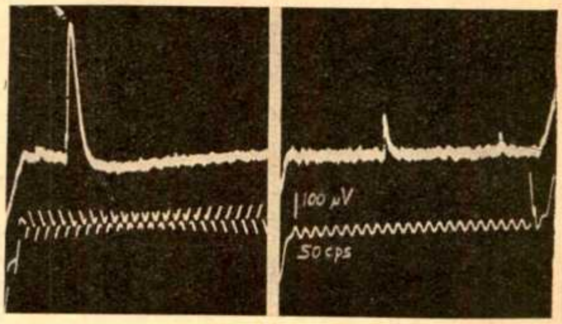
To discover whether the serum of myasthenic patients might contain any substance with blocking properties similar to those of HC3, serum from two patients was added to the mouse muscle preparations. Pooled serum from both patients was dialysed against the nutrient solution and a portion heated to 57° C. for 15 min. Neither sample produced the slightest change in the miniature end-plate potentials, even in an amount sufficient to replace nearly all the solution in the 15-ml. bath.

We infer from these investigations that HC3 may act at both limbs of the mammalian neuromuscular junction when applied *in vitro*. In fact, one may observe in the records from the same cell effects on quantal output frequency and, later, on amplitude, while the 'active state' is extended with other agents. On the other hand, parenteral administration of the compound seems without effect on motor nerve end-



A *B*

Fig. 2. Mouse intercostal muscle. *A*, control miniature end-plate potentials; *B*, 80 sec. after 0·6 mgm. HC3 added to bath. Average amplitude after HC3 only slightly reduced



A *B*

Fig. 3. Mouse intercostal muscle. *A*, 'giant' potential 2 min. after 1 ml. neostigmine 0·5 mgm./ml. added to bath; *B*, 70 sec. after *A* and 20 sec. after 2 ml. HC3, 0·1 mgm./ml. added to bath. *B*, shows highest voltage obtained after HC3

ings, *in vivo*, even when it kills the animal. We thus agree with Schueler, who feels that neuromuscular block is not the only cause of HC3 toxicity[11]. The alterations in frequency observed when HC3 is added to the bath occur too soon to be ascribed to depletion or contamination of transmitter stores and are more probably the result of some interference with the quantal release mechanism. Since miniature end-plate potentials of substantial size (equal to pre-treatment signals) could be found when the frequency dropped, it seems unlikely that the change in frequency is due to artefact[7]. The post-junctional effects, as illustrated in suppression of the neostigmine response, develop without gross changes in the resting membrane potential and may, perhaps, be viewed as competitive inhibition. The results reported earlier in the cat might well be related to such post-junctional effects[1]. In any event, neither pre- nor post-junctional blocking properties have been observed in human subjects given HC3, and it would seem unlikely that myasthenic weakness is directly the result of the presence of this particular type of compound unless it be in greater concentration than we have used. The preliminary tests with myasthenic serum do not support the latter possibility.

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ANTIGEN POLYMORPHISM OF A LOW-DENSITY BETA-LIPOPROTEIN. ALLOTYPY IN HUMAN SERUM

By Dr. B. S. BLUMBERG, Dr. S. DRAY and Dr. J. C. ROBINSON

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It is now known that there are several families of inherited serum protein variants which constitute polymorphic systems[1]. These include the haptoglobins, transferrins, γ-globulin (Gm.) groups, group-specific components and others (for review see ref. 2). In such systems variations in the protein structures are inherited in simple Mendelian fashion, and two or more forms of the phenotype are common in the population. Individuals receiving multiple transfusions of blood of compatible red cell type would probably receive some transfusions containing serum proteins of a different phenotype from their own. Although there is no evidence that the known differences in the polymorphic serum protein systems are sufficient to lead to the development of antibodies, it was considered possible that some multiply transfused individuals would develop antibodies against phenotypes of the known polymorphic systems or against others not yet detected. Such a reaction has been examined in rabbits. Oudin[3], Dray and Young[4], and Dubiski *et al.*[5] have shown that serum proteins from individual rabbits are antigenic in other rabbits. Isoprecipitins have been found to proteins with α, β and γ mobility in agar gel[4]. As few as three or four intravenous injections of serum can lead to the production of precipitating antibodies (Dray, S., unpublished observations). This phenomenon of individual differences in antigenic specificity of proteins within a species was called 'allotypy' by Oudin[3].

Allison and Blumberg[6], using the Ouchterlony technique, studied the sera of patients who had received multiple transfusions, in an effort to detect the presence of antibodies. A precipitating antibody was found in the serum of a patient (Mr. C. de B.) with a refractory anæmia of unknown ætiology, who had received approximately fifty transfusions during the course of three years. The antibody was a 7*S* γ-globulin and it reacted with some but not all human sera to form a well-defined precipitin line. The protein with which the antibody reacted travelled as an α-globulin protein (probably of high molecular weight) in agar-gel. Family and twin studies[6] demonstrated that the presence or absence of the reacting α-globulin is under genetic control. Individuals with a single or double dose of the dominant gene designated Ag^A (phenotype $Ag(a+)$; genotypes Ag^A/Ag; Ag^A/Ag^A) reacted; those who were homozygous for the alternate allele *Ag* did not react (phenotype $Ag(a\text{-})$; genotype Ag/Ag). The polymorphism appears to be independent of the haptoglobins, transferrins, group-specific components, γ-globulin types, and of the rheumatoid factor. It appeared, therefore, that the antibody present in the blood of patient C. de B. defined a previously unknown human serum polymorphism which was designated the isoprecipitin Ag(a) system. However, the Ag(a+) protein had not been further identified.

In the present article evidence is presented that the Ag(a+) protein is a low-density β-lipoprotein. (Lipoproteins are classified as low or high density on the basis of their flotation or sedimentation in an ultracentrifuge. The low-density lipoprotein migrates as a β-globulin on paper and an α-globulin on agar-gel electrophoresis; it is usually referred to as the low-density β-lipoprotein[7].)

The gel-diffusion precipitation technique (Ouchterlony procedure) was carried out in 5 cm. Petri dishes containing 8 ml. of 0·9 per cent Oxoid 'Ionagar' (w/v) in 0·07 sodium phosphate buffer, *p*H 7·0. The method used is described elsewhere[6]. Immunoelectrophoresis was done by the micromethod of Grabar and Williams[8] and Scheidegger[9], using standard lantern slide plates coated with agar made up in barbitone buffer, 0·01 *M*, *p*H 8·4. Staining was done by a method modified from that described by Uriel and Grabar[10]. Sudan

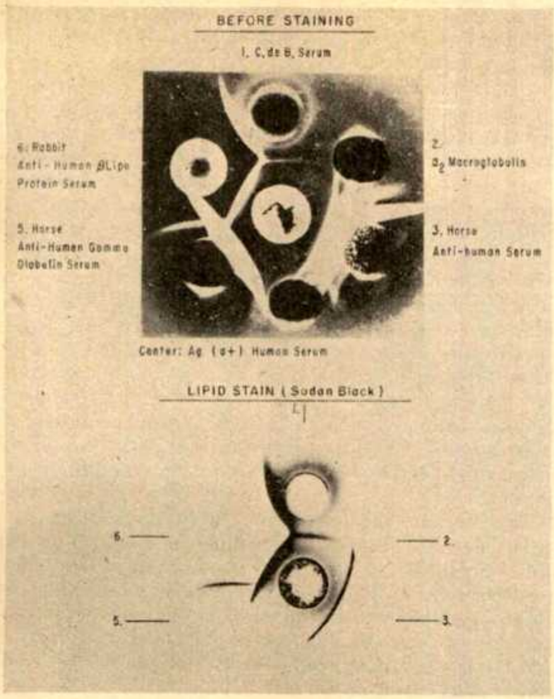
BEFORE STAINING
1. C. de B. Serum
6. Rabbit Anti-Human β Lipo Protein Serum
2. α2 Macroglobulin
5. Horse Anti-Human Gamma Globulin Serum
3. Horse Anti-human Serum
Center: Ag (a+) Human Serum
LIPID STAIN (Sudan Black)
6
2
5
3

Fig. 1. Ouchterlony experiment designed to show that the precipitin line formed between the serum of Mr. C. de B. (well 1) and an Ag(a+) individual (centre well) stains with the fat stain sudan black. Top, photograph of Ouchterlony plate before staining. Bottom, same after staining

black *B* (National Aniline) was used for the lipoprotein and azocarmine (National Aniline) for the protein stain. Single-dimension starch-gel electrophoresis was by the method of Smithies[11] using the *tris*-(hydroxymethyl)-aminomethane discontinuous buffer system of Poulik[12] and filter paper insertion. Two-dimensional starch-gel electrophoresis was by the method of Poulik and Smithies[13], in which the first dimension was on paper using an acetate-barbital buffer (3·74 gm. sodium acetate and 11·04 gm. sodium barbital in 1 litre of distilled water) and the second dimension was in gel using the *tris* buffer. Agar-gel electrophoresis was performed on standard lantern slides using the same conditions as for immunoelectrophoresis. The isolation of $7S$ γ-globulin was by the method of Levy and Sober[14]. The low- and high-density lipoproteins were separated from each other by ultracentrifugation in a high-density salt medium using whole serum and the method of Havel *et al.*[15]. Two fractions were separated, one of a density lower than 1·063 (low-density lipoprotein) and the second greater than 1·063 (high-density).

Fig. 1 is a photograph of an Ouchterlony experiment before and after staining with sudan black. It was designed to show that the precipitin line formed between the serum of Mr. C. de B. (well 1) and an Ag(a+) individual (centre well) stains with sudan black. A positive control was obtained by reacting rabbit anti-β-lipoprotein (well 6) with the serum of Mr. C. de B. and the Ag(a+) individual; the two precipitin lines coalesce and both also coalesce with the precipitin line formed between the serum of Mr. C. de B. and the Ag(a+) individual (first and centre well). It appears that the rabbit anti-β-lipoprotein and the serum of Mr. C. de B. precipitate the same protein. The precipitin line between horse anti-human serum (well 3) and the Ag(a+) serum (centre well) served as another positive control. Two precipitin systems were used for negative controls: (1) between horse anti-human serum (well 3) and isolated α-macroglobulin (which contains no lipoprotein) (well 2); and (2) between horse anti-γ-globulin (well 5) and the Ag(a+) serum (centre well). Neither of these lines stained with sudan black.

Fig. 2 is a photograph of an Ouchterlony plate designed to compare the reactions of low- and high-density lipoproteins prepared from the serum of Ag(a+) and Ag(a−) individuals. Mr. C. de B.'s serum reacts only with the whole serum and the low-density β-lipoprotein of the Ag(a+) serum but not with the high-density lipoprotein. As expected, Mr. C. de B.'s serum does not react with the Ag(a−) whole serum or the fractions thereof. The sera of 8 other Ag(a+) and 8 other Ag(a−) individuals were tested in the same way and the same results as mentioned here were obtained.

Fig. 3 is a photograph of the results of a starch-gel electrophoresis experiment which compares whole serum and the low-density β-lipoprotein fraction from the serum of the same individual. The lipoprotein was approximately three times its concentration in whole serum. It migrates a single well-defined band (broken arrow).

Fig. 4 is a photograph of an immunoelectrophoresis experiment designed to show the reaction between the concentrated $7S$ γ-globulin (isolated from the serum of Mr. C. de B.) with whole serum, and with high- and low-density lipoprotein. As seen in Fig. 2, the whole serum and the low-density lipoprotein from an Ag(a+) individual reacted to give precipitin lines, but the high-density lipoprotein did not. (As in the

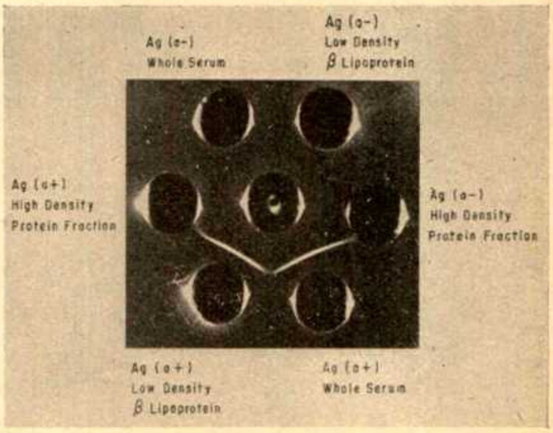
Ag (a−) Whole Serum
Ag (a−) Low Density β Lipoprotein
Ag (a+) High Density Protein Fraction
Ag (a−) High Density Protein Fraction
Ag (a+) Low Density β Lipoprotein
Ag (a+) Whole Serum

Fig. 2. Photograph of Ouchterlony experiment designed to show that the C. de B. serum (centre well) reacts only with the low-density β-lipoprotein fraction of the serum of an Ag(a+) individual. Photograph of unstained plate

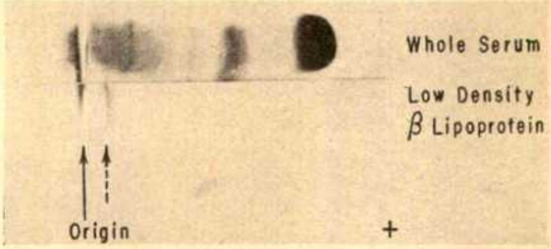

Fig. 3. Photograph of a starch-gel electrophoresis experiment in which the low-density β-lipoprotein fraction is compared with the whole serum from which it was isolated. The longer arrow shows the position of the origin, and the broken arrow, the position of the single lipoprotein band. Amido black stain

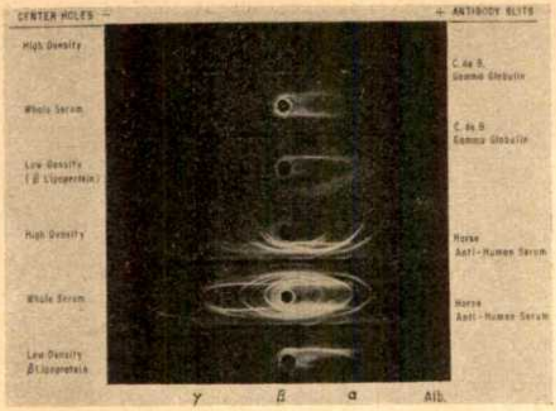

Fig. 4. Immunoelectrophoresis experiment designed to show the reaction between the γ-globulin isolated from the serum of Mr. C. de B. and the serum of an Ag(a+) individual and the high-density and low-density fractions separated from the serum. The reactions between horse anti-human serum and the same antigens are shown for comparison. The approximate position of the major protein bands are shown. Photograph of unstained plate

Ouchterlony plates, the precipitin lines stained intensely with sudan black (not shown).) Horse anti-human serum also reacted with the whole serum and high- and low-density lipoproteins. By comparison with the horse anti-human serum, it is seen that the low density β-lipoprotein migrates in the agar gel with the electrophoretic mobility of an α-globulin as previously reported[6].

The experiments summarized here have shown that the low- but not the high-density ultracentrifuge fraction of the serum of an Ag(a+) individual precipitates in agar gel with the 7*S* β-globulin antibody of Mr. C. de B. (Fig. 2). The precipitin line stains with the lipid stain, sudan black B; it coalesces with a precipitin line found in rabbit anti-human β-lipoprotein and human serum (Fig. 1). The antigen has the mobility of an α-globulin on agar gel (Fig. 4) and migrates as a single band between the origin and the slow α-two globulin on starch-gel electrophoresis (Fig. 3). These experiments identify the antigen as the low-density β-lipoprotein.

The whole serum, low-density and high-density fractions of 45 Ag(a+) and 35 Ag(a−) subjects were analysed for total cholesterol, total phospholipid and the total cholesterol/phospholipid ratio. (We are indebted to Dr. D. S. Fredrickson for supplying these data.) There were no significant differences in these broad lipid classes between the Ag(a+) and Ag(a−) individuals. It would be of interest if chemical differences in the β-lipoproteins of Ag(a+) and Ag(a−) individuals could be found.

In an experiment designed for other purposes, two chimpanzees were immunized with a single injection of whole human serum of an Ag(a−) individual plus complete Freund's adjuvant. Serum was obtained four weeks later. Both the animals developed precipitating antibody to human low-density β-lipoprotein as evidenced by a single band in agar gel. The chimpanzee antibody reacted with 225 normal American human sera tested and did not distinguish between Ag(a+) and Ag(a−) individuals. When the chimpanzee sera were used instead of rabbit anti-β-lipoprotein serum, coalescence was seen between the three precipitin lines similar to that observed among the centre, first and sixth wells of Fig. 1. Further immunization experiments with lower primates are in progress. We hope to obtain antibody which will distinguish individual differences in human β-lipoproteins.

This article, in conjunction with the genetic data presented elsewhere[6,16], establishes that a genetic polymorphism exists in the human low-density β-lipoprotein. The factors which maintain this polymorphism are unknown, although they may be related to diseases in which the lipoproteins are involved, such as some kinds of arteriosclerosis, diabetes and coronary heart disease. It would be of interest to determine if antigenic polymorphisms of β-lipoproteins (allotypy) occur in species other than man; for example, in rabbits, where allotypy for the α- and β-globulin has already been reported.

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VISION IN INSECTS IN TERMS OF THE ELECTRICAL ACTIVITIES OF THE DESCENDING NERVE FIBRES

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SINCE Frisch's work[1], good colour discrimination in a honey-bee has been demonstrated by behavioural and electrophysiological studies. The behaviour of an insect to light stimuli must be activated by the descending fibres from the brain to the thoracic ganglia. Examining the response patterns and the spectral sensitivity curves of the descending fibres from the ocelli and compound eyes to the thoracic ganglia is a simple way to understand the ability of the colour discrimination in an insect rather than studying the receptor mechanism. With this view, we tried to record the impulse discharges of the descending fibres from the eyes with such a purpose. The material used was a species (male) of grasshopper, *Gampsocleis buergeri* (Orthoptera).

In order to learn what information is sent out over the brain, impulse discharges were recorded of descending fibres at the connective immediately

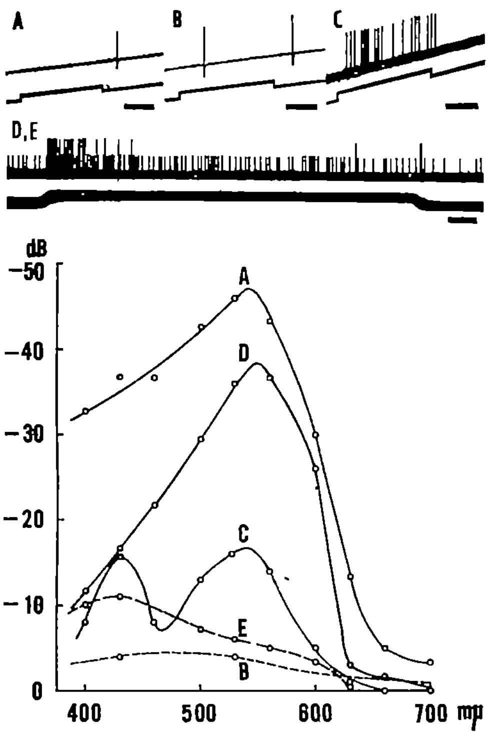

Fig. 1. Top, responses (upper beam) to white light (lower beam) of five descending fibres from the ocellus (*A*) and the compound eye (*B*, *C*, *D* and *E*). The horizontal line in each photograph shows 0·1 sec in *A*, *B* and *C* and 1 sec. in *D*, *E*. Bottom, spectral sensitivity curves of five descending fibres. Curves *A–E* correspond to the responses (*A–E*) shown in the top figure

before the thoracic ganglia connecting with the motor nerves to legs and wings, that is, the sub-œsophageal-prothoracic connective. Ten monochromatic lights with equal energy were produced from neutral density and interference filters and a tungsten filament lamp. The intensity of light was attenuated by neutral density filters. A micro-electrode filled with 3 *M* potassium chloride was inserted into the connective after destroying the prothoracic ganglion. The ocelli or compound eyes were cauterized with a platinum wire heated electrically in order to decide from which eye impulses descended.

A descending fibre from the lateral ocellus run in the contralateral nerve cord, because the response to the illumination on the lateral ocellus was recorded from the cord opposite the stimulated eye. The fibre spontaneously discharged impulses in the dark at the rate of 40–80/min. Spontaneous discharges of this fibre vanished when the lateral ocellus was cauterized. When the lateral ocellus was placed in the light, spontaneous discharges were completely inhibited. A few impulses appeared in a group after the cessation of a light stimulus. Thereafter, spontaneous discharges were suppressed for several hundred msec. With a long illumination period off discharge was enhanced and the latency became short. The after-effect of a stimulus remained for a long time, and it took several seconds after off response in order to return to the original rate of spontaneous discharges. When the lateral ocellus was regularly exposed to light stimuli at the rate of 1 flash/3 sec., long after-effects summed up one another, and no spontaneous discharges, but off responses, were observed (Fig. 1*A*, top). Off response was evoked even by a very short flash lasting 4 msec. Flash-light delivered on the median ocellus evoked off response on the descending fibre from the lateral one. Spontaneous discharges of this fibre in the dark were almost uninfluenced by cauterizing the median ocellus. The number of impulses in off response was less by about one-third after cauterizing the median ocellus than that in normal conditions. Therefore, the neural activity of the median ocellus will be similar to that of the lateral one and will be divided into a pair of descending fibres from the lateral ones. The spectral sensitivity curve shown by off response was on the wave-length of less than 400–700 mμ, the most sensitive spectrum being about 540 mμ (Fig. 1*A*, bottom). The curve did not change even if it was measured after cauterizing either the lateral or median ocellus and the threshold of the response to a light stimulus was always lower than those of the descending fibres from the compound eye.

Descending fibres from the compound eye were classified into four types. Each of them was characterized by the spectral sensitivity curve and also the discharge pattern. One of them showed on–off response. This neuron discharged only two spikes at the onset and cessation of a light stimulus when its intensity was strong. The off response was unstable compared with the on response and disappeared at first by decreasing the light intensity. The neuron had a very high threshold in spite of the fact that the neuron was activated by the light of 400–700 mμ and was more sensitive to the light of 430–530 mμ (Fig. 1*B*, top and bottom). The second type of the neuron was characterized by the spectral sensitivity curve, which extended from less than 400 to 630 mμ and had two peaks at about 430 and 540 mμ. The neuron discharged many impulses by a flash light-stimulation (Fig. 1*C*, top and bottom), but it adapted quickly to a long illumination. The discharge pattern of the third type of the neuron to the short flash light was mainly the on type. When the compound eye was illuminated for a long time, the discharge-rate of the neuron decreased gradually to zero and a few impulses were found on cessation of the stimulus. The neuron was characterized by the spectral sensitivity curve with a peak at 540 mμ (Fig. 1*D*, top and bottom, large spikes). The fourth type of the neuron showed the strong response to the light stimuli with short wave-length. The most effective spectrum seemed to be found at 420 mμ. This neuron often produced many spontaneous discharges and showed on response. When a light stimulus was applied on the compound eye, the discharge-rate of the neuron increased at first and thereafter decreased slowly to the original level of spontaneous discharges (Fig. 1*E*, top and bottom, small spikes). The results obtained with microelectrodes did not reveal how many descending fibres there were, if they showed similar discharge patterns and spectral sensitivity curves. The external recording from the whole connective was made by hooking it up in the air by a fine silver wire electrode. In favourable cases, the responses of four descending fibres from the compound eyes as described here were recognized by their different spike heights. More than four descending fibres were not found. By cauterizing the contralateral compound eye, almost all their impulses disappeared and a few impulses remained there. They seemed to be those of the second and the third type of neuron. Therefore, descending impulses from the compound eye were mainly transmitted to the contralateral fibres.

Quite recently, it was revealed that on and on–off responses of neurons were recorded at the optic lobe of an insect, but no off response[2]. The off response is, however, known to be evoked by light stimulation on ocelli[3]. It is an interesting fact that three types of responses similar to those in vertebrate eyes are elicited by light stimulation on two kinds of the eyes in the insect. The descending fibre from the ocelli showed pure off response to a light stimulation, while those from the compound eyes were characterized by on and on–off responses though off in on–off response was very faint as compared with on. We cannot interpret the meaning of the difference in those response patterns. It may, however, be suggested that three kinds of the response patterns (on, on–off, and off responses) are not concerned with the colour discrimination, but the pattern discrimination, because the response patterns of the axons of all primary sensory cells are on type in *Limulus*[4] and insects[5], but the other types of the responses are recorded from the region beyond synapses[2,6].

The ocellus seems to correspond to the rods in a vertebrate eye, because its descending fibre has the lowest threshold, while the compound eye with several kinds of descending fibres seems to correspond to the cones and have a more complex function than the ocelli. Though the response pattern results from intensity, pattern, and colour discriminations by the complex neural network in the optic lobe and the brain, each of the descending fibres from the ocelli and compound eyes is characterized by a different kind of a response pattern and a different spectral sensitivity curve. Therefore, it may be supposed that five descending fibres from the eyes evoke different kinds of neural activities and the insect is able to discriminate colours to some extent. The spectral sensitivity curves are limited to the red colour; but they seem to extend toward the ultraviolet, and are thus different from those of vertebrate eyes. If the thresholds of the descending neurons for ultra-violet are measured, a more conclusive answer to colour discrimination may be obtained. Phototaxis in insects is well interpreted by the fact that almost all impulses from two kinds of the eyes are transmitted to the contralateral descending fibres and activate the contralateral motor organs, the wings and the legs.

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EFFECTS OF HETEROGENEOUS POPULATIONS ON RADIATION SURVIVAL CURVES

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MANY sigmoid or multi-hit curves representing the survival of cells exposed to radiation have appeared in the literature. Examples are those of Puck[1] and Elkind and Sutton[2], showing curves of the type described by the equation:

$$S = 1 - (1 - e^{-D/D_0})^n \qquad (1)$$

where S is the fraction of the population surviving a radiation dose, D, and $n \geqslant 1$. The value of n, called the extrapolation number[3], is determined by extrapolating the straight-line portion of the plot of log survival versus dose to $D = 0$. The mean lethal dose, D_0, is the increment of dose on this straight line portion corresponding to an incremental reduction of survival to 37 per cent (that is, $1/e$) of an initial value. In practice, the accuracy in graphically estimating values of n and D_0 from a plot of equation (1) depends on the portion of the curve used for making the extrapolation and the magnitude of n. If the extrapolation of the slope of the curve is made at survival values between 0·1 and 0·01, the value for D_0 will be in error less than + 4 per cent for $n < 100$, and the value for n will be in error about: − 8 per cent for $n = 2$, − 13 per cent for $n = 10$, and − 25 per cent for $n = 100$. The accuracy improves at lower survival values. The value of n is sometimes interpreted as the number of targets which must be inactivated in the cell in order to produce lethality, and the value of D_0 is sometimes interpreted as the dose required to inactivate one target. Implicit in these interpretations, however, is the assumption that the population of cells being irradiated is a homogeneous group with regard to radiosensitivity.

Atwood and Norman[4] considered the effect of heterogeneity on the shape of the survival curves for the situation in which a population consisted of several groups, each of which has the same D but$_0$ a different value for n. The resultant survival curve has the same D_0, but the value for n is an arithmetical average of the individual n's. In such a case, the difference between the survival of a homogeneous group and a heterogeneous population could be detected only if a plot of $-\log\,(1 - S)$ versus D could be accurately determined.

Examples of heterogeneities in radiation sensitivity have been reported for yeast cells[5], *Escherichia coli*[6], and animal cells[7-9]. For *E. coli* and animal cells, the radiation sensitivity depends on the stage of the cell in its mitotic cycle. For example, animal cells in the process of synthesizing deoxyribonucleic acid (DNA) are about twice as radiosensitive as those in the period preceding DNA synthesis. Therefore, if information is to be deduced from shape of survival curve, it is important to consider further the effect of such heterogeneities.

In contrast to Atwood and Norman's analysis, in which the population is divided into groups having different values of n, our analysis is made for a population which is divided into groups with different values of D_0. Theoretical survival curves are calculated for a population of cells divided into several groups, A, B, . . ., i . . ., which have the same extrapolation number, n, but which have different values for D_0. The general equation for the survival curve is:

$$S = 1 - C_A(1 - e^{-D/D_A})^n - C_B(1 - e^{-D/D_B})^n \ldots - C_i(1 - e^{-D/D_i})^n \ldots \quad (2)$$

where C_A, C_B, . . . C_i . . ., are the fractions of cells in each group, and the values of D_0 for each group are D_A, D_B, . . . D_i . . ., respectively. The first term binomial expansion of this equation is :

$$S = C_A n e^{-D/D_A} + C_B n e^{-D/D_B} \ldots + C_i n e^{-D/D_i} \ldots \quad (3)$$

When the fractional survival of each group is less than 0·1, equation (3) differs from the complete expression, equation (2), by only a small amount, that is, 0 for $n = 1$, less than 4 per cent for n up to 6, and less than 11 per cent for n up to 100. For $D_A > D_B$, . . . D_i . . . and for doses sufficiently large where all but the first term becomes negligible:

$$S = C_A n e^{-D/D_A} \quad (4)$$

The extrapolation number for this curve is $C_A n$, rather than n, and the value for D_0 is equal to D_A, that is, the mean lethal dose for the most resistant group.

In Fig. 1, the survival curves as calculated from equation (1) are shown for a population divided into two groups with $n = 2$, and with $D_0 = 100$ for one group (curve B), and $D_0 = 200$ for the other group (curve A). The composite curve (C), as calculated from equation (2), for a population divided equally into the two groups is also shown. Curve (C) shows a D_0 of 200 corresponding to the D_0 of the resistant group and an apparent extrapolation number, as determined by the shape of the curve below the first decade, equal to 1·0, that is, half the value of n for the individual groups. However, in the first decade a slight inflexion is noted. The greatest difference between the survival curve (C) for the heterogeneous population and one for a homogeneous population with $n = 1$ and $D_0 = 200$ (not shown) is 18 per cent; this occurs at a dose value of 100, that is, $\frac{1}{2}D_0$ for the resistant group. Therefore, in order to distinguish experimentally the survival curve obtained for a heterogeneous population consisting of two such groups with $n = 2$ from an exponential survival curve expected for a homogeneous 'single-hit' population, a detailed experimental study of the first decade survival would be needed.

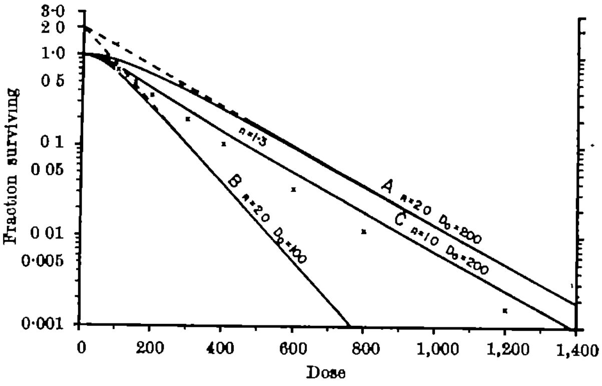

Fig. 1. Theoretical survival curve (C) for a heterogeneous population of cells divided equally into two homogeneous groups, A and B, with $n=2$ and values for D_0 as indicated. The straight line portions are extrapolated to $D=0$ to obtain n. Curve (C) differs from a purely exponential response (that is, $n=1$) by a maximum of 18 per cent. The value of 1·3 for n would be obtained in curve (C) if the extrapolation were made from the first decade of the curve. The points, X, are for a population consisting of 0·3 of the cells with the radiosensitivity of group A and 0·7 with the radiosensitivity of group B

Survival points (the X's) are also shown in Fig. 1 for the population of cells divided into two unequal groups by a ratio of 3 : 7, the larger and more sensitive group having a D_0 of 100 and the smaller and more resistant group having a D_0 of 200. In this case, the extrapolation number is reduced to 0·6 and in the first decade the survival curve is concave upward.

The curves in Fig. 2 are calculated for two equal groups on the same assumptions as those in Fig. 1 except that $n = 10$. Again, the composite curve (C) for the heterogeneous population has an apparent extrapolation number half the value of n for the individual groups and a mean lethal dose identical with that of the resistant group. Survival points are also shown for a homogeneous population with an extrapolation number of 5 and a D_0 of 200, that is, values corresponding to the apparent values resulting from the heterogeneous population. The greatest difference (8·6 per cent) between the survival curves for the homogeneous and heterogeneous populations occurs at a dose of about 400, that is, $2D_0$ for the resistant population. Again, the small difference in survival curve shape between a homogeneous and a heterogeneous population could be resolved only if precise experimental observations were made in the first decade of the survival curve.

The part of the survival curve which should be investigated carefully in order to detect heterogeneities depends on the fraction of the population composed of the resistant group, the mean lethal dose of the resistant group compared with that of the other groups, and the extrapolation number. By using equation (2), it is possible to determine the survival level below which only one group in the population provides a major contribution to the survivors. The same information can be obtained from equation (3), the approximation to the survival curve. If the

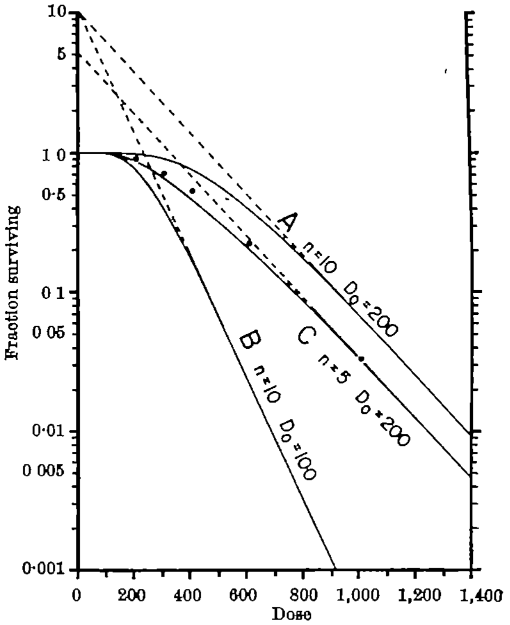

Fig. 2. Theoretical survival curve (C) for a heterogeneous population of two equal groups, A and B, as illustrated in Fig. 1, but with $n=10$. Curve (C) has an apparent n equal to 5. The dots shown indicate the response of a homogeneous population with $n=5$ and $D_0=200$ (equation (1)) and differ from (C) by a maximum of 9 per cent

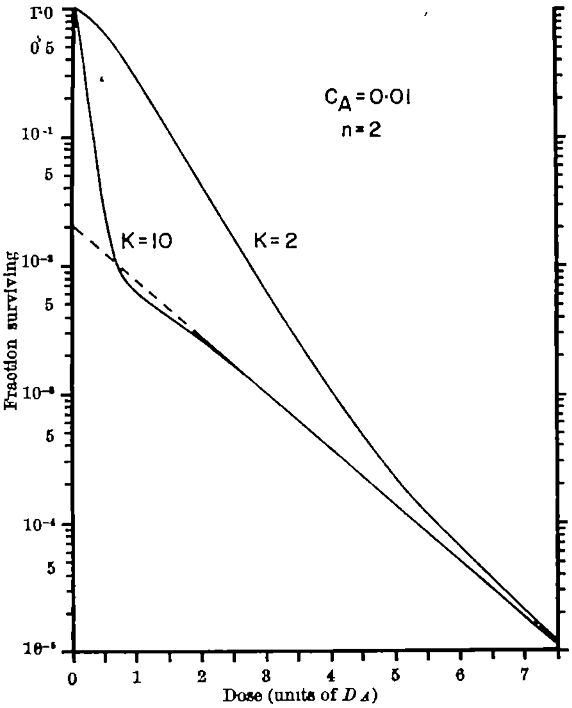

Fig. 3. Theoretical survival curves for populations of cells in which 0·99 of the cells are in a radiosensitive group and 0·01 ($C_A = 0{\cdot}01$) of the cells are in a radioresistant group. For both groups $n = 2$, but the mean lethal dose, D_A, of the radioresistant group is K times the mean lethal dose of the radiosensitive group

population is divided into two groups, a resistant fraction A and a sensitive fraction B, with $D_A = KD_B$ where ($K \geqslant 2$), it can be determined at what dose value the sensitive fraction becomes negligible by setting the second term in equation (3) equal to 0·1 of the first term. The dose when this occurs will be:

$$D = \frac{D_A}{K - 1} \ln \frac{10(1 - C_A)}{C_A} \qquad (5)$$

The fractional survival for this dose can be determined by substituting this value of D into equation (2). This approximate method for estimating D leads to an error in S, which is greatest when n is large; however, when $1 \leqslant n \leqslant 10$ the value of S will be accurate to within a factor of 2. In extreme cases with n and K approaching 100, the estimate of S, using the value of D from equation (5), approaches a factor of 10 higher than an exact solution using equation (2). This is still sufficient to define the decade of the fractional survival during which a resistant group dominates the remaining population.

When at least one-half of the cells are in the resistant group ($C_A \geqslant 0{\cdot}5$) with $1 \leqslant n \leqslant 100$ and where K varies from 2 to 100, the number of cells in the sensitive group will become negligible during the first decade of the survival curve. Therefore, a heterogeneity produced by a sensitive group, consisting of less than one-half of the population, would lead to a survival curve that would differ in the first decade only from a typical survival curve for a homogeneous population.

Conversely, when the resistant cells compose only a small fraction of the population, the survival curve approaches that of the resistant cells at survival values less than 0·1. For example, when only 0·01 of the cells are in the resistant group ($C_A = 0{\cdot}01$) with $1 \leqslant n \leqslant 100$ where K varies from 10 to 100, the number of sensitive cells will become negligible when the survival decreases to about 0·01 ($S \cong C_A$), as shown in Fig. 3. However, for $K < 10$, the sensitive cells will drop out at lower survival values depending on n. For example, for $K = 2$, the relevant survival values are 10^{-5} for $n = 2$ as shown in Fig. 3, 10^{-4} for $n = 10$, and 10^{-3} for $n = 100$. Therefore, in order to detect resistant cells which compose less than one-tenth of the population, it is necessary to investigate the survival curve below the first decade.

Based on the previous discussion, it is apparent that if a population of cells is heterogeneous with regard to radiosensitivity, the observed survival curve may appear to have an extrapolation number and a mean lethal dose quite different from those applicable to each group of the population. When the survival curves are used for interpretations of the mechanism of radiation action, these considerations are important.

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INTERACTION OF THE OXYGEN EFFECT AND RADIOSENSITIZATION PRODUCED BY BASE ANALOGUES INCORPORATED INTO DEOXYRIBONUCLEIC ACID

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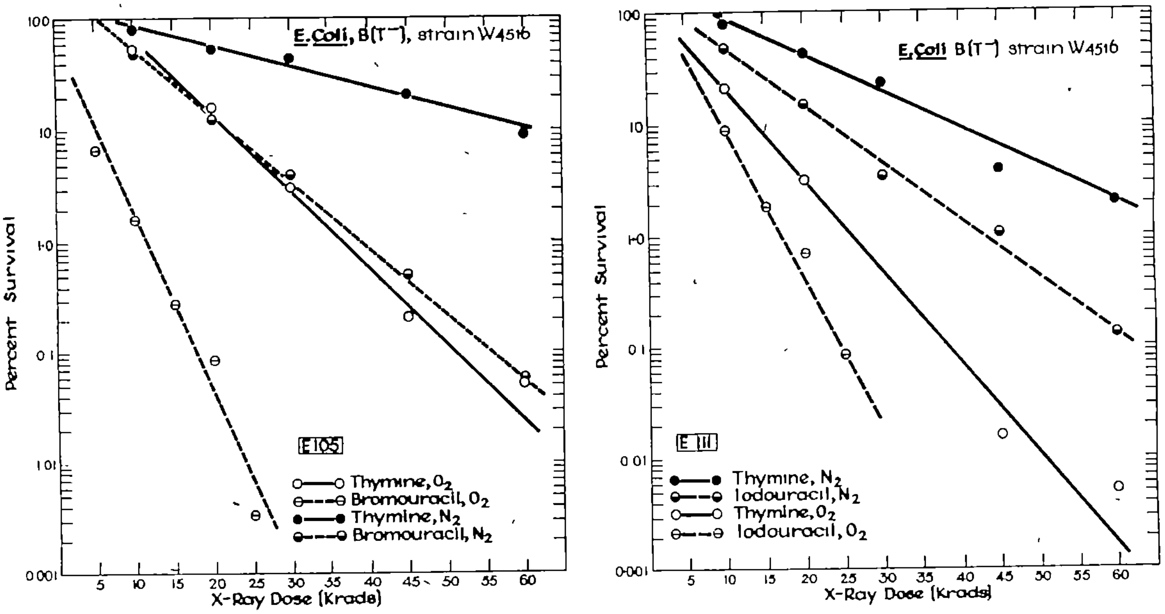
When bacterial or mammalian cells are grown under appropriate conditions in the presence of certain pyrimidine or purine analogues, they incorporate into their newly synthesized deoxyribonucleic acid (DNA) varying amounts of these analogues instead of the corresponding natural bases[1–6]. Cells the DNA of which contains appreciable amounts of such analogues are abnormally sensitive to both ultra-violet and ionizing radiations[7–10]. The increase in X-ray sensitivity is most conveniently expressed by the ratio of slopes of the dose–log survival curves for analogue versus natural-base-grown cells. Slope ratios of approximately 2·0 have been obtained with the halogenated pyrimidines, 5-bromo- and 5-iodouracil acid (BU, IU) and their deoxyribosides (BUDR, IUDR)[8–10] as well as with the purine anal-



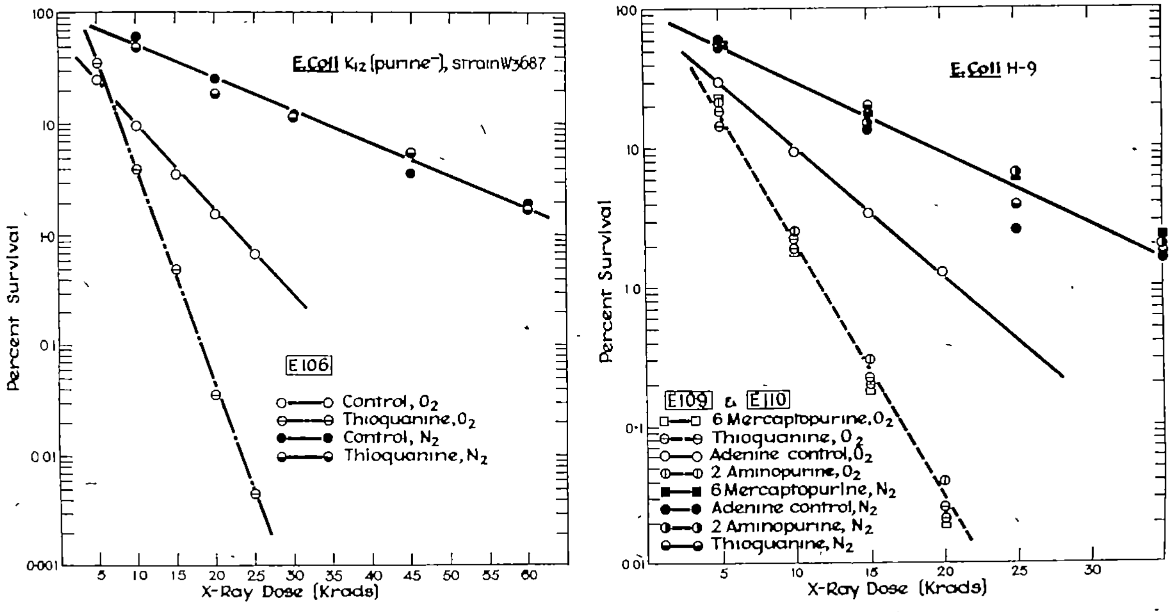
(a) (b)

Fig. 1

ogues 6-thioguanine (TG)[9], 6-mercaptopurine (6-MP), 2-aminopurine (2-AP), 8-azaguanine, 6-methylmercaptopurine and 2-mercapto-6-aminopurine[11].

It has long been known that oxygen sensitizes virtually all biological systems, and many chemical reactions as well, to inactivation or alteration by ionizing radiations[12]. This enhanced response has been termed the 'oxygen effect'. This article reports a series of experiments undertaken to study the interaction between the oxygen effect and the radiosensitization produced by the base analogues.

Several mutants of *Escherichia coli* were used. These included a thymine-deficient mutant of strain *B* (*W*4516) and a purine-deficient mutant of strain *K*12 (*W*3687), provided by Dr. Esther Lederberg of the Department of Genetics, Stanford University, and nitrosoguanidine-induced[13] purine-deficient mutants of strain *H* (*H*-9, *H*-515) and strain *S* (*Sng* 1–14), provided by Dr. Joseph Greenberg of the Palo Alto Medical Research Foundation. Pre-irradiation culture conditions were as previously described[9,10]. X-irradiation was carried out at 250 kV., 15 m.amp., 17·8 cm. distance, no added filter, average dose-rate 1,190 rads/min. The X-ray beam was directed vertically upwards into four buffer suspensions of bacteria (two analogue-grown, two control) contained in 30-ml. flat-bottomed glass vials fixed to the centre of a 'Plexiglass' plate, which was held in the cone-holder of the X-ray tube housing. Each vial was provided with a green rubber stopper through which a short (outlet) and a long (inlet) capillary glass tube passed; the latter extended almost



(a) (b)

Fig. 2

Table 1. Slope Ratios of Dose–Log Survival Curves of *E. coli* after X-irradiation in Nitrogen or in Oxygen

Analogue	Analogue (O_2) / Control (O_2)	Analogue (N_2) / Control (N_2)	Control (O_2) / Control (N_2)	Analogue (O_2) / Analogue (N_2)
BU	2·07	3·08	3·33	2·36
IU	1·60	1·53	2·54	2·67
TG	2·02	0·9–1·1	1·85–2·36	4·0
6-MP	2·02	0·9–1·1	1·85–2·36	4·0
2-AP	1·93	0·9–1·1	1·85–2·36	3·8

to the bottom of the vial and was bent at its tip so that the emerging fine bubbles of gas would impart a swirling, mixing motion to the bacterial suspension. Oxygen (medical grade) was bubbled through two vials (one analogue, one control), nitrogen through the other two. The nitrogen (pre-purified grade, Mathison Co.), which was stated to have a maximum of 20 p.p.m. of oxygen, was passed through a heated 4·5 × 80 cm. glass tube packed with copper wire to remove the last traces of oxygen. Both gases were humidified by passage through water, and flow-rates were adjusted to approximately 0·5–1·0 l./min. The humidifying flasks and tubing were equilibrated for 30 min. and the bacterial suspensions for 20 min. prior to irradiation. Immediately prior to irradiation and at each desired X-ray dose-level, an aliquot of each bacterial suspension was aspirated without interruption of gas flow, after which irradiation of the remaining organisms was resumed. The bacterial samples were appropriately diluted, plated on yeast extract agar (purine-deficient mutants) or on nutrient agar fortified with 200 μgm./ml. of thymine (thymine-deficient mutants), and incubated at 37° C. overnight. Survival curves were plotted on semilog paper from the viable colony count at each dose-level (average of 4–8 plates per point) as a fraction of the viable count of the corresponding unirradiated sample. Results are summarized in Table 1, and representative dose–log survival curves are presented in Figs. 1 and 2, respectively. The two halogenated pyrimidine analogues tested, BU and IU, exerted the same radiosensitizing effect whether X-irradiation was carried out in oxygen or in nitrogen. In contrast, the radiosensitization noted under oxygenated conditions with three purine analogues tested to date (TG, 6-MP, 2-AP) completely disappeared when X-irradiation was carried out in the absence of oxygen.

No obvious difference between purines and pyrimidines with respect to the oxygen dependence of their radiochemical degradation has been apparent when aqueous solutions of the natural bases were X-irradiated *in vitro*[14,15]. Perhaps comparable studies with aqueous solutions of the base analogues will elucidate the mechanism of the present unanticipated but unambiguous *in vivo* results. Parallel *in vivo* experiments with neutrons and other high-LET radiations might also be illuminating.

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EVIDENCE FOR A PARTICULAR BASE ALTERATION IN TWO *T*4 BACTERIOPHAGE MUTANTS

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THE particular nucleotide which is altered by ultra-violet light in producing a mutation can theoretically be determined by action spectra. An action spectrum should reflect the absorption spectrum of the affected nucleotide, provided the deoxyribonucleic acid (DNA) is irradiated in the disordered condition, when there is little transfer of energy from one base to another[1,2]. The DNA of *T*4 is in the disordered state 7 min. after infection, as judged by the action spectrum for killing (Fig. 1). This action spectrum is similar to that reported for *T*2 (ref. 3). Point mutants for the r_{II} region of *T*4 apparently differ from wild-type by a small number of nucleotide pairs[4], possibly one. Thus, a reversion might require a change in only one nucleotide. The results presented here favour this approach in that action spectra for reversion of two r_{II} mutants irradiated at time 7 min. suggest a particular nucleotide change.

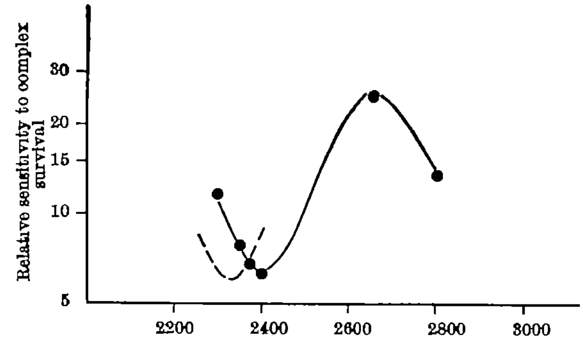

Fig. 1. Action spectrum of ultra-violet killing of *T4-E. coli* complexes (multiplicity of 7) at time 7 min. in the latent period. Each point represents the slope of the straight line portion of a semilogarithmically plotted survival curve with survival-levels down at least to 10^{-3}. The dashed line is from an action spectrum for ordered DNA[2]

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* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

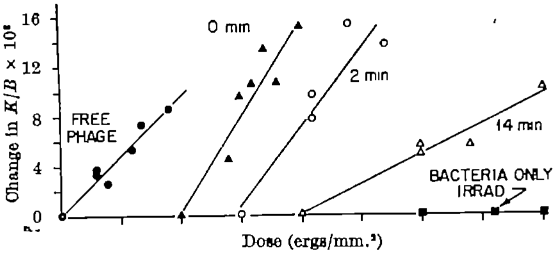

Fig. 2. Reversion of AP156 with 2652 Å. radiation at different times in the latent period. Straight lines were fitted by least squares. Each increment of dose is 500 ergs/mm.². The first point for each time represents zero dose. The ordinate is the change in the ratio of plaque counts on K to plaque counts on B

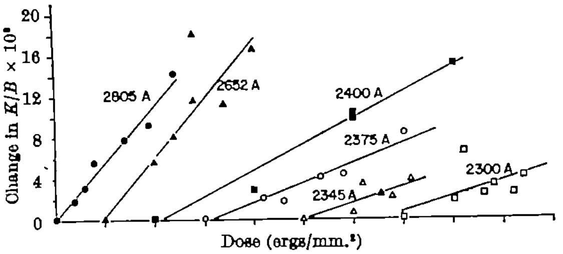

Fig. 3. Reversion of AP156 with irradiation at time 7 min. in the latent period. Straight lines were fitted by least squares. Each increment of dose is 500 ergs/mm.². The first point for each wave-length represents zero dose. The ordinate is the change in the ratio of plaque counts on K to plaque counts on B

Measurement of reversion was made, in accordance with a suggestion by Krieg[5], by assaying progeny of phage grown and irradiated in a non-selective host. The two r_{II} mutants, AP61 and AP156 (ref. 6), had been originally induced by 2-aminopurine. The host bacteria (*Escherichia coli* strain *Bb*) were grown with aeration to a concentration of 10^8/ml. in M9 medium[7] with added 0·05 per cent casamino acids. The cells were centrifuged, re-suspended at 10^9/ml. in M9 containing 0·0025 mgm. 1-tryptophan/ml. and 10^{-3} M sodium cyanide, and r_{II} virus was adsorbed at 37° C. for 3 min. This procedure yielded a mean multiplicity of seven viruses per bacterium. Cold medium was then added and the mixture was centrifuged in the cold to remove cyanide and most of the unattached phage. Less than 3 per cent of the cells were uninfected, as judged by colony counts. About 10^9 cells were re-suspended in a small volume of cold medium and added to 10 ml. of M9 aerated at 37° C. without tryptophan or casamino acids. Development of the complexes was stopped at the desired stage by rapid chilling. Before irradiation, the transmission of the infected cell suspension was adjusted to a minimum of 15 per cent by adding cold medium. 2-ml. samples at about 10° C. were irradiated at six different wave-lengths with a Hilger quartz prism monochromator. Intensities were measured and corrected for absorption as in previous work[4]. Following irradiation, 1 ml. of suspension was added to 8 ml. of M9 medium containing casamino acids. (Samples were removed and assayed for the number of infective centres. Each control mixture usually contained a total of around 6 × 10^7 infective centres. For the irradiated complexes the survival was usually no lower than about 25 per cent.) The mixtures were aerated for 2 hr. at 37° C. and treated with chloroform for complete lysis of bacteria. The progeny virus was plated on strain K, which permits plaque formation by wild-type but not r_{II} phage, and on strain B, which yields plaques from both.

Ultra-violet irradiation of extracellular phage can also induce mutations[8], including reversion of certain other r_{II} mutants[9]. In such experiments with AP156 (Fig. 2), the numbers of phage and bacteria used for attachment and final progeny growth were the same as in the experiments with irradiated complexes.

The progeny plaques on K from irradiated complexes were indistinguishable from wild-type. The ratio of final total phage to unirradiated infective centre counts was around 400, and this number was somewhat lower for irradiated complexes. The progeny from unirradiated complexes showed a ratio of revertant plaques on K to total plaques on B of about $2/10^8$ for AP156 and about $20/10^8$ for AP61.

The dose relation for reversion appears to be linear (Figs. 2 and 3) for different wave-lengths as well as for irradiation at various times in the latent period. This result may be interpreted to mean that a change in a single base causes a mutation, as has been suggested for the similar kinetics of chemical mutagenesis in T4 (ref. 10). The slope of each of these lines is a measure of sensitivity to reversion by radiation of the particular wave-length at a given time in the latent period.

A convenient way to compare two spectra is to plot their ratio as a function of wave-length. If the two spectra have the same shape, the points may be expected to fall on a straight line of zero slope. The deviation from such a line is an indication of lack of correspondence. The plots of the ratios in Fig. 4 show that the action spectra for reversion of the two mutants: (1) differ markedly from the absorption spectra of hydroxymethylcytosine (HMC) and adenosine; (2) differ less markedly from the absorption spectrum of guanosine and the action spectrum for killing; but (3) bear more resemblance to the thymidine absorption spectrum. The spread in the ratio is less than a factor of two for the thymidine comparison, but more than a factor of two (AP61) or three (AP156) for the comparison with killing. Unfortunately, the scatter in the mutation data is so great that one cannot

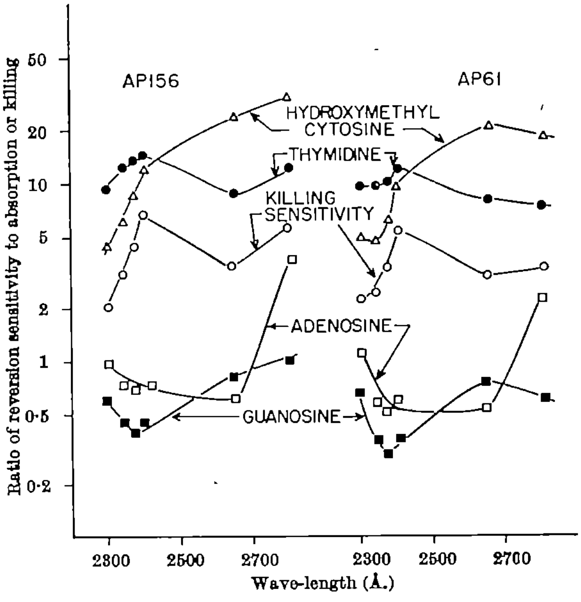

Fig. 4. Ratios of reversion action spectra to other spectra, in arbitrary units. Absorption data for *HMC* were kindly supplied by Dr. R. A. Deering. Absorption data for the other compounds at pH7 are taken from Beaven *et al.*[17]. Killing data are those of Fig 1

conclude that the reversion action spectrum is or is not the same shape as the thymidine absorption spectrum, although the coincidence is striking (Fig. 5). The data tend to support the hypothesis that these two mutants revert to apparent wild-type as a result of an ultra-violet-affected thymine in phage DNA.

Doudney and Haas[11] have proposed the theory that ultra-violet mutagenesis involves rather stable alteration of nucleic acid precursors in the bacterial cell. If alteration of nucleic acid precursors plays an important part in ultra-violet reversion of irradiated phage complexes, then one would expect to find little difference between the amount of reversion produced by irradiating the complexes just after phage DNA injection (time 0 in the latent period), when the phage has not yet had time either to use or to alter any of the available precursors, and the amount of reversion produced by irradiating the bacteria just before infection. However, reversion was highest at time 0 (Fig. 6). No reversion could be detected when the bacteria alone were irradiated (Fig. 2), in agreement with other results on irradiated starved bacteria[9]. The amount of reversion per unit of ultra-violet dose delivered to extracellular phage, where the precursor theory cannot apply, is not very different from the intracellular case (Fig. 6), an extraordinary coincidence if the mechanisms are entirely different. It is concluded that reversion by ultra-violet irradiation of both extra- and intra-cellular phage is the direct effect of alteration of viral DNA.

A mechanism for such mutagenesis is suggested by data on altered base incorporation after ultra-violet irradiation of primer in calf-thymus DNA-polymerase experiments[12]. The ultra-violet-produced dimerization of a *T–T* sequence at the mutant site of the viral primer might result in incorporation of another base instead of an adenine in the newly formed polynucleotide chain, perhaps because of a random base addition rather than the usual base pairing. In accordance with Freese's hypothesis that 2-aminopurine induces transitions[13], which are substitutions of one purine–pyrimidine pair for another, a reverse mutation in *AP*61 and *AP*156 would also require a transition. Thus a wild-type base sequence could result from a transition of a *T–A* base pair in *AP*61 and *AP*156 to an *HMC–G*.

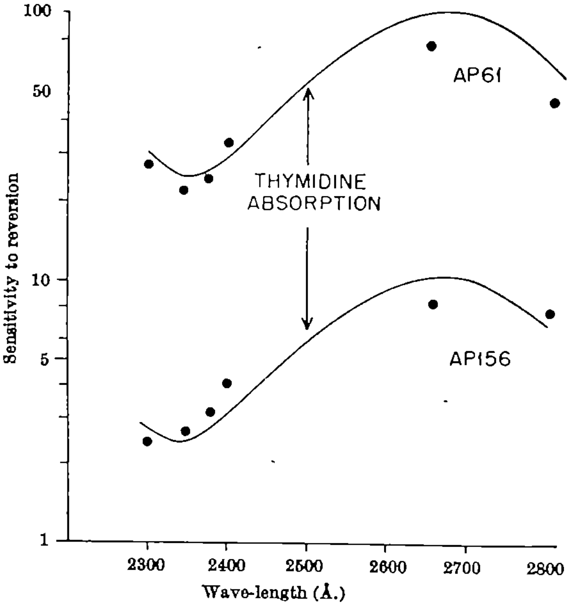

Fig. 5. Action spectra for reversion (points) compared with thymidine absorption (solid curve). Each point represents the slope of a line, such as in Fig. 3, corrected for change in photon energy with wave-length

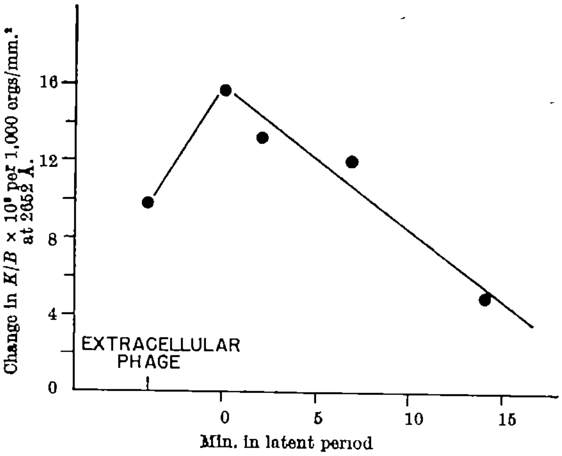

Fig. 6. Sensitivity to reversion of *AP*156 at different times during viral development. Each point represents the slope of a line in Fig. 2 or 3

Induced reversion of *AP*61 and *AP*156 has also been studied for a number of other mutagens. The amount of reversion of these mutants induced by hydroxylamine[14] and low *p*H[15] places them in the relatively unrevertible group of base-analogue induced mutants. The ethyl ethanesulphonate data[16] place *AP*156 in a medium category, and *AP*61 again in the low reversion group. However, in another study with the similar mutagen ethyl methanesulphonate[5], there is very little induced reversion in either *AP*61 or *AP*156. All these mutagenic agents are considered to induce transitions and to have their greatest effect on either *G* or *HMC*.

These results suggested that the forward and reverse mutations at the mutant site involved transitions between *A–T* and *G–HMC* pairs, but the tentative conclusion that the mutant gene contained the *A–T* pair was drawn from indirect evidence. The evidence from ultra-violet action spectra provides direct support for this hypothesis.

I thank Dr. Marvin Kastenbaum of the Mathematics Panel for fitting straight lines to the reversion data.

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LETTERS TO THE EDITOR

RADIOPHYSICS

Radiation from Jupiter at 4·8 Mc./s.

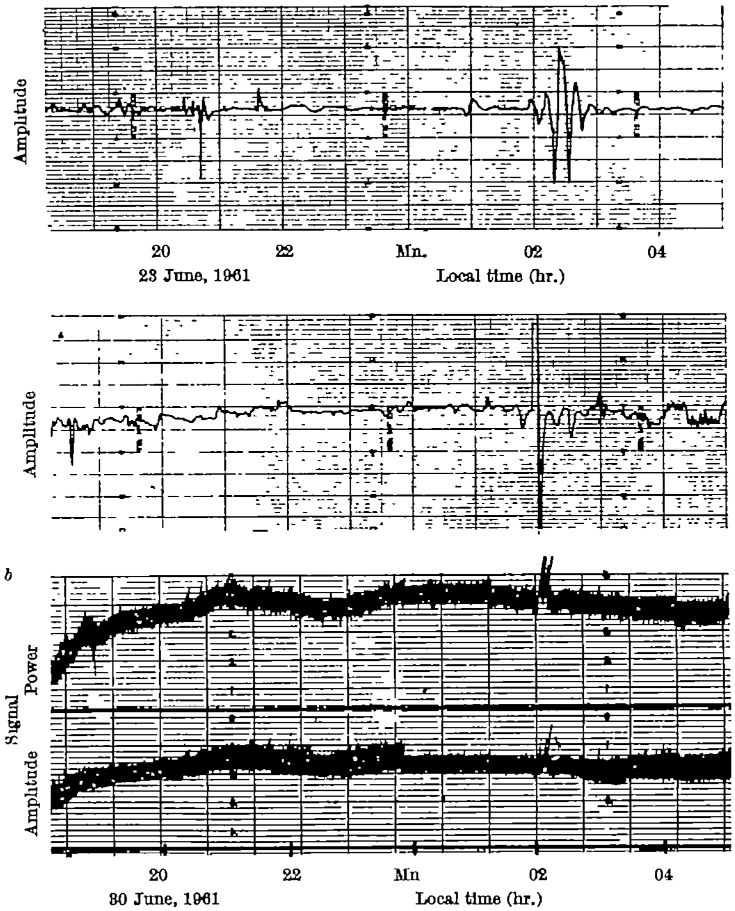
Early observations of the decametric radio emissions of Jupiter appeared to show that they had a narrow spectrum with a peak in the vicinity of 20 Mc./s. (refs. 1 and 2). As a result there were attempts to explain the radiation in terms of some appropriate single frequency mechanism such as plasma oscillations in a Jovian ionosphere[2]. More recently, however, with the passage of the solar activity maximum and consequent improving terrestrial observing conditions at low frequencies, the radiation has been detected at 10 Mc./s. (ref. 3). Here the results of observations at 4·8 Mc./s. will be reported.

They were made at Hobart, Tasmania, during June–August 1961. The equipment used was a phase-switching interferometer with an antenna primary beam 45° wide in the north–south direction and 12° wide east–west. The fringe spacing was 3°. The bandwidth of the receiver was 6 kc./s. There were three types of recorders. The phase-switching interferometer record was made with a post-detection time constant of 3 min. and hence averaged the shorter bursts of radiation. Records were made concurrently of the total signal power from both interferometer antennæ with a post-detection time constant of 0·2 sec. and of the total signal amplitude with a minimum recorder using time-constants of 0·1 sec. and 30 sec.

Identification of the Jupiter signals was made in all cases by inspection of the interferometer record. An average signal power of 10^{-24} W.m.$^{-2}$(c./s.)$^{-1}$ could positively be identified. Figs. 1*a* and *b* show sample records. There was frequently a fairly constant weak background signal on which the stronger bursts were superimposed. This is illustrated in Fig. 1*b*. The similarity of the three records of the burst in Fig. 1*b* indicates that it was essentially continuous without large variations of amplitude for several minutes. The peak intensity of the strongest bursts was 2×10^{-20} W.m.$^{-2}$(c./s.)$^{-1}$ while the average intensity of all bursts was 10^{-22} W.m.$^{-2}$(c./s.)$^{-1}$.

All observations were made at night when the ionospheric critical frequency f_0F_2 was in the vicinity of 2 Mc./s. Sporadic *E* and spread *F* ionospheric phenomena appeared on many occasions to be associated with the suppression of the interferometer records of cosmic radio sources. Assessed by the presence of these sources there were 40 nights of usable observations. On 29 of these Jupiter was recorded. Because some records were confused by transmitting station interference, this number should be regarded as a lower limit and it is considered likely that the Jupiter radiation was present on most nights.

Determination of the intensity spectrum of the radiation was difficult since very few observers have specified either the peak intensity or the average intensity of the bursts, but discuss instead the variation of the probability of their occurrence with frequency. However, the peak intensity recorded in the observations reported here did not differ significantly from the peak intensity of 10^{-19} W.m.$^{-2}$(c./s.)$^{-1}$ reported by Gardner and Shain[1] at 19·6 Mc./s., particularly when allowance is made for the fact that there was an interval of 5 years between the two observations. It seems likely, therefore, that the spectrum extends downwards in frequency below 4·8 Mc./s. possibly to much lower frequencies and is therefore not a narrow spectrum as had been thought earlier.



Figs. 1*a* and 1*b*. Sample records of Jupiter at 4·8 Mc./s. The interferometer records were made with an integration time of 3 min. The middle and lower records of Fig. 1*b* were made with a minimum recorder and with a simple integrator (time-constant 0 2 sec.) respectively

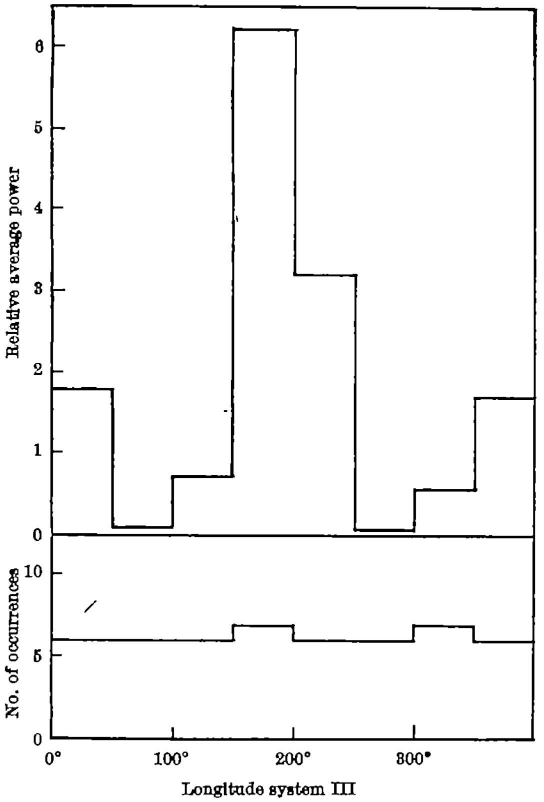


Fig. 2. Variation of the mean power of the 4·8 Mc./s. noise bursts and of the number of bursts with Jupiter longitude

When the probability of occurrence of the bursts was plotted as a function of Jupiter longitude very little variation was found, confirming the results of Carr *et al.*[3] at 10 Mc./s. However, as shown in Fig. 2 there was a pronounced variation in their average intensity with longitude. Using Longitude System III proposed by Smith and Carr[2] with a rotational period of 9 hr. 55 min. 28·8 sec. the two maxima occurred between 150° and 250° and between 000° and 050°. The former of these corresponds with the peak in the probability of occurrence found by Shain[4] in his 1951 records.

These results indicate that at least at the lower frequencies the average burst intensity is a much more powerful indicator of the variation of the radiation with the planet's rotation than is the probability of occurrence used by other investigators.

The existence of two peaks with a longitude separation of about 180° suggests that the radiation is associated with a Jovian magnetic field the axis of which is inclined to the rotational axis. It might be expected, if this were so, that the polarization of the radiation would be of opposite sign at the longitudes of the two maxima.

This work is partly financed by a grant from the Australian Radio Research Board.

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ASTROPHYSICS

Distribution of Elements over the Surface of a Magnetic Star

The anomalous abundances observed in magnetic stars have been attributed to nuclear processes occurring in hot spots in the surface layers. Induced electric fields set up by changes in the magnetic field with time are assumed to heat the spots and thus provide the reacting particles with sufficiently high energy[1]. Both in the magnetic stars and in the related spectrum variables the equivalent widths of certain classes of lines show periodic variations with time. On the oblique rotator model this is interpreted as an aspect effect, corresponding to a 'patchy' distribution of elements over the stellar surface[2]. It does not appear easy to account for this uneven distribution on the hypothesis of surface reactions alone.

There is, however, another effect which, under favourable conditions, might lead to or preserve such a distribution. In a magnetic field, B, that is not strictly homogeneous, each atom and ion is acted on by a force, $\mu\nabla B$, along the field lines. The magnetic moment, μ, is equal to the product of Bohr's magneton, μ_B ($0{\cdot}927 \times 10^{-20}$ erg. gauss^{-1}), the magnetic quantum number, M, and Landé's g-factor.

In the case of the rare-earth elements, which is the group showing the most striking non-uniform distribution in the magnetic stars, the magnetic moments are particularly large. Due to the only partly filled N- and O-shells of these atoms all stages of ionization that may exist in stellar atmospheres have large magnetic moments. In the case of the neutral atoms of europium, samarium and gadolinium, for example, the configurations of the ground-states are ^8S, ^7F and ^9D respectively.

The corresponding maximum magnetic moments in terms of $\mu_B g$ are 3½ for the first one and 6 for the last two. Comparable values for the magnetic moments are found in the iron group, the elements of which also show variations in line strength in magnetic stars. The value of the magnetic moment which is most important for the distribution of a particular element is clearly that corresponding to the stage of ionization which is dominating in the region considered. This mechanism will only work in the atmospheres of the stars where the alignment of the particles is not too much disturbed by collisions.

We may make an estimate of the order of magnitude of the forces involved and of the resulting scale in the density distribution. The maximum observed field strength in a magnetic star runs as high as $3{\cdot}4 \times 10^4$ gauss[3]. This is the field integrated over the stellar surface so it does not seem unreasonable to assume that the actual field strength in local regions may exceed this average value by one order of magnitude. Assuming a field strength of 10^5 gauss and a characteristic length determining the gradient in the magnetic field of 10^9 cm., the magnetic force amounts to 10^{-3}–10^{-2} of the weight of the particles. If we further assume that the lines of force are roughly horizontal, the equilibrium equation is:

$$\nabla n/n = \mu\nabla B/kT \sim 1/L$$

where n is the partial density. Taking $T \sim 10^4$ we obtain $L \sim 10^{12}$ cm., which is of the order of magnitude of the radius of an A-star.

Even in the photospheric layers a net paramagnetic moment will result, since the Zeeman levels are here populated according to Boltzmann's Law.

For this mechanism to work the magnetic field should not change too violently with time. With the oblique rotator model the intrinsic changes in the field come out relatively small. If this model is representative the mechanism described here may well play an important part in determining the distribution of elements in the atmospheres of the magnetic stars.

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PHYSICS

Cæsium-137 in the Seas off the Cape of Good Hope

MEASUREMENTS of fall-out cæsium-137 in surface sea-water in the northern hemisphere have been reported by two groups of workers[1,2]. With the view of obtaining an estimate of the fall-out contamination in the southern oceans, a programme of cæsium-137 determination in the waters off the Cape of Good Hope was begun in July 1961 in collaboration with the Oceanography Department of this University. Samples were collected by the University of Cape Town research vessel, the *John D. Gilchrist*, and five had been obtained by the middle of September 1961. At this stage the recent series of Russian nuclear tests was in progress, and it was decided to extend the programme on a semi-permanent basis. This communication provides an estimate (based unfortunately on a limited number of samples) of the cæsium-137 level in sea-water in southern latitudes due to earlier (pre-1961) nuclear explosions.

Our detection techniques in general followed those of Folsom *et al.*[2]. Thus we collected 200-litre samples in polythene drums, and we used the extremely simple and effective method of cæsium co-precipitation with potassium by the addition of sodium cobaltinitrite to the sea-water in the drums. Further, we detected the 0·66-MeV. γ-ray of cæsium-137 utilizing a large (4-in.) sodium iodide (thallium) low-background crystal assembly. In our case[2], however, only a two-channel and not a multichannel pulse height analyser was available. We accordingly thought it desirable to perform further chemical reactions to remove potassium from the precipitate. The following procedure as recommended by Sugihara *et al.*[3] was used. The precipitate was dissolved in 6 *N* hydrochloric acid, the solution filtered, and 1 gm. cæsium chloride and 60 gm. dodecatungstosilicic acid were added. The solution was heated on a water-bath for 1 hr. before being allowed to stand overnight. The resultant cæsium silicotungstate precipitate was then well washed by successively slurrying up with hydrochloric acid and distilled water, and decanting the supernate after the precipitate had settled. This was repeated until the supernate was free of the pink colour from the original hydrochloric acid solution, and the precipitate was then transferred to a standard polythene bottle for counting. The size of precipitate obtained from the original 200-litre sea-water sample was about 10 c.c.; trial experiments with tracer cæsium-137 showed that it contained 74 ± 7 per cent of the cæsium in the sea-water. This yield was independent of the temperature of the sea-water during the cobaltinitrite precipitation process provided this temperature was kept below 19° C. The sample as counted was virtually free of potassium, thus reducing the predominant source of interference when performing γ-spectrometry in the cæsium channel. Possible sources of interference from other radioactive isotopes were considered by Mohanrao and Folsom (private communication), who concluded that they were likely to be negligible. To check the efficacy of the tungstosilicic acid stage of our chemistry, and to guard in general against possible sources of contamination giving γ-rays of a higher energy than that from cæsium-137, we used the second channel of our double channel spectrometer to monitor an energy window just above the cæsium-137 0·660-MeV. peak. Counts in this channel were found to be on the average only 3 per cent of those in the cæsium peak, and were never higher than 12 per cent for any of the samples tested by us. The additional stage in the chemical process would thus appear to be fulfilling its purpose.

Table 1

Sample	Date collected	Position	Distance from coast (miles)	^{137}Cs (μμc./kl.)
1	8/8/61	Off Cape Point	7	161
2	14/8/61	36° 22′ S., 21° 27′ E.	139	87
3	26/8/61	34° 38′ S., 18° 38′ E.	12	92
4	13/9/61	34° 23′ S., 17° 50′ E.	28	29
5	13/9/61	34° 23′ S., 17° 50′ E.	28	79

The results are presented in Table 1. Each individual determination has a 95 per cent confidence limit of ± 25 μμc./kl. This is the error due to counting statistics alone, which in general dwarfs the other sources of error due to variations in the chemical yield and uncertainties in the calibration process. The cæsium-137 content of the waters around Cape Town just prior to the 1961 nuclear tests was calculated on the basis of our five samples to be 90 ± 47 μμc./kl. This is to be compared with the values of 138 ± 40 μμc./kl. obtained off the coast of California during 1959–60 by Folsom *et al.*[2], and 380 ± 136 μμc./kl. obtained in 1959 by Yamagata[1] in Japanese waters. The level of contamination in the ocean would thus appear to be lower in the southern than in the northern hemisphere. This is, of course, to be expected from general fall-out measurements[4]. It will be of interest to follow the cæsium-137 level over the next few years; to this end, we are continuing to collect samples at regular intervals.

We thank Messrs. P. van Reenen and J. Engelbrecht for help in collecting our samples. Financial aid was given by the University of Cape Town Oceanography Department and the South African Atomic Energy Board. We thank Dr. R. Arnold and Dr. R. Edge for advice on chemical matters, and Dr. T. R. Folsom for supplying us with details of his chemical methods prior to publication.

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Some Consequences of Ion Slip in a Plasma flowing through a Magnetic Field

Direct generation of electrical power can be achieved by driving a conducting gas through a magnetic field transverse to the direction of flow. The necessary conductivity is obtained by heating the gas to a temperature at which appreciable thermal ionization occurs, the latter in accordance with the Saha equation. The conducting gas will be considered to consist of neutral particles and charged particles, no distinction being made between ions and electrons. It is the purpose of this communication to discuss the behaviour of this system as it flows along a duct (neglecting wall effects) through a uniform transverse magnetic field as shown in Fig. 1.

In region 1 the various particle species have a common flow velocity, V_f. In region 2 the respective flow velocities of neutrals and charged particles are V_{fn} and V_{fc}. If no collisions occurred and provided that the Larmor radii were very much less than the field dimensions the charged particles within the field would gyrate about the magnetic field lines and their flow velocity through the field would be zero. However, collisions result in momentum transfer between the neutral particles and the charged particles to produce the flow velocity V_{fc}. If slip of charged particles with respect to the neutrals is present, V_f is not the velocity to use in calculating the electric field induced by the flow of charged particles. Unfortunately, the data to calculate V_{fc} are not available but a discussion of some of the consequences of the fact that V_{fc} is less than V_f may be useful.

In region 1 an equilibrium condition exists between thermal ionization and recombination events. When the flow enters region 2 the charged particles present in the system cannot penetrate the magnetic field to a distance greater than their Larmor radii unless momentum transfer collisions with neutrals occur. The result is that a region of increased charged particle density is created at the magnetic field boundary between regions 1 and 2 and is continuously replenished by charged particles from the flowing system. The loss processes operative in this region of enhanced ionization are diffusion into regions 1 and 2, recombination, and flow into region 2 to produce an increased charged particle density as indicated in Fig. 2. When the flow leaves region 2 and enters 3 a decrease in charged particle density occurs.

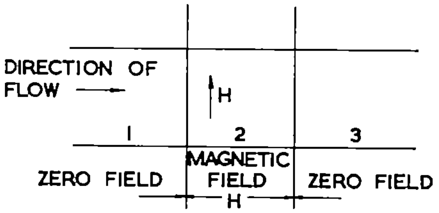

Fig. 1. Regions considered in flow through a uniform transverse magnetic field

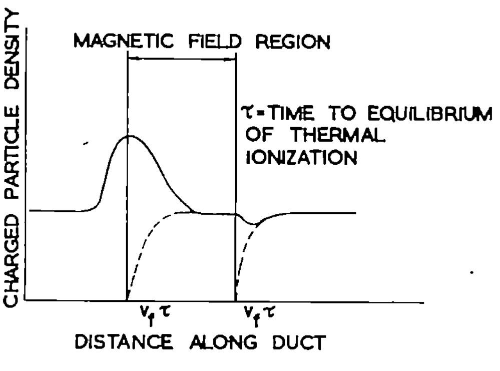

Fig. 2. Variation in charged particle density produced when plasma flows through a magnetic field region

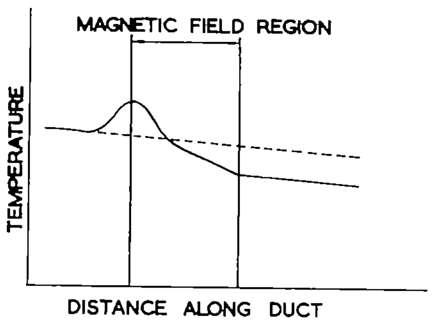

Fig. 3. Variation of temperature along duct

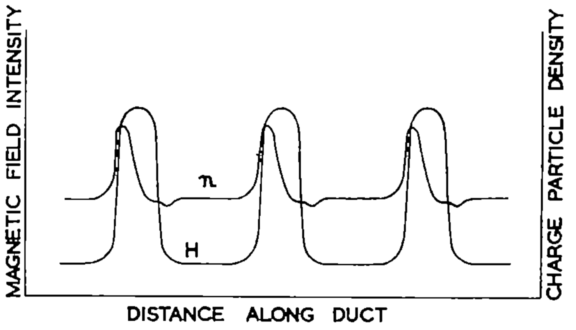

Fig. 4. Suggested non-uniform magnetic field designed to increase the local density of charged particles

Consider a slab of gas flowing through the three regions; when the charged particles are removed by the magnetic field, the ionization energy is also removed, and thus the region of increased charged particle density also becomes one of increased temperature when the ionization energy is released during the recombination processes. As the slab moves through the magnetic field, the continuous removal of charged particles progressively cools the gas. The temperature distribution along the duct resulting from this process is shown in Fig. 3.

The charged particle flow velocity is produced by collisions which displace the centres of gyration, and the charged particles progress through the magnetic field in a series of arcs of the Larmor radii r_L rather than the trajectories characteristic of a field-free system. Consequently, V_{fc} is a function of r_L and the collision frequency ν_c appropriate to the species in a magnetic field. Since $r_L \propto 1/H$ and since ν_c is probably dependent on H, it is clear that the usual assumption that the electric field induced by the charge flow is a linear function of H is unlikely to be valid.

It has been shown that an increased charged particle density may be anticipated when a flowing plasma enters a magnetic field. It may be possible to increase the power output of a d.c. electrical power generator based on magnetoplasmadynamic principles by using a non-uniform magnetic field as shown in Fig. 4 instead of the more conventional uniform field. Such a field would be designed to produce and take advantage of the predicted increased charged particle density.

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Vacuum Ultra-Violet Absorption Spectra of Flash-heated Lead reacting with Various Gases

Recent investigations of the hot atom chemistry of lead-212 recoiling from α-decay of polonium-216 have shown that energetic lead atoms can react with gases such as methane to form volatile compounds[1]. In the recoil reactions, the lead species originate with high kinetic energy and are afterwards degraded by collisions, eventually reacting to form stable compounds.

In an effort to determine whether gaseous lead atoms could react at essentially thermal energies (<1 eV.) to give products similar to those observed in the recoil studies, the present investigations were undertaken.

The techniques of flash heating lend themselves ideally to the study of thermalized metal atoms in the gas phase because of the ability to vaporize fairly significant quantities of material in the presence of a surrounding medium that is only slightly heated[2]. Combination of flash heating with kinetic absorption spectroscopy allows the detection and examination of short-lived species in the vapour-phase during the reaction[3].

Using the techniques previously described[3], about 8 or 10 mgm. of lead was vaporized per flash. The metallic lead in the form of a thin ribbon (0·0005 in. thick, 1/8 in. wide) was wound on a quartz support so as to pass through the optical path within the cell and replaced the carbon grids used in the earlier work.

The usual spectral region photographed extended from 1300 to 3300 Å. This region includes the Rydberg series leading to the ionization limit of PbI at 1671·65 Å., corresponding to transitions from the ground-state ($6p^2\ {}^3P_0$) (ref. 4).

All spectra taken within a few milliseconds after the beginning of the preparative photoflash showed absorption lines due to lead atoms in the ground-state. Transitions from the first and second excited states were also observed. These lie 7,819·35 and 10,650·47 cm.$^{-1}$, respectively, above the ground-state and correspond to the configurations $6p^2\ {}^3P_1$ and $6p^2\ {}^3P_2$ (ref. 4). No evidence for ionization of the vaporized lead was obtained.

Reactions of lead vaporized in the presence of CF_4 or SF_6 resulted entirely in fluorine abstraction from the parent fluorinated molecule by the metal atoms, yielding lead monofluoride. The monofluoride was identified from the band systems near 2100, 2500 and 2800 Å.[5,6]. The PbF could be observed from the time of maximum light intensity until about 700μ later. After this period, light scattered by particles formed by further reaction of PbF caused a heavy continuous absorption and prevented subsequent spectral observations. Many new vibrational bands and an apparently new system in the 1800–1900 Å. region have been observed in association with PbF in these studies. Rotational fine structure was clearly observed, particularly in the 2800 Å. region.

Table 1. Species Observed by Flash Heating and Kinetic Spectroscopy of Lead in Various Gases

Short-lived atom or molecule	Gaseous matrix	*Delay times (μs.)	Wave-lengths (Å.)
Pb ($6p^2\ {}^3P_{0,1,2}$)	All	0–1300	1600–3300
Pb	,,	,,	bands at 2136, 2143, 2161, 2167
PbF	SF_6 CF_4	0–1050	band systems at 1900, 2100, 2500, 2800
PbO	CD_4+air	45–1000	band systems from 2800 to 3700
Unidentified	O_2 C_2H_6 CH_4 D_2 (2900 Å. only)	180–420	band systems at 2400, 2900
CO (excited)	CO	1000	1700
O_2 (excited)	O_2	180	2000–2500
Emission lines	vacuum SF_6 CF_4 Ar CO	0–1000	1587, 1634, 1661

*Delay times refer to time elapsed between peak intensity of preparative flash and peak intensity of spectroscopic flash.

PbO was produced by reactions of flash-heated lead with oxygen or with air present as an impurity in methane. Band systems for PbO lie mostly in the wave-length regions above 2800 Å. (ref. 5).

In the presence of methane or ethane, unidentified diffuse transient systems were observed in the regions near 2400 and 2900 Å. The system at 2900 Å. was faintly visible when the flash heating was carried out in a deuterium atmosphere, indicating a possible hydride formation.

Since it was desired to limit these studies as much as possible to thermally initiated reactions, gases were chosen so as to be transparent to the light used and to minimize photochemical processes. Table 1 summarizes the gases used and the absorptions observed at various delay times following peak intensity of the preparative flash.

Of special interest are the diffuse bands observed at 2136, 2143, 2161 and 2167 Å. These occurred in all the gases and in vacuum and have tentatively been assigned to the $Pb_{2(g)}$ molecule.

The absorption spectra were supported by mass spectrographic analyses in showing that no volatile compounds analogous to those reported for the recoil lead-212 studies[1] were produced by reactions in methane, methane and air, carbon monoxide or argon.

Complete analyses of the spectra are in progress in an effort to determine electronic temperatures of the vaporized lead atoms and vibrational and rotational temperatures of the product molecules. Additional efforts are being made to identify the emission lines (Table 1) and the unidentified systems near 2400 and 2900 Å.

This work was done in the Bell Telephone Laboratories, Inc.. Murray Hill, New Jersey.

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Prevention of Uptake of Strontium Ions on Glass

THE uptake of metal ions by the walls of glass vessels is a frequently encountered phenomenon in analytical chemistry and radiochemistry, and is responsible for the difficulty of maintaining dilute solutions at a known concentration during storage. In general this problem is overcome by making such solutions acid. and neutralizing immediately before use. This technique is not applicable, however, when it is necessary to use the same solution at neutral *p*H for long periods of time.

We have been investigating the uptake, by bone, of strontium ions (labelled with strontium-89) at approximately physiological *p*H (7·3), and here the effect of uptake by the walls of the glass tubes used is quite serious. Attempts were made to overcome this by using polythene tubes; but these were found to be even less suitable than glass.

The difficulty has been overcome by coating the inside surfaces of the glass tubes with a non-adsorbent material—a proprietary polyester resin. Though this is satisfactory in preventing uptake, the vigorous shaking required for our experiments caused breakdown of the coating after 60–70 hr. The life of the coating can be extended by first coating the glass tubes with vinyltrichlorosilane and then with the polyester resin. Coating surfaces with non-adsorbent materials has already been investigated for the purpose of easing the decontamination of active surfaces[1,2]; but there appears to have been no application of this to the prevention of uptake in reaction vessels.

Labelled solutions of strontium chloride (*p*H ~ 7) were vigorously shaken for 24 hr. in tubes of the following materials: (*A*) glass, (*B*) polythene, (*C*) glass coated with polyester resin, (*D*) glass coated first with vinyltrichlorosilane and then polyester resin.

Afterwards, an assay was made of the radioactivity remaining in solution. Table 1 shows the percentage uptake for each series at varying concentrations of metal ion. The number of tubes examined is shown in parentheses.

Table 1

$[Sr^{2+}]$ (moles/l.)	*A*	*B*	*C*	*D*
1×10^{-3}	12 ± 8 (8 tubes)	66 ± 6 (6 tubes)	0 (36 tubes)	1 (12 tubes)
1×10^{-6}				0 (6 tubes)
Carrier-free ^{89}Sr	15 (5 tubes)		7 (10 tubes)	8 (12 tubes)

Within the limits of experimental error the results show that polyethylene is quite unsuitable, but that the use of this coating introduces a significant improvement compared with glass.

The use of this, or other similar coatings, was suggested by Mr. J. R Morris of this Establishment, and may be applicable to other systems of metal ions.

We thank the U.K. Atomic Energy Authority for permission to publish this communication.

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THE finding that polyethylene adsorbs about five times as much strontium-89 as does glass is rather unexpected considering that in the case of glass, possible adsorption mechanisms include powerful ion exchange processes, while with polyethylene the possible mode of attachment is not evident. On this finding the widespread use of polyethylene instead of glass or ceramics for radioactive effluent tank linings, pipelines, etc., could conceivably lead to a build-up of activity if conditions of *p*H were suitable.

The assumption by Boocock *et al.* that the strontium-89 is in ionic form may not be justified. At the physiological *p*H of 7·3 it is not unlikely that the strontium is partially, at least, in the form of a radiocolloid of strontium hydroxide, the adsorption mechanism for which is dependent on interfacial zeta potential, rather than ionic bonding.

It is hoped that the authors can improve further the adhesion of their polyester film, as the technique might well greatly ease the problems of the decontamination of vessels or equipment which have been in contact with radioactive solutions.

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CHEMISTRY

Isotope Effects in the Infra-red Spectrum of 1-^{13}C-Propene-1

THE abundance and location of isotopically labelled carbon atoms have been investigated by numerous techniques, but no use has been reported of vibrational spectra for this purpose. This communication describes how the infra-red spectrum of a sample of propene prepared with carbon-13 is consistent with all the labelled isotope being in the 1-position.

The sample was prepared from barium carbonate enriched with 55 per cent carbon-13, using a modification of the method of Fries and Calvin[1]. These authors provide radiochemical evidence for the specific preparation of 1-^{14}C-propene-1 by this route. Analysis by mass spectroscopy gave good value for the percentage enrichment but it could not locate the carbon-13 with certainty. There seemed to be rearrangement in the ionization chamber, possibly due to the formation of a cyclic intermediate. The use of nuclear magnetic resonance spectra in the examination of carbon-13 resonances in a variety of organic compounds has been discussed by Lauterbur[2]. However, insufficient sample was available for a nuclear magnetic resonance investigation in the present work.

The infra-red spectrum of a labelled propene sample at 32 mm. mercury pressure in a 9·5 cm. single path cell was recorded between 400 and 3,650 cm.$^{-1}$ on a Unicam *SP*100 spectrometer fitted with the *SP*130 grating accessory. The quantity of sample used was 2·4 mgm. The spectrum differs from unlabelled propene in two respects: first the C=C stretching frequency at 1,652 cm.$^{-1}$ is decreased by approximately 10 cm.$^{-1}$; secondly, the out-of-plane frequency at 912·3 cm.$^{-1}$ is shifted to 903·6 cm.$^{-1}$.

A shift in the C=C stretching frequency can arise from a labelled carbon atom in either the 1- or 2-position. Unfortunately the precise shift and the relative strengths of the carbon-13 and carbon-12

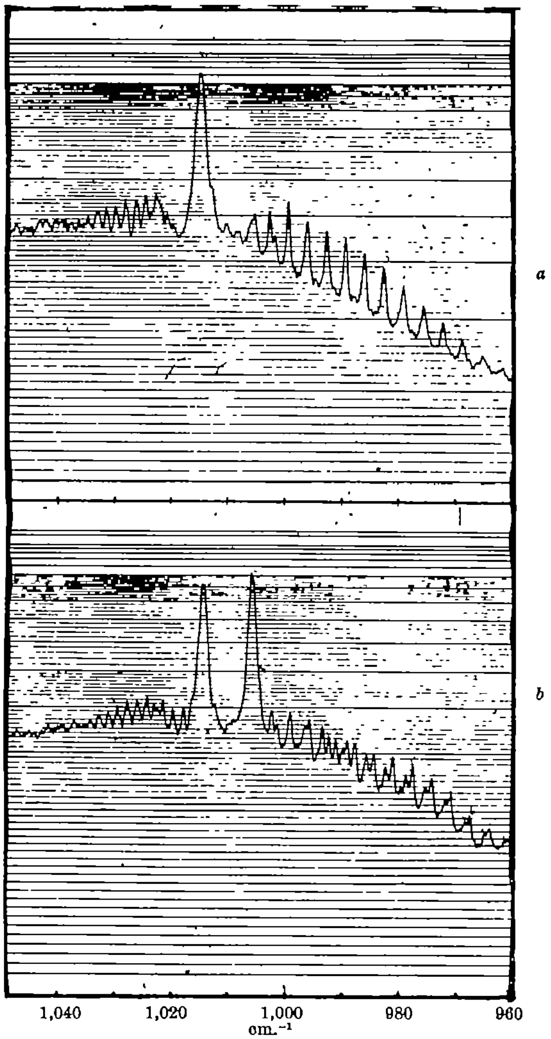

Fig. 1. Methylene wagging mode. *a*, Propene; *b*, labelled propene

components are difficult to measure owing to the complex PQR structure of this hybrid parallel-pendicular band.

The 912·3 cm.$^{-1}$ band has usually been assigned to an out-of-plane deformation of the hydrogen atoms of the $=CH_2$ group[3]. On this simple picture a change in the mass of the 1-carbon atom would not be expected to affect the frequency of this mode. However, a recent normal co-ordinate analysis of out-of-plane vinyl modes by Scherer and Potts[4] describes this as a methylene wagging mode in which the 1-carbon atom participates appreciably.

The spectrum of the methylene wagging frequency for propene and labelled propene is shown in Figs. 1*a* and *b*. The shifted band at 903·6 cm.$^{-1}$ implies that the carbon-13 is in the 1-position; furthermore, the measured strength of this absorption shows that 55 per cent of the propene is in this form. This correspondence with the enrichment ratio of the starting material proves that all the labelled carbon is in the 1-position.

The rotational fine structure of the 903·6 cm.$^{-1}$ carbon-13 methylene wagging mode shows some measurable differences from the corresponding 912·3 cm.$^{-1}$ carbon-12 band. These differences and the shifts in fundamental frequencies with isotopic substitution are at present being investigated theoretically for propene and some simple related molecules.

I thank Mr. G. Gough for the mass spectroscopic data and Dr. C. C. McCain for making this compound available.

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Oxygen Adsorption and the Magnetic Susceptibility of Ice at Low Temperatures

When dealing with the magnetic susceptibility of tumour tissue[1], we reported the magnetic susceptibility of ice at various temperatures from 273° K. down to 77° K. Since this publication, the authors have made many susceptibility measurements of ice, using the same equipment, and have obtained similar results, that is, a relatively large increase in diamagnetism below 150° K. Normal diamagnetism is not dependent on temperature, and hence further experiments were made of the magnetic susceptibility of ice at low temperatures in an attempt to determine some extraneous cause of the increased diamagnetism.

In the work reported in ref. 1 the ice was prepared by immersion of distilled water in liquid nitrogen. Both the weight and the magnetic susceptibility were measured on the same quartz helical spring balance[2]. Just prior to making the measurements, the balance case was flushed out with helium using a flow method, and the system was sealed at atmospheric pressure. The specimen was then cooled to 77° K., and measurements were taken as the temperature was raised to 235° K.

Recent tests using the system under a slight positive pressure revealed a small leak in a faulty thermocouple seal, which allowed a small amount of air into the balance. By using a new leak-free system, about 0·01 atmosphere of oxygen was admitted into the helium atmosphere, and this resulted in a marked increase in the diamagnetism when the sample was cooled to about 100° K. The oxygen in the small amount of air which leaked into the system was adsorbed on to the ice below 150° K. The fact that the oxygen increased the diamagnetism shows that the phenomenon is one of chemical adsorption[3]. Additional investigations of the adsorption of oxygen on ice at low temperatures are being made to show the role of oxygen in the previous tumour measurements.

After determining the cause of the anomalous diamagnetism of ice, the true magnetic susceptibility of ice at low temperatures was determined. Although there are several recorded measurements[4–6], no other measurements appear to have been made below about 220° K. Thus, the magnetic susceptibility of ice was remeasured, using the following technique. A drop of approximately 16 mgm. of deionized distilled water was suspended from the quartz helix by a very fine 'Pyrex' loop. The balance case was flushed and filled with helium, and the specimen was frozen by passing liquid nitrogen

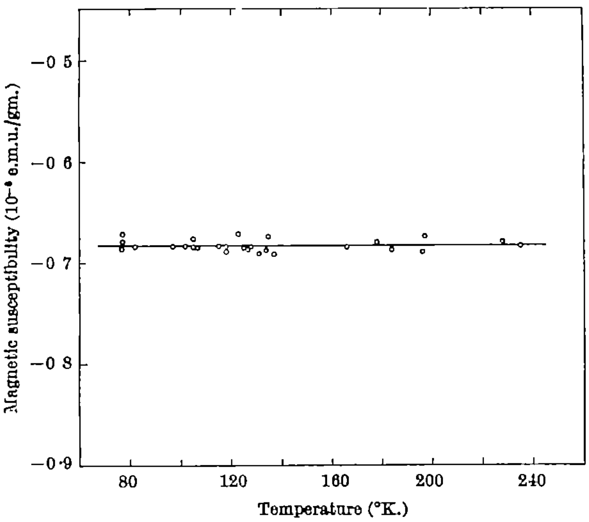

Fig. 1. Magnetic susceptibility of ice as a function of temperature

under pressure through a double-wall jacket of the balance case. With the specimen at 77° K., the sample chamber was evacuated and refilled with helium to ensure the removal of all oxygen. Susceptibility measurements taken at different temperatures for six different specimens of ice are shown in Fig. 1. The mean value for the magnetic susceptibility of ice is:

$$(-0{\cdot}683 \pm 0{\cdot}001) \times 10^{-6}\,\text{e.m.u./gm. from } 77°\text{ K. to } 235°\text{ K.}$$

Publication of this communication is authorized by the Director of the U.S. Geological Survey.

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Relation between Partition Coefficients and Properties of Solvents

PUBLISHED data on the partition of non-electrolytes between water and different organic solvents are abundant; but no successful attempt has been made to correlate the partition coefficients with the properties of solvents. No macroscopic property such as dielectric constant, refractive index or dipole moment can be correlated with partition coefficients in a simple manner. I have found the correlation between Hildebrand's solubility parameters of solvents and partition coefficients to be the most satisfactory.

The partition coefficient of a solute z corrected, if necessary, for dissociation in aqueous phase, extrapolated to infinite dilution, and expressed in mole fraction units $({}^{x}k^{\infty}_{z})$, is simply related to standard chemical potentials (μ^{θ}_{z}) of the solute in both phases, namely: $-RT \ln {}^{x}k^{\infty}_{z} = \mu^{\theta}_{z(i)} - \mu^{\theta}_{z(H_2O)} \equiv \Delta\mu^{\theta}_{z}$. In Fig. 1 $\Delta\mu^{\theta}_{z}$ for acetic acid[1] (I), acetone[2] (II) and *o*-nitro-aniline[3] (III) are plotted against solubility parameters[4] (δ_i) of the solvents listed below Fig. 1. As can be seen, the experimental points clearly follow two different curves, which in Fig. 1 are denoted by numbers 1 and 2. The curves of type 1 have been calculated by the following equation:

$$\Delta\mu^{\theta}_{z} = v^{0}_{z}\,(\delta_z - \delta_i)^2 + C_z \qquad (1)$$

where v^{0}_{z} and δ_z are the molar volume and solubility parameter of the solute, respectively, and C_z is a constant. In deriving equation (1), two assumptions on the thermodynamic properties of the organic phase are made: 1° $\lim\limits_{x_z \to 0} \dfrac{\partial s^E}{\partial x_z}$ is independent of the solvent, and 2° $\lim\limits_{x_z \to 0} \dfrac{\partial h^E}{\partial x_z}$ may be calculated by the Hildebrand–Scatchard equation.

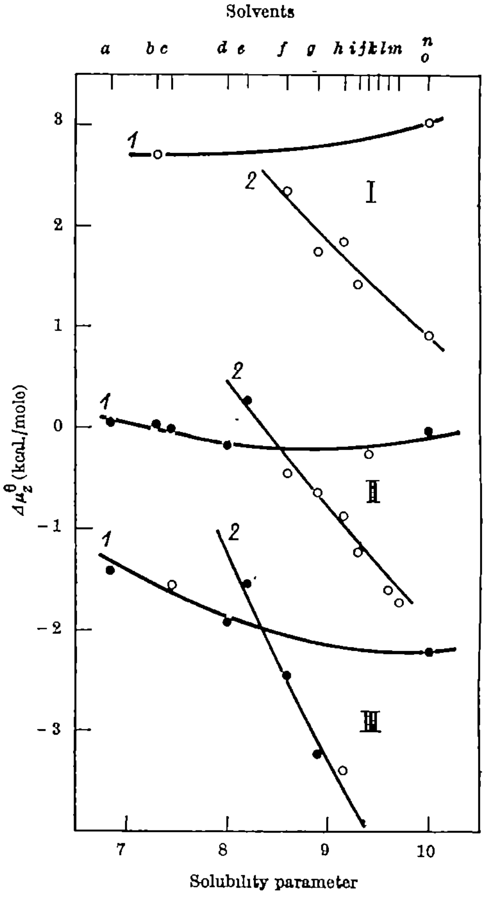

Fig 1. Plot of $\Delta\mu^{\theta}_{z} = -RT \ln {}^{x}k^{\infty}_{z}$, for acetic acid (I), acetone (II) and *o*-nitroaniline (III), versus solubility parameter of *a*, isooctane; *b*, *n*-hexane; *c*, *n*-heptane; *d*, hexadecane; *e*, *cyclo*hexane; *f*, carbon tetrachloride; *g*, toluene; *h*, benzene; *i*, chloroform, *j*, tetrachloro-ethylene; *k*, chlorobenzene; *l*, 1,1,2-trichloroethane; *m*, 1,1,2,2-tetrachloroethane; *n*, carbon disulphide and *o*, nitrobenzene. Curves 1 and 2 are calculated from equations (1) and (2), respectively, with the following values of the parameters: $v^{0}_{\text{I}} = 0{\cdot}0575$, $\delta_{\text{I}} = 7{\cdot}60$, $C_{\text{I}} = 2{\cdot}70$; $v^{0}_{\text{II}} = 0{\cdot}0785$, $\delta_{\text{II}} = 8{\cdot}80$, $C_{\text{II}} = -0{\cdot}19$, and $v^{0}_{\text{III}} = 0{\cdot}106$, $\delta_{\text{III}} = 9{\cdot}75$, $C_{\text{III}} = -2{\cdot}20$. $v^{0}_{\text{I}*} = 0{\cdot}061$, $\delta_{\text{I}*} = 17{\cdot}26$; $v^{0}_{\text{II}*} = 0{\cdot}085$, $\delta_{\text{II}*} = 15{\cdot}87$, and $v^{0}_{\text{III}*} = 0{\cdot}144$, $\delta_{\text{III}*} = 15{\cdot}42$. ○, Published data; ●, own measurements

The extension of these assumptions to the aqueous phase as well leads to the following equation:

$$\Delta\mu^{\theta}_{z*} = v^{0}_{z*}\,(23\cdot4 - \delta_{l})\,(2\delta_{z*} - \delta_{l} - 23\cdot4) \qquad (2)$$

where 23·4 is the solubility parameter of water. The curves of type 2 agree well with equation (2). The constants v^{0}_{z*} and δ_{z*} of equation (2) can be calculated easily, since this equation divided by $(23\cdot4 - \delta_{l})$ gives an equation linear in δ_{l}. Experimental values of v^{0}_{z*} are larger than the molar volumes of pure solutes, v^{0}_{z}, and δ_{z*} are larger than δ_{z} in equation (1).

The observed dual behaviour of the solute, depending on the organic solvent, seems to be quite general, and is not restricted to the solutes described here. Many other substances, such as formic and propionic acids, phenol and nitrophenols, aniline, *m*- and *p*-nitroaniline, *o*-, *m*-, and *p*-toluidine, nitropropane and nicotine, behave in a similar manner. The appearance of the two curves of partition for each solute is probably due to a difference in the degree of hydration of the solute molecules. In solvents with rod-like molecules (as aliphatic hydrocarbons or carbon disulphide) the curves of type 1 are characteristic, for the non-hydrated molecules probably predominate, whereas in other solvents the degree of hydration is high.

I thank Prof. Wiktor Kemula for advice.

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Tri*cyclo*quinazoline and Combustion Products

Tri*cyclo*quinazoline (TCQ), which can be produced by the pyrolysis of methyl anthranilate, is a stable compound which has been shown to be carcinogenic[1,2]. Since methyl anthranilate is present in plants[3], there was therefore good reason to look for TCQ in the products of combustion of fuels derived from vegetation.

From a sample of pure TCQ, kindly given by Prof. G. T. Cunningham (Royal College of Surgeons), its ultra-violet and fluorescent spectra were determined (see Figs. 1 and 2), and it was by these characteristics that its presence was sought in samples of particulate matter which pollutes urban air. The samples were collected by filtration or electrostatic precipitation.

Four samples were examined: one was of pure coal smoke collected from an experimental chimney; two were of air solids collected on the roof of St. Bartholomew's Hospital and from the air-conditioned filter of the Royal Festival Hall; another was from the air in the Rotherhithe Tunnel, which is polluted by the exhausts of petrol and Diesel traffic. Because the amounts and nature of the samples varied there were minor variations in the techniques of analysis; but the following account gives the essential details.

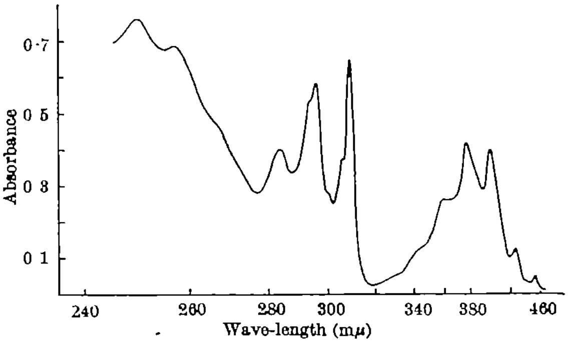

Fig. 1. Absorption spectrum of tri*cyclo*quinazoline in *cyclo*hexane. (Concentration, 4 3 μgm./ml.)

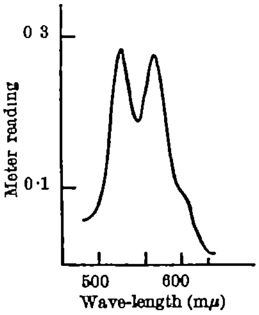

Fig. 2. Fluorescence spectrum of tri*cyclo*quinazoline in *cyclo*hexane (Concentration, 4 3 μgm./ml.)

5 gm. of collected air solids were extracted with hot benzene for 14 hr. The extract was shaken with caustic soda, acid and water in sequence to remove unwanted material. The benzene solution was then distilled to small bulk, divided into two equal portions, and one portion labelled with about 0·2 mgm. of TCQ.

Two chromatographic columns were prepared from a slurry of *cyclo*hexane and partially deactivated alumina. The two portions of the extract were separated on the columns, which were developed initially with *cyclo*hexane and then with benzene. The position of TCQ in the labelled column could be seen as a pale yellow-green fluorescent band and the fractions eluted which contained TCQ could be found by looking for an absorption peak at 310 mμ. The corresponding fractions in the test column did not indicate the presence of TCQ. The TCQ from the labelled column and the corresponding fractions in the second column were each separately bulked. M. W. Partridge (private communication) found the TCQ was little affected by treatment with hot alkaline potassium permanganate and this property of the compound was exploited to enable the removal by oxidation of other compounds present in the same chromatographic fractions suspected of containing TCQ.

The bulked fractions after evaporation to dryness were each oxidized for 1 hr. by boiling with alkaline permanganate, and after removing the excess oxidizing agent with sulphur dioxide were extracted with benzene. Each was again separated chromatographically on an alumina column and again the eluted fractions from the column were examined for the presence of TCQ. It was not found in the unlabelled column. The TCQ fractions were again bulked and re-oxidized, and separated chromatographically.

In none of the four samples examined was the presence of TCQ detected by these methods.

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Confirmation of the Gibbs Theory of Elasticity of Soap Films

As long ago as 1878 Gibbs gave an explanation for the fact that liquid soap films behave as elastic membranes, whereas pure liquids do not; he assumed that as a result of adsorption on the surfaces, the solution becomes depleted in surface-active solute, and therefore the surface tension increases with extension of the lamella. The modulus of elasticity, E, of a lamella is defined by:

$$E = 2A\frac{d\gamma}{dA} = -2\frac{d\gamma}{d\ln L} \qquad (1)$$

where γ is the surface tension of the lamella at an area A and thickness L.

Until recently, it was not possible to decide how far this theory is valid for real soap films. On one hand, no measurements of elasticity had been made. On the other, it was known that several other effects contribute to foaming—notably, the dynamic (Marangoni) elasticity, the damping effect of viscous and plastic adsorption layers and the long-range forces between the two parallel adsorption layers, which become important with very thin films ($< 0 \cdot 1\mu$) [1].

Recently, the first experimental determinations of the modulus of elasticity of films have been reported by Mysels, Cox and Skewis[2]. They devised an ingenious method of measuring the increased tension in a soap film while it was stretched to a measured extent, as determined by displacement of the interference bands. As the time-scale of the experiment was several seconds, there should have been negligible contribution from the Marangoni effect because equilibrium between the surfaces and the substrate would be reached within 0·01–0·1 sec. for these films, which were presumably 1–10μ thick. For 0·01 M solutions of sodium dodecyl sulphate, Mysels *et al.* recorded approximate values of the modulus of elasticity in the range 8–20 (mean 15 ± 4) dynes cm.$^{-1}$. The purpose of the present communication is to show, by calculating values of E from surface-tension data, that these results confirm the validity of the Gibbs theory.

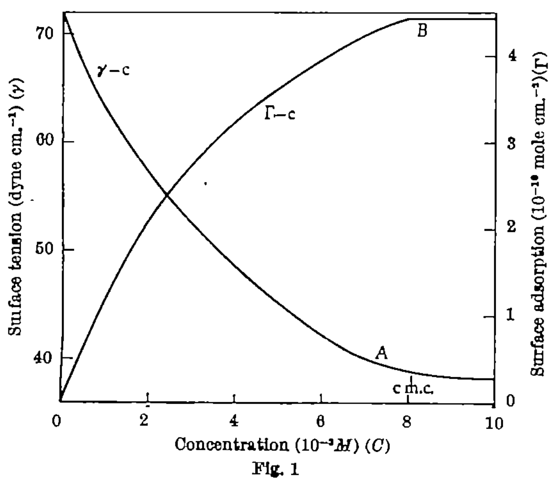

Fig. 1

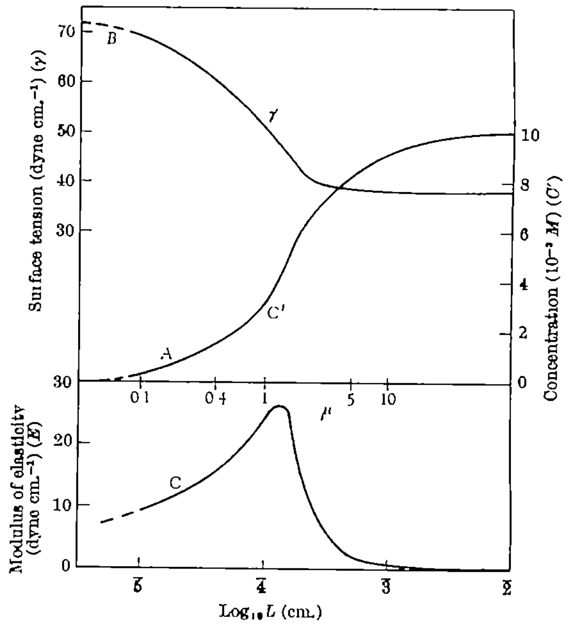

Fig 2

Fig. 1 (curve A) shows the results reported by Clayfield and Matthews[3] for the surface tension of solutions of pure sodium dodecyl sulphate. Curve B gives the corresponding adsorption values, Γ, calculated from curve A by means of the Gibbs adsorption equation, namely:

$$\Gamma = \frac{-1}{2RT}\cdot\frac{d\gamma}{d\ln(Cf_{\pm})} \qquad (2)$$

in which C is the concentration in moles l.$^{-1}$ and $f_{\pm}$ the activity coefficient of solute. (In absence of activity coefficients between the critical micelle concentration (c.m.c.) (0·008 M) and 0·01 M, Γ was assumed constant over this range, any error being negligible.)

If a solution of original concentration C_0 is extended into a lamella of thickness L cm., the depleted bulk concentration C' in equilibrium with the two adsorption layers containing Γ moles cm.$^{-2}$ is given by:

$$C' = (C_0 - 2 \times 10^3\,\Gamma/L) \qquad (3)$$

Curve A in Fig. 2 shows C' as a function of L for a solution starting at $C_0 = 0 \cdot 01\,M$: the values were obtained by solving equation (3) by successive approximations. From these values the corresponding surface tensions of the depleted solutions were read off from Fig. 1A and are plotted in Fig. 2B. Finally, the modulus of elasticity at different film thicknesses was obtained by graphical differentiation of 2B and use of equation (1). The final results are given in Fig. 2C.

It is seen that the Gibbs theory predicts elasticities to develop in this solution at lamella thicknesses in the range 5–0·1μ, with a maximum modulus of about 26 dynes cm.$^{-1}$ at a thickness of 1–2μ. The experimental value of 15 ± 4 obtained by Mysels, Cox and Skewis for films in the range 1–10μ is therefore in satisfactory agreement with the Gibbs theory.

This concord between theory and experiment confirms that the elasticity of thick soap films under quasi-static conditions is governed by the Gibbs effect. It does not mean, however, that the other effects mentioned here are unimportant in foams under other conditions; the evidence for long-range forces in very thin films, for example, is compelling

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Photochromism of o-Nitrotoluenes

THE crystals of 2-(2′,4′-dinitrobenzyl)-pyridine (I), which are colourless when kept in the dark, turn blue when exposed to light[1]. Hardwick, Mosher and Passailaigue[1] by using a cryoscopic technique have demonstrated that the change in colour is not confined to the crystals but occurs in dilute solutions as well. A similar change in colour has also been observed with 4-(2′,4′-dinitrobenzyl)-pyridine (II)[2].

Now, by using the flash photolysis technique, I have established the fact that *o*-nitrotoluene and a variety of compounds, all derivatives of *o*-nitrotoluene, are photochromic. In this work, a light pulse of high intensity was obtained by the discharge of 1,800 joules (9 μF., 20 kV.) through four straight quartz tubes (Lindqvist, L., Institute of Physical Chemistry, University of Uppsala) giving a flash duration of 5 μsec. (1/*e* time). Light emitted from the lamps of wave-lengths shorter than 250 mμ was absorbed in a filter surrounding the test solution. The transmission of 20 cm. of the solutions was recorded photoelectrically. All investigations were performed at room temperature (24° ± 1° C.) with the concentration of the compound being about 10^{-4} *M*. No detectable stray light from the exciting flash entered the recording system and the absorption spectra of the coloured solutions were determined at maximum optical density during the flash. The kinetics of the fading reaction were evaluated from the change with time of the light transmission of the coloured solution after the flash.

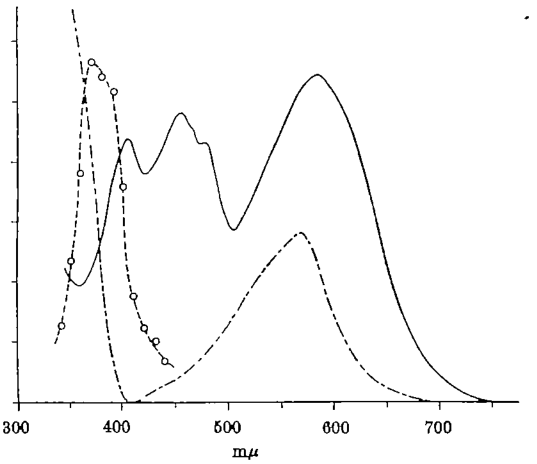


Fig. 1. Absorption spectra of ethanol solutions of *o*-nitrotoluene (– – –), 2-(2′,4′-dinitrobenzyl)-pyridine (–·–·–), and 2-(2′-nitro, 4′-cyanobenzyl)-pyridine (——) after exposure to ultra-violet light. The ordinate is proportional to optical density with different proportionality constants for the different solutions

Compounds examined were *o*-nitrotoluene (III), 2,4-dinitrotoluene (IV), 2-(2′-nitro,4′-aminobenzyl)-pyridine (V) and 2-(2′-nitro,4′-cyanobenzyl)-pyridine (VI) as well as I. It was shown that all these compounds have the property of becoming coloured on exposure to light and then becoming colourless in the dark.

The colourless compounds do not appreciably absorb light of wave-lengths longer than 330 mμ.

The absorption spectra of three of the coloured solutions are presented in Fig. 1. The irradiated solutions of I and VI maintained their colour long enough at – 70° C. to measure the spectra by means of a cryoscopic technique using a Cary 14 spectrophotometer[3]. The spectrum of the irradiated solution of III was obtained at room temperature by means of flash photolysis using a small Bausch and Lomb grating monochromator.

In absolute ethanol the fading reaction in all cases followed the kinetics of a first-order reaction. Rate constants are given in Table 1.

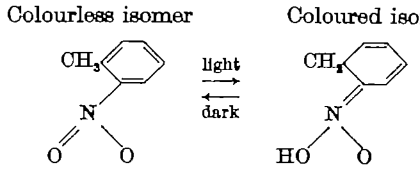
Table 1. RATE CONSTANTS FOR THE FIRST-ORDER FADING REACTIONS IN ABSOLUTE ETHANOL AT ROOM TEMPERATURE

Compound	Rate constant (sec.$^{-1}$)
o-Nitrotoluene	$1{\cdot}3 \times 10^{2}$
2-(2′,4′-Dinitrobenzyl)-pyridine	$1{\cdot}2 \times 10^{-1}$
2-(2′-Nitro,4′-cyanobenzyl)-pyridine	$3\ 7 \times 10^{-1}$

The cycle of becoming coloured and then reverting to the colourless state could be repeated several times without noticeable change in the behaviour of the solution. After 5–10 cycles, however, a change in the rates of the fading reaction was observed; in the cases of I and VI the rates are increased, but in the case of *o*-nitrotoluene the rate is decreased. This behaviour appears to be due to irreversible side reactions which render the solutions acidic since the same phenomena were observed when acid is added to the solution.

A detailed study has been made[4] of the fading reaction of I in water solutions under different conditions of *p*H and the rate of the fading reaction has been found to be increased by a factor of 10^5 when going from *p*H 12 to 2.

A probable mechanism for the change in colour is as follows:



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CRYSTALLOGRAPHY

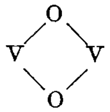
Structure of Vanadium Dioxide

GOLDSCHMIDT[1] came to the conclusion that vanadium dioxide had a rutile structure in which, in its idealized form, each vanadium would be surrounded by a regular octahedral arrangement of oxygen atoms. In fact, in actual rutile structures, the regular octahedral arrangement must always be lost[2] (as is in fact observed), though the unit cell will remain tetragonal, because four of the metal oxygen (or halogen) distances will remain equal. The other two could be different without any loss of symmetry, but it is found that usually they have virtually the same length as the other four (for example, in titanium dioxide four of 1·94 Å. and two of 1·99 Å. and in magnesium fluoride four of 2·00 Å. and two of 1·98 Å.)[3]. The arrangement of the six cations around a metal atom in a tetragonal rutile structure may therefore be visualized as "one at the north pole, one at the south, and four on the equator as a rectangle".

The structure of vanadium dioxide is sufficiently like this for Goldschmidt to have believed that it had a true rutile structure. However, it has been found more recently that the structure is distorted and is, in fact, monoclinic[4]. A detailed examination shows that the oxygen atoms lie very close to what may be described as the 'rutile positions' but that the vanadium atoms are somewhat displaced, the six VO distances being 2·03, 2·01, 2·05, 1·87, 1·86, 1·76 Å. Considerable stress has been placed on the fact that while in a rutile structure the metal atoms lie, equally spaced, on straight lines, the vanadium atoms in vanadium dioxide are spaced alternately 2·65 Å. and 3·12 Å. apart (instead of at regular intervals of about 2·87 Å.; the vanadium atoms are no longer quite on one straight line). As a result it has been proposed that there is metal–metal bonding[5], the vanadium atoms being bonded in pairs.

It has been claimed that evidence for this type of bonding is provided by the correlation of: (*a*) the axial ratios for rutile type phases; (*b*) the metal–metal bond-lengths, with the number of valence electrons supposedly available for each metal–metal bond. It is of course very possible that the degree of distortion may be dependent on the number of valence electrons present on each metal centre—but this may or may not be related directly with metal–metal bonding. It seems to us that there is no evidence that it is so related and in fact that metal–metal bonding does not appear likely to occur in vanadium dioxide or to provide a probable reason for the particular structural distortion that occurs.

The two close vanadium atoms are in a situation which can be represented by:



the four atoms being coplanar. Moreover these two oxygen atoms shown are particularly close to one another. Consequently, the space between the two vanadium nuclei is very much occupied by the electron clouds of the two oxygen atoms, and it is difficult to suppose that there is direct bonding between them in any ordinary sense. Moreover, if metal–metal bonding were the cause of the distortion it would be expected that the two vanadium atoms would be displaced directly towards one another. This is quite definitely not so. The vanadium atoms are displaced partly towards one another, or alternatively towards the oxygens lying between them, but even more towards one of the other oxygen atoms. It is as if there is a displacement of the vanadium atoms from their rutile positions so that a V_2O_4 molecule is beginning to be formed, with the structure shown in Fig. 1. The four oxygen atoms are approximately in a single plane, being close packed in that plane, the two vanadium atoms being placed so that each forms three pyramidally directed bonds, one vanadium being on one side of the oxygen plane and the other vanadium being on the other side. The whole structure is then a particular interlocking pattern of these V_2O_4 units. However, it must be stressed that the structure is really intermediate between this and a true rutile structure since the other three distances (2·01, 2·03, 2·05) are only a little greater than those in the V_2O_4 groupings (1·76, 1·86, 1·87), the incipient V_2O_4 units representing the way in which the true rutile structure is distorted.

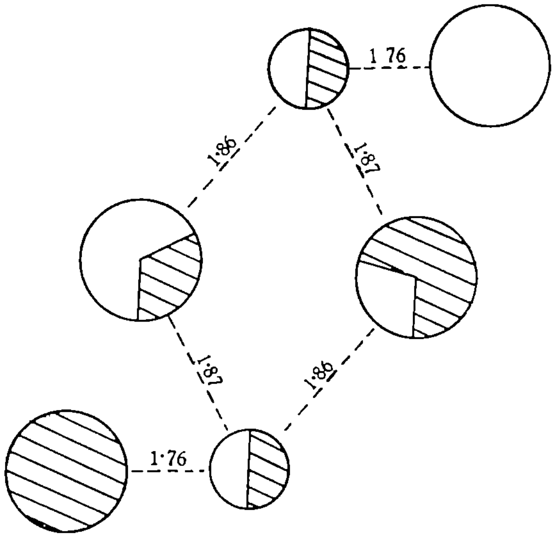


Fig. 1. Diagram showing the arrangement of atoms in the 'V_2O_4 units'. Large circles represent oxygen atoms, small ones vanadium atoms. The degree of shading represents the relative distances of the centres of the circles below the plane of the paper. The numbers give distances between centres (in Å.)

This structure will be analysed in more detail elsewhere and a comparison with the structures of VO, V_2O_3 and V_2O_5 will then be made.

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Crystal Structure of β-TiCl₃

β-$TiCl_3$ is usually prepared according to the reaction:

$$(C_2H_5)_2AlCl + TiCl_4 \rightarrow (C_2H_5)AlCl_2 + TiCl_3 + \tfrac{1}{2} C_2H_4 + \tfrac{1}{2} C_2H_6$$

by mixing heptane solutions of the reactants. The

Table 1

hkl	d^0_A own results	d^0_A Natta exp.	d^0_A calc	I own results	I Natta exp.	I calc Natta	hkl	d^0_A own results	d^0_A Natta exp	d^0_A calc.	I own results	I Natta exp.	I calc. Natta
100	5·41	5 43	5 48	*s*⁺	*s*	1222	114	—	—	1 32			0
110	—	—	3 14			1	204	*1 27*	—	1 28	*vw*		11
002	2·90	2 89	2 91	*w*⁺	*vw*	82	320	—	—	1 25			5
111	2 74	2 76	2 76	*s*⁻	*ms*	1017	402	*1 22*	—	1 23	*vvw*		2
200	—	—	2 71			70	321			1·22			9
102	2·56	2 57	2·57	*w*⁺	*vw*	173	223			1·22			58
112	2 12	2 14	2 13	*m*	*m*	577	214	*1 18*	—	1·19	*vw*		27
210	2 06	—	2 06	*w*		92	313			1 19			3
202	1 98	1 97	1 99	*w*⁺	*vw*	178	410	1 16		1 19			0
211	*1 92*	—	1 94	*vw*		30	411		1 16	1 16	*vvw*	*vvw*	96
300	1·80	1·81	1 81	*m*⁻	*w*	346	322			1·15			69
212	1·66	1 68	1 68	*w*	*vw*	144	304	1 14	1·14	1 14	*w*⁻	*vvw*	148
113	1·64	1 68	1 65	*w*⁺		183	412	1 09		1 10			110
220	—	—	1·57			0	115		1 09	1 09	*w*⁻	*vvw*	50
302	*1 53*	—	1 54	*w*⁻		40	500			1 09			1
221	1·50	1 52	1 51	*w*⁺	*vw*	128	224	—	—	1 07			0
310			1·51			75	314	*1 05*	—	1 05	*vw*		49
311	1·45	1 45	1·46	*w*⁺	*vvw*	7	323						6
004			1·46			56							
104	*1 40*	—	1·41	*w*⁻		41							
213			1·41			9							
222	1 38	1 39	1·38	*w*⁻	*vvw*	116							
400	*1 35*	—	1·36			39							
312			1 34	*vw*		20							

product thus obtained, however, is not suitable for determinations of X-ray structure, because of the poor quality of the powder patterns. Natta *et al.*[1] obtained better X-ray photographs from samples prepared by thermal décomposition of CH_3TiCl_3 (ref. 2). From these photographs they arrived at a crystal structure which is very similar to the structures of the needle-shaped modification of $TiBr_3$, TiI_3 and the zirconium trihalogenides[3].

However, a number of weaker lines calculated from this structure model were not observed, probably owing to the still insufficient quality of the X-ray patterns.

I obtained well-crystallized β-$TiCl_3$ by a very slow reaction according to the above equation using the apparatus shown in Fig. 1. Tubes *A* and *B* were filled with 0·1 molar solutions of $TiCl_4$ and $(C_2H_5)_2AlCl$ in heptane respectively. The apparatus was sealed under vacuum at *P* and *Q* and kept at room temperature for a month. During this period $TiCl_4$ slowly diffused to tube *B*, where butterfly-shaped crystal deposits were formed. These crystals were isolated under nitrogen and used for making X-ray powder photographs.

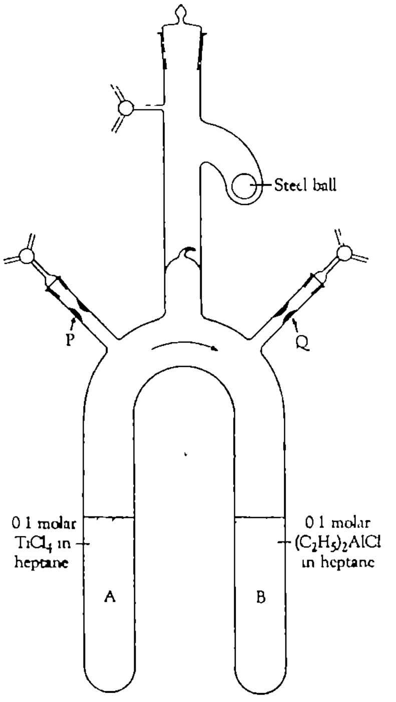
Steel ball
P
Q
0 1 molar $TiCl_4$ in heptane
0 1 molar $(C_2H_5)_2AlCl$ in heptane
A
B

Fig. 1

Table 1 gives the X-ray data of this compound and these are compared with those published by Natta *et al.* Columns 2, 5, and 7 show that the weaker lines, which were calculated but not observed by Natta *et al.*, were present in our photographs.

In the structure the *x*-parameter of the chlorine atoms features as the unknown. Natta found its value to be 0·315. Although the number of crystallographic data has been extended by this investigation, it is still too small to permit judgment on the reliability of the value mentioned. However, in view of the agreement between the experimental and calculated values of the intensities of the new reflexions found, it seems justified to conclude that the model of this β-modification is correct.

I thank Dr. Hn. de Vries for help with the apparatus.

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Crystal and Molecular Structure of *Cyclo*tetradecaheptaene

The crystallographic constants at room temperature of the alternant hydrocarbon $C_{14}H_{14}$, kindly supplied by Prof. F. Sondheimer and Dr. Y. Gaoni of the Organic Chemistry Department of this Institute[1], are: $a = 15{\cdot}05$ Å., $b = 4{\cdot}35$ Å., $c = 8{\cdot}63$ Å., $\beta = 106{\cdot}5°$, space group $P2_1/a$, X-ray density 1·12; $n = 2$, indicated molecular symmetry $\bar{1}$. If the molecular centre of symmetry is not an artefact due

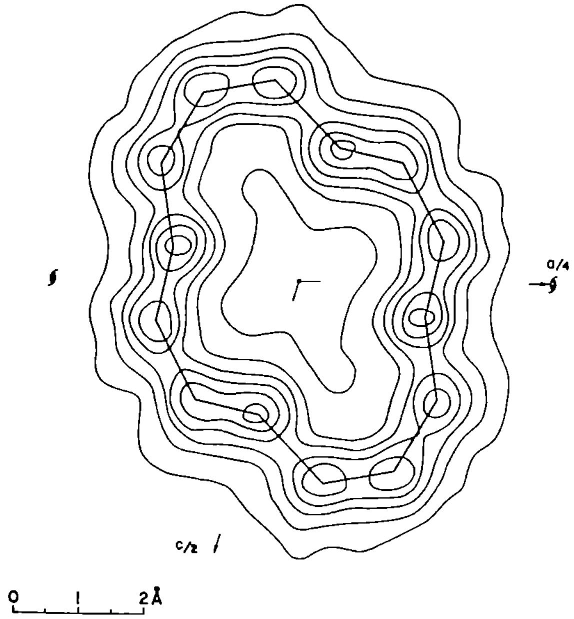

Fig. 1 $C_{14}H_{14}$, $\rho(x,z)$ at room temperature. Contours at arbitrary intervals

to packing disorder, it precludes the presence of alternating single and double bonds around the carbon ring, here as in $C_{18}H_{18}$ (ref. 2).

The intensities of the ($h0l$) reflexions (110 observed) were measured visually from zero-level Weissenberg photographs taken at room temperature, and corrected in the usual manner. The approximate molecular orientation of the molecules centred at (0,0) and ($\frac{1}{2}$,0) was determined by the permutation Fourier technique[3] from nine strong low-order reflexions (two reflexions with arbitrary signs, and seven reflexions permuted sixteen times). Trial co-ordinates derived from the most acceptable projection were refined on 'Weizac' by a least-square procedure, allowing one isotropic thermal parameter per atom, to a conventional R factor of 16 per cent. The final electron density projection is shown in Fig. 1.

The molecule is seen to correspond to a distorted pyrene structure. The projected distance between carbon atoms that in pyrene are bonded to the same interior carbon atom is 2·9 Å., already as long as the closest carbon-carbon approach reported in other overcrowded hydrocarbons[4]. It is probable that a major part of the overcrowding of the carbon atoms has been relieved by in-plane distortion of the carbon ring.

The full structure will be refined from three-dimensional low-temperature data when the possibility of lattice disorder will also be considered.

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BIOCHEMISTRY

Action of Chloretone on Reduced Cozymaze Oxidation by Cytochrome c

It has long been known that anæsthetics, including barbiturates and chloretone, suppress brain respiration *in vitro*[1-3], the suppression occurring at concentrations causing narcosis when brain respiration is stimulated by increasing the potassium ion concentration of the medium in which the brain tissue is incubated[4]. Michaelis and Quastel[5] concluded that anæsthetics, as represented by chloretone, suppress in the respiratory chain the activity of a flavoprotein forming a link between reduced cozymase (DPNH) and cytochrome oxidase. It was later shown[6], in accordance with this conclusion, that 'Amytal' suppresses the oxidation, by brain mitochondria, of DPNH and its oxidative phosphorylations. In view of the increasing use of 'Amytal' as a tool in the investigation of respiratory systems, we have carried out experiments to observe whether chloretone is effective in the suppression of DPNH oxidation by cytochrome *c* in a tissue homogenate or mitochondrial system. Chloretone offers a definite advantage over sodium amytal in not being ionized in solution and hence in not interfering with electrolyte equilibria.

Two sources of the DPNH cytochrome *c* reductase system[7] have been used.

(*a*) Rat brain homogenate. The animals were decapitated, the brains excised and homogenized in a Potter-Elvehjem homogenizer, under ice cooling, in 0·25 *M* sucrose with 0·001 *M* sodium versenate added. For each gram of fresh brain 10 ml. of the medium were taken.

(*b*) Dog liver mitochondria. A biopsy sample, weighing 15 gm., was taken under light 'Nembutal' anæsthesia and homogenized in an ice-cooled de Virtis homogenizer for approximately 1 min., in 10 × v/w in 0·25 *M* sucrose containing 0·001 *M* sodium versenate. The mitochondria were isolated in a refrigerated centrifuge at 0° C. The suspension was centrifuged at 700 *g* for 10 min. and the supernatant centrifuged again at 700 *g* for another 10 min. This was repeated once more. The sediment was discarded and the supernatant was centrifuged at 5,000 *g* for 10 min. The precipitate was washed in the centrifuge with the sucrose-versenate solution using a Potter-Elvehjem homogenizer. This is a modification of Hogeboom's method[8].

Table 1. EFFECTS OF CHLORETONE ON OXIDATION OF DPNH

Values give changes of optical density (at 550 mμ) per min. per mgm. protein

Tissue system	Control	With chloretone	
		0·67 m*M*	2·0 m*M*
Dog liver mitochondria	61·6	47·1	—
Rat brain	14·9	11·3	5·9

The assay was carried out with the Beckman *DU* spectrophotometer at 550 mμ. Protein was determined by the method of Folin–Ciocalteau using the Beckman *DU* spectrophotometer at 750 mμ. Chemicals were of analytical grade and were dissolved in triple distilled water. Into each cuvette the components were pipetted in the sequence given: 0·2 *M* *p*H 7·4 phosphate buffer, 1·0 ml.; cytochrome *c* (Sigma) 0·6 m*M*, 0·2 ml.; DPNH (Sigma) 6 m*M*, 0·1 ml.; sodium cyanide 7·5 m*M*, 0·2 ml.; sodium chloride 0·85 per cent to give a total volume of 3·0 ml.; chloretone 10 m*M*, 0·2–0·6 ml.; and enzyme suspension 0·2 ml. Temperature was 25° C. (thermospacer). Readings of optical density were taken either manually at 1-min. intervals or with the

Gilford optical density converter, and recorded with a Texas Servoriter No. 500. Units of activity are the changes in optical density/min./mgm. protein. Typical results are shown in Table 1. These results show that chloretone, like sodium amytal, exerts a considerable inhibition of DPNH cytochrome *c* reductase in a brain homogenate or in a preparation of dog liver mitochondria.

This work was supported by a grant from Abbott Laboratories. We thank Prof. J. H. Quastel for help in this work.

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Terminal Amino-Acid Analysis of Vasculokinase-activated Fibrin

IN recent experiments in this laboratory, a substance has been demonstrated in human aortæ which is capable of coagulating fibrinogen independently of thrombin[1]. This factor, vasculokinase, appears to have no esterase, amidase or gelatinase activity. Nevertheless, its capability of catalysing fibrinogen to fibrin suggests that it may be a proteolytic enzyme. In previous experiments by Lorand and Middlebrook[2], it has been shown that the *N*-terminal amino-acids of the fibrin monomer catalysed by thrombin are tyrosine and glycine. It has been suggested that there is a specific peptide site in the fibrinogen molecule which must be split in order for the monomers to polymerize[3-4].

In order to test this hypothesis, various fibrins were formed and coupled with 2,4-dinitrofluorobenzene according to Sanger's technique[5]. Thrombin-catalysed fibrin was made from Parke-Davis thrombin and partially purified fraction I (Armour). A similar product was prepared from Parke-Davis thrombin and a purified fibrinogen prepared by the Blomback's technique[6] (kindly provided by Dr. U. Westphal, Ft. Knox, Kentucky). Similarly, two vasculokinase-catalysed fibrins were prepared by the activation of Armour fibrinogen and Blomback fibrinogen with purified vasculokinase[7].

Various digestion processes of the coupled fibrin were utilized. These included digestion with 6 *N* hydrochloric acid under nitrogen, digestion with perchloric and acetic acids under nitrogen and reflux digestion with 6 *N* hydrochloric acid.

The coupled amino-acids were subjected to paper chromatography on Whatman No. 1 filter paper. 2 chloroethanol, pyridine, toluene and ammonia (6 : 3 : 10 : 6) were used in the organic dimension and phosphate buffer, 1·5 *M*, was used in the polar dimension.

The observation that the *N*-terminal amino-acids of thrombin-catalysed fibrin are tyrosine and glycine was confirmed. The *N*-terminal amino-acid of vasculokinase, however, was found to be phenylalanine. Inasmuch as the *N*-terminal amino-acids of fibrinogen are glutamic acid and tyrosine[8], it must be concluded that vasculokinase splits the fibrinogen molecule at a different site from thrombin. Although the liberated peptide has not as yet been recovered and evaluated for its size, these results suggest that vasculokinase is indeed a proteolytic enzyme. The results also suggest that there is more than one locus in the fibrinogen molecule which may be cleaved and still permit polymerization to fibrin fibrils.

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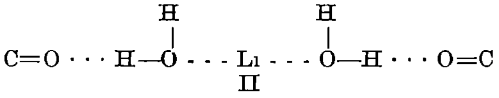
Evidence from Model Peptides relating to the Denaturation of Proteins by Lithium Salts

IN an earlier communication[1] we reported that mixtures of *N*-methylacetamide (NMA) or *N,N*-dimethylacetamide (DMA) with strong aqueous lithium bromide show viscosities much higher than those calculated on the assumption of ideal mixing. This was termed 'relative viscosity', which is different from the usual use of this term. It was proposed that the high viscosity is caused by aggregation arising from carbonyl–lithium (I) and carbonyl–water–lithium (II) associations.

$$\mathrm{C{=}O\ -\ -\ -\ Li\ -\ -\ -\ O{=}C}$$

I



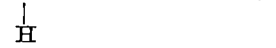
$$\mathrm{C{=}O\cdots H{-}\overset{\overset{\displaystyle H}{|}}{O}\ -\ -\ -\ Li\ -\ -\ -\ \overset{\overset{\displaystyle H}{|}}{O}{-}H\cdots O{=}C}$$

II

Dots indicate hydrogen bonds, and dashes ion-dipole interactions. These associations were also considered to occur in protein denaturation. We now report evidence for the existence of both types of association, as well as peptide-anion associations.

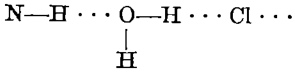
We had reported[1] that on mixing NMA and 13 *M* lithium bromide (incorrectly reported as 12 *M* before) in the volume ratio 4 : 1 (also molar ratio), respectively, a crystalline precipitate was obtained. The crystals dissolve in their mother liquor at about 53° C. The crystals contain NMA and bromide in the molar ratio of 4 : 1, by analysis. The structure of the crystal has been determined by means of X-ray diffraction by Dr. David Harker and Mr. David Haas, to whom we are grateful for permission to describe some of their results. The detailed structure will be published elsewhere after additional refinement. The main features of the structure that are of interest with regard to protein denaturation are as follows: each lithium is surrounded by four carbonyl oxygens and two water oxygens; each bromide is surrounded by hydrogens of four N—H groups and of two water molecules. Each water molecule forms a bridge between a lithium and a bromide. Bromide to H—N association has recently been proposed by Bufalini and Stern[2] to account for infra-red data obtained from dilute solutions of NMA and tetrabutylammonium bromide in benzene. We have not been able to apply our viscosity method with tetramethylammonium bromide due to immiscibility with the amides at the high concentrations required.

The participation of water in the aggregates was investigated by the use of deuterium oxide (all viscosity data are corrected for density). A mixture of 64 volume per cent DMA and 36 volume per cent 10 *M* lithium chloride in deuterium oxide is 1·13 times as viscous as a similar mixture made with water. For mixtures of NMA and lithium chloride the deuterium oxide system is 1·16 times as viscous as the water system, using NMA in which the N—H previously had been exchanged to N—D. The viscosity of 10 *M* lithium chloride in deuterium oxide is 1·16 times as great as that of 10 *M* lithium chloride in water. The amide–lithium salt system has only 0·36 of the water content of 10 *M* lithium chloride alone. Therefore, in the above mixtures of amide and 10 *M* lithium chloride the viscosity increase due to deuterium is 2·3–2·8 times the increase due to deuterium in 10 *M* lithium chloride without amide. These results show a significant participation by water, for which formulations II–IV are reasonable. Lithium–chloride ion pairs or higher ion aggregates may also participate.



$$\mathrm{C{=}O \cdots Li \cdots \underset{\substack{|\\ H}}{O}{-}H \cdots Cl \cdots H{-}N{-}}$$

III



$$\mathrm{N{-}H \cdots \underset{\substack{|\\ H}}{O}{-}H \cdots Cl \cdots}$$

IV

In comparing DMA–deuterium oxide with DMA–water, the viscosity increment due to deuterium is 11 per cent compared with 13 per cent given above for the complete system, DMA–salt–water. Correcting for the lower water content in 10 *M* lithium chloride (43 *M*) than in pure water (55 *M*), the deuterium increment for DMA–lithium chloride–water is 30 per cent greater than for DMA–water. It thus appears that the potency of water in aggregating amides is augmented by its association with lithium ions, which would be expected to increase the hydrogen bonding ability of water by decreasing the shielding of the proton. Also a group of water molecules around a lithium ion has many possibilities for hydrogen bonding, leading to large aggregates.

There may also be interactions between the halide ion and the peptide dipole. This is of interest especially in connexion with peptides of imino-acids. No crystalline complex of DMA and lithium bromide could be formed under our conditions; but Kumler[3] has prepared the solid hydrochloride of DMA, the structure of which would be of interest. The higher viscosity of DMA-lithium bromide compared with NMA-lithium bromide[1] may be only apparent, the resultant of an increase due to the salt and a decrease due to disruption of the structure of liquid NMA, which is more viscous than DMA.

That both ions are involved in the aggregation is suggested not only by X-ray diffraction, but also by the fact that 7·4 *M* sodium bromide raises the viscosity of NMA half as much as does 8 *M* lithium bromide, but about three times as much as does water. Lithium bromide is somewhat more effective than lithium chloride, the peak relative viscosities for 10 *M* salt with DMA being 14 and 12, respectively. (The value of 8 for the peak viscosity previously reported was obtained with unpurified DMA; ion exchange followed by distillation through a 60-cm. column packed with stainless steel mesh resulted in the new value of 14.) The greater effect of bromide is in accord with the well-known fact that bromides are more effective denaturants than chlorides, although the hydrogen bonding and ion-dipole interactions of chloride are expected to be greater than for bromide. Fluoride may be an anti-denaturant; it raises the melting point of a gelatin gel[4].

The various peptide-ion interactions proposed may be involved in protein denaturation. It is possible that unfolding of a particular protein may occur by another mechanism, for example, disruption of the water structure. The unfolded protein may enter into the associations proposed here. At the low protein concentrations normally examined there would be little aggregation. In concentrated protein solutions the salt and water may cause aggregation as indicated by the following observations. The relative viscosity (in the usual sense as applied to polymers) of 1 per cent gelatin in 7 *M* lithium bromide is about 10 per cent higher than in dilute buffer, while at 35 per cent gelatin concentration the relative viscosity in lithium bromide is six times as great as in water.

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Deoxyriboside Control and Synchronization of Mitosis

In seeking simple physiological methods for blocking followed by synchronization of cell division, materials were sought which, applied for prolonged periods, permitted synthesis of ribonucleic acid (RNA) and protein but inhibited cells immediately prior to DNA replication. During this I used excess (2 m*M*) thymidine as a blocking-synchronizing agent.

Monolayers of genetically heterogeneous Chang-appendix cells were grown in medium 199 supplemented with 20 per cent bovine serum in sloping 20 ml. tubes with 3 c.c. medium. The shortest doubling times observed were about 20–23 hr. After 4 days growth (at 36°–37° C.) the tubes contained about 300,000 cells, and were beyond conditions permitting logarithmic growth. Normal cultures showed a mitotic index varying from about 3–7 per cent. At 4 days the medium was decanted and excess thymidine introduced in fresh medium. After 24 hr. (at time 0 hr.) at 36°–37° C. the tubes were washed with two lots of 3 c.c. Hanks's balanced salt solution before addition of fresh growth medium. The monolayers were inspected periodically (× 75 using × 10 objective) in the constant-temperature room. Direct counts of mitoses per field were made in 5–8 fields in a standard region of the tubes. The average number per field was used as the index of mitotic activity.

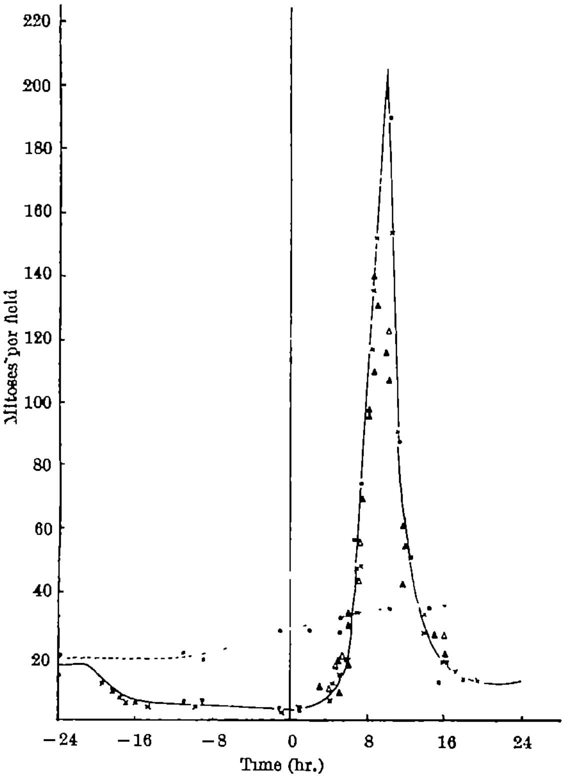

Fig. 1. Mitoses in Chang-appendix monolayer in 199–20 per cent bovine serum. The continuous line shows the inhibition of mitoses during 2 m*M* thymidine blockage (thymidine applied at −24 hr. and washed off at 0 hr.), and the parasynchronous burst of mitoses with maximum at 10 hr. post-wash. The broken line, based on a limited number of counts, indicates the level of mitotic activity in control tubes subject to similar washing at 0 hr.

The curve in Fig. 1 showing the mitotic counts per field over the 24 hr. before and after the removal of thymidine is drawn to the readings of Exps. 1 and 2 performed in successive weeks. The readings made in Exps. 3*a* and 3*b* (begun on the 5th day and with less cells per tube) were added to the graph later. No toxic effects of the 2 m*M* thymidine either during treatment or during the following week were observed. Mitoses appeared normal throughout and were roughly estimated to take about 2 hr. in my material. After removing the block the minimal mitotic index observed in its presence persisted for about 4 hr. The parasynchronized burst began at about 5 hr. and reached a maximum mitotic index at about 10 hr., declining rapidly thereafter. Between 1 hr. and 7 hr. about 7–8 per cent *C*-mitoses accumulated (0·00001 per cent colchicine). Accumulated *C*-mitoses in the 6¼–12¼ hr. period were about 44 per cent, and in the 6¾–12¾ hr. period 40 per cent. 6 per cent *C*-mitoses accumulated in the 17–23 hr. period. Hæmocytometer counts of acetocarmine preparations gave a mitotic index of 11 per cent at 8¼ hr. (Exp. 3*b*) and 19 per cent at 9¾ and 10 hr. (Exp. 2) when counts per field were 90 and more than 180 respectively.

To obtain several successive spikes of near 100 per cent synchronous mitoses clones of selected material in log-phase growth conditions need to be used. The present culture conditions are designed only to facilitate detection of possible physiological blocking-synchronizing agents.

In Exp. 3*b*, 2 m*M* thymidine was re-applied at ½, 1, 1½, 2, 3, 4, and 5 hr. or not at all. All tubes showed a peak of mitoses at about 9¾ hr., the peak itself being lowest (about 60 per field) in those to which the block was re-applied at ½–3 hr., and more than 130 per field in the tube to which the block was not re-applied. The earlier the block was re-applied, however, the slower was the decline of the mitotic count in the post-peak period and the higher the level at 15 hr. The percentage *C*-mitoses accumulated between 6¾ hr. and 12¾ hr. in one tube of each of the eight treatments was 26, 21, 21, 20, 22, 20, 26, and 40 respectively. A few tubes were left in 2 m*M* thymidine for up to 2½ days, with fresh medium containing the same concentration of thymidine being supplied at 40 hr. Mitotic activity began to rise after 30–34 hr. and gradually over the next 24 hr. reached the level (mitoses per field) of the normal controls at 0 hr.

The explanation of the thymidine block to mitosis must be sought largely in the cells' enzymatic mechanisms for the synthesis of deoxycytidine triphosphate from cytidine-5′-phosphate and the inhibition of this synthesis by excess thymidine. Reichard[1] found in cell-free extracts of chick embryo that deoxycytidilate synthesis was strongly inhibited not only by thymidine, but also by the triphosphates of deoxyadenosine and deoxyguanosine. Deoxyadenosine has been reported to inhibit synthesis of DNA in Ehrlich ascites tumour cells[2,3] and in chick embryo mince[4].

In experiments on the Chang-appendix cell system 2 m*M* deoxycytidine did not act as a brake on cell proliferation. A burst of parasynchronous mitoses similar to that obtained by washing was observed in cultures blocked for 24 hr. with 2 m*M* thymidine after simply adding 2 m*M* deoxycytidine to the tubes. The close agreement of the two curves suggests that excess thymidine acts simply, preventing synthesis of DNA by inhibiting the formation of deoxycytidine. In further experiments 2·5 m*M* deoxyadenosine and 1·5 m*M* deoxyguanosine (24 hr. application) each blocked mitosis and their inhibition was released by washing, resulting in delayed parasynchronized bursts of mitoses. Thymidine (2 m*M*, 24 hr. application) appeared not to be toxic to the cells to any significant extent, and has so far proved a more suitable blocking synchronizing agent than the other two deoxyribosides. Since prolonged exposure to thymidine did not produce giant cells it would appear that synthesis of RNA and protein ceased at the point normally appropriate to replication of DNA. It may be supposed that mobilization of spindle protein and spiralization of the chromosomes, etc., awaited replication of DNA and the release of some activator from the doubling chromosomes. The thymidine-blocked system may be useful in attempts to obtain controlled synchronous meiosis. The relevance of the results to the further study of the mitotic cycle and its changing properties is obvious. The use of excess thymidine, and the other deoxyribosides as a possible means of distinguishing DNA from RNA viruses, and for their possible therapeutic effect in cell-virus systems, may be noted.

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Identification of the Main Ethylenediamine Condensate of Noradrenaline with that of Catechol

My attention has been directed to a recent communication[1] by T. Nagatsu and K. Yagi dealing with the identification of the main ethylenediamine condensate of noradrenaline with that of catechol. The authors report some experiments, quote an infra-red spectrum and two microphotographs of crystals. Also, the sentence occurs: "However, the precise identification of these compounds in the pure form has not yet been reported".

In fact, not only "the precise identification of these compounds", but also a rigorous proof of the chemical structure and an independent synthesis of these compounds was reported in a detailed paper by J. Harley-Mason and A. H. Laird more than two years ago[2], and all the experiments reported by the Japanese authors are contained in this paper.

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[1] *Nature*, 193, 484 (1962).

[2] *Tetrahedron*, 7, 70 (1959).

Though we missed the report by J. Harley-Mason and A. H. Laird (*Tetrahedron*, 7, 70; 1959), we realize that the results reported in our communication are identical with a part of the results reported by them. Therefore, as a result, our communication serves to confirm their results.

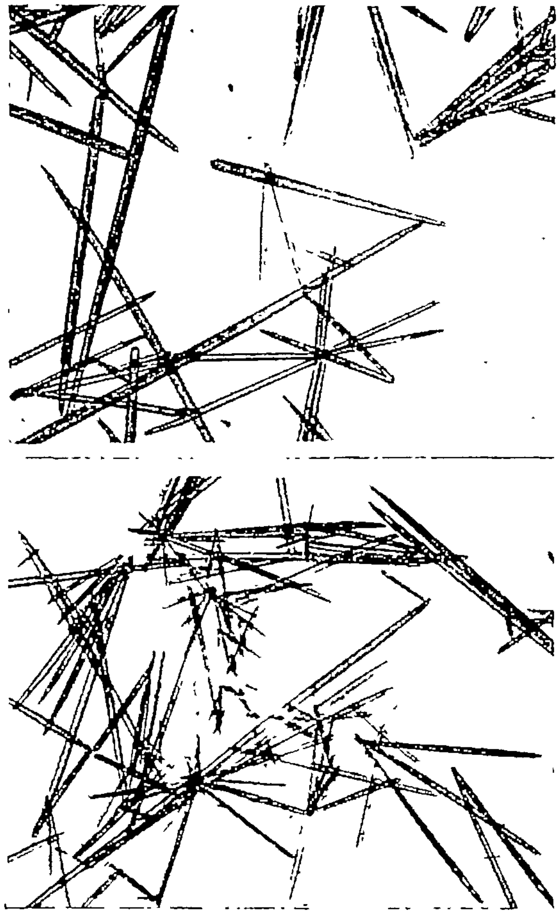

Fig. 1. Picrate crystal of a main ethylenediamine condensate of catechol (above); noradrenaline (below) (× 1,000)

We may add another fact confirming the identification of both the main condensates. We isolated both substances as picrates. Both picrates formed similar, needle-shaped, orange red crystals (Fig. 1)

The infra-red spectra of both picrates were identical. The result of elementary analysis of picrate crystals of the main noradrenaline–ethylenediamine condensate was as follows: calc. for $C_{10}H_{10}N_4 \cdot 2C_6H_3N_3O_7$, C: 41·00, H: 2·500, N: 21·74, O: 34·76; found, C: 40·96, H: 2·695, N : 21·67, O : 34·55 per cent.

This result corresponds with the dipicrate of tetrahydro-tetra-aza-anthracene, confirming the result of Harley-Mason and Laird.

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Determination of Free Amino-Acids in Human Ejaculate by Ion Exchange Chromatography

Reports on investigations concerning the content of free amino-acids in human ejaculates are rare. Sakar, Luecke and Duncan[1] estimated the amino-acid composition by microbiological methods. Adam and Korting[2], Jacobsson[3] and Lundquist[4] separated amino-acids from human sperm liquor qualitatively by paper chromatography. Keutel and Gabsch used paper chromatography for qualitative and quantitative determinations of amino-acids from ejaculates[5].

We analysed quantitatively the content of free amino-acids in sperm liquor by ion exchange chromatography[6] using an automatic recording equipment[7]. The method is capable of giving reproducible recoveries of better than 100 ± 3 per cent for quantities ranging from 0·25 to 2 μmoles of amino-acid. One complete analysis required 2 days.

Four to six hours after ejaculation, a 10-fold amount of 1 per cent picric acid according to Hamilton's method[8] was added to the ejaculate. The picric acid precipitate was removed promptly by centrifugation. To remove excess picric acid from the protein-free ejaculate, the solutions were passed through columns of 'Dowex 2' (2 cm. diam.)[9]; a bed 2 cm. in height was used. The colourless effluents were filtered with 'Celite' if cloudy and hydrolysed for 24 hr. with 100 ml. 6 *N* hydrochloric acid under reflux to split peptides and conjugated amino-acids, if present. The hydrolysate was dehydrated three times in a rotary evaporator, treated with 'Celite', when necessary and made up to a volume of 5–10 ml. with a 0·2 *N* sodium citrate buffer, *p*H 2·2 [10]. All solutions were kept frozen prior to chromatography. An amount of the hydrolysate corresponding to 0·5 ml. of the original ejaculate was chromatographed in most instances. The samples were analysed on columns of 'Amberlite *IR*-120' using a self-designed amino-acid analyser[11].

Table 1 shows the average values of 10 patients with normospermia, 5 with azoospermia combined with Leydig cell insufficiency and one patient with aspermia due to deformity of accessory sexual glands. Fig. 1 demonstrates a typical effluent curve of free amino-acids from an ejaculate of a case of normospermia.

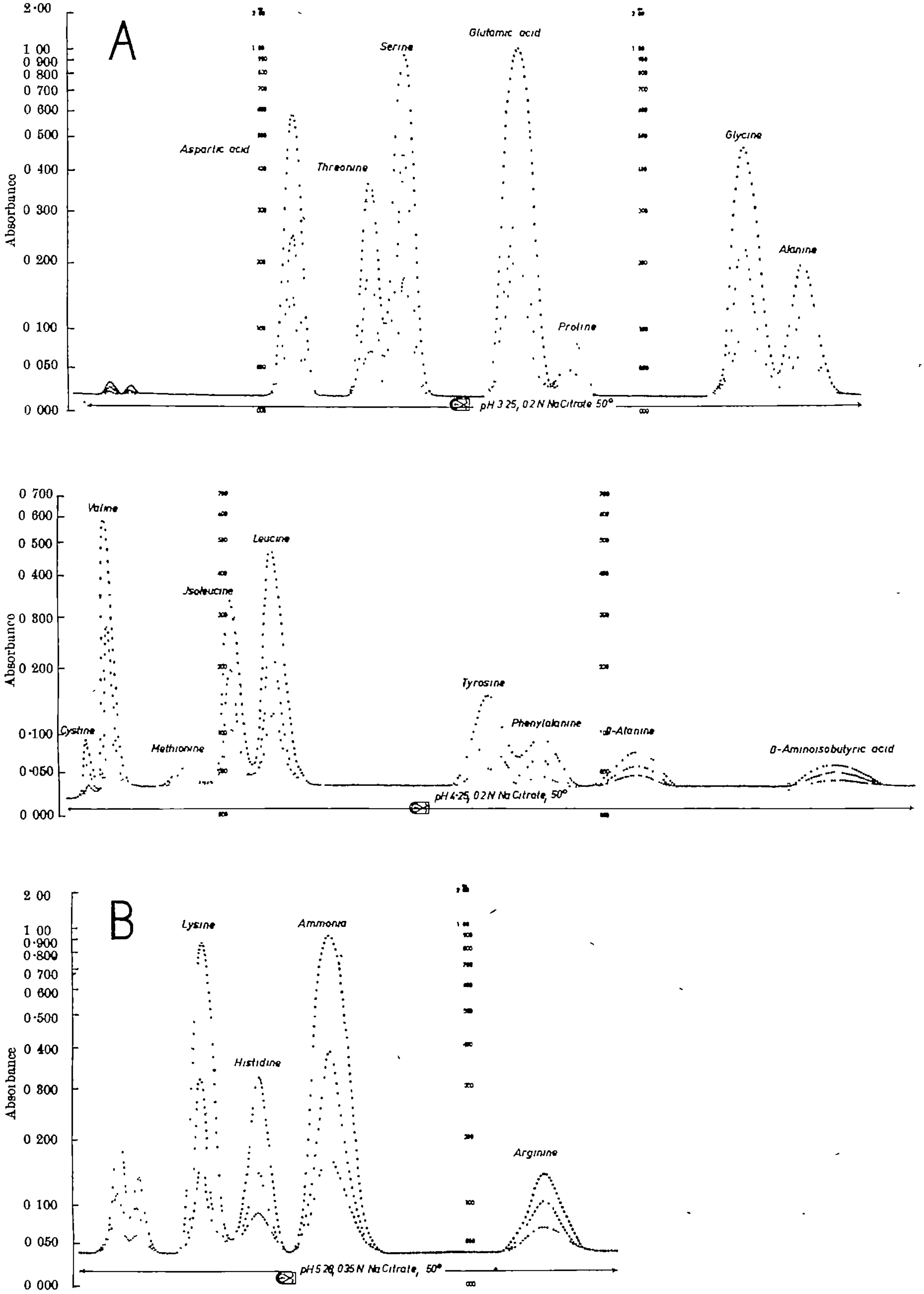

Fig. 1. Typical effluent curve of free amino-acids from an ejaculate of a case of normospermia. *A*, 150-cm. column 'Amberlite *IR*-120' at 50°, *B*, 15-cm. column of 'Amberlite *IR*-120' at 50°

Table 1. Amino-Acids in Human Ejaculate and Blood Plasma Determined by Ion Exchange Chromatography

Neutral amino-acids	Normo-spermia (mgm /ml.)	Azoo-spermia (mgm./ml.)	Aspermia with deformity of accessory sexual glands (mgm./ml.)	Blood plasma (mgm./ml.)
Methionine	0·038	0·007	0·011	0·0008
Cystine	0·034	0·006	0·015	0·010
Glycine	0·589	0·230	0·071	0·018
Alanine	0·291	0·096	0·043	0·036
Proline	0·269	0·139	—	0·016
Tyrosine	0·514	0·232	0·101	0·009
Valine	0·498	0·134	0·100	0·037
Phenylalanine	0·282	0·164	0·112	0·009
Threonine	0·477	0·190	0·115	0·012
Serine	1·116	0·429	0·104	0·010
β-Alanine	0·360	0·158	0·152	—
β-Aminobutyric acid	0·317	0·053	—	—
Isoleucine	0·624	0·191	0·087	0·023
Leucine	0·967	0·332	0·228	0·013
Total	6·376	2·361	1·139	0·1938
Acidic amino-acids				
Aspartic acid	0·998	0·336	0·187	0·007
Glutamic acid	1·797	1·552	0·397	0·094
Total	2·795	1·888	0·584	0·101
Basic amino-acids				
Arginine	0·790	0·295	0·094	0·014
Lysine	1·521	0·580	0·183	0·027
Histidine	1·091	0·398	0·079	0·014
Total	3·402	1·273	0·356	0·055

It is remarkable, however, in those cases having Leydig cell insufficiency and aspermia with normal function of Leydig cells and deformity of accessory sexual glands, the values were essentially lower than in cases of normospermia.

It could be observed that the amino-acid content in the semen was higher than in the blood plasma.

Normal and pathologically changed values of initial fructose did not run parallel with the amino-acid values. One patient with azoospermia due to a stoppage of spermiogenesis and normal function of Leydig cells showed no particular difference from normal values.

It was seen that the content of amino-acids from an individual ejaculate was rather constant when picric acid was added at the time after ejaculation. An increase in the values of amino-acids after ejaculation could be observed in ejaculates of only some of the tested patients.

The content of amino-acids as well as fructose and inositol in human ejaculates probably depends to a high degree on the normal function of the Leydig cells. Owing to the expensive and time-consuming method, this type of amino-acid determination in human ejaculate is not suitable for routine analysis such as the estimation of fructose.

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PHYSIOLOGY

Permanent Fibrin Film on the Intima of the Vessels

According to Copley, the intima of blood vessels is lined with a thin fibrin film which protects the surface from becoming wet and facilitates the flow of blood[1]. There are indications that fibrin forms at the sites of physiological defects of the vessels[2]. Fibrin is being continuously formed during fissure in the vessels under the influence of the blood's fibrinolytic activity[1,2]. These views have so far not been checked. The possibility of continuous fibrin formation is denied on the basis of investigations of fibrinogen iodine-131 turn-over. It was found that the half-life of fibrinogen in dogs is not changed when the animals were given heparin or coumadil, the drugs that inhibit the transformation of fibrinogen into fibrin[3]. Our experiments testify against the existence of a permanent fibrin film on the intima of vessels.

The presence of a fibrin film on the intima of the vessels would inhibit the passage of albumins through the vascular wall[4]. Evans blue forms complexes with plasma albumins, and consequently the rate of its excretion from the blood is an indicator of the speed at which the plasma albumin passes through the vascular wall[5]. An investigation was carried out to find the rate of excretion of Evans blue from the blood of 9 normal dogs and 19 animals of which the blood fibrinolytic activity had been stimulated by heparin[6] or nicotinic acid[3]. A 0·2 per cent solution of Evans blue was administered (0·6 ml./kgm.) intravenously in dogs 30 min. following the intravenous administrations of heparin (5 μ/ml. of blood) or nicotinic acid (1 mgm./kgm.) solutions. An increase of the blood's fibrinolytic activity should, presumably, create conditions for the formation of the fibrin film.

The concentration of Evans blue in the plasma was determined by photometry at 610 mm. with 30-, 60-, 90-, 120-, 150- and 180-min. intervals following the injection of the dye into the blood stream. The exponential curves (the axis of the ordinate showing concentration of Evans blue, and that of abscissa indicating time) were used for calculating the time of the semi-excretion of the dye from the organism. The fibrinolytic activity of the blood was determined by the partial lysis time (3 hr.) of a fibrin clot[7] and by the time of lysis of the euglobulin fraction (euglobulin time[8]). The experiments were conducted simultaneously on experimental and control dogs.

As a result of Evans blue injection, the fibrinolytic activity of the blood in heparin experiments was increased on the average by 25 per cent, while coagulation took 300 min. (5–8 min. in normal animals). The semi-excretion time of Evans blue in heparin-fed dogs (205, 8 ± 21 min.) and that in the animals which did not receive the drug (205, 8 ± 26 min.) proved the same.

In those animals which received nicotinic acid the blood coagulation-rate was increased somewhat by administration of Evans blue, and the fibrinolytic activity of the blood showed an abrupt rise (Table 1).

The difference probability (P) was calculated by the Student's t test with respect to the initial data.

Nicotinic acid also failed to change the rate of Evans blue excretion from the blood (Table 2).

Heparin and nicotinic acid were administered in doses which accelerated fibrinolysis. In this process conditions were created for dissolving the fibrin film, had it been on the intima of blood vessels. Moreover,

Table 1. Fibrinolytic Activity and Coagulation-Rate of Blood following the Administration of Nicotinic Acid

	Statistical indices	Prior to administration	Following administration
Coagulation-rate (sec.)	M	336	234
	P	18	0 001
Partial lysis time of fibrin (per cent)	M		41
	P		0 001
Euglobulin time (min.)	M	322	179
	P		0 001

Table 2. Semi-excretion Time (min.) of Evans Blue from the Blood of Animals during the Administration of Nicotinic Acid

Statistical indices	Experimental dogs	Controls
M	160	160
$\delta \pm$	24·5	14 4
$m \pm$	7 6	6 5

in experiments with heparin, the formation of fibrin from fibrinogen anew was quite impossible. In spite of this, the rate of passage of Evans blue, and hence plasma albumin, through the walls of blood vessels, did not change. This last observation in particular casts doubts on the existence of a fibrin film on the vascular intima as well as on the participation of fibrin in normal vascular permeability.

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Arterial and Tonometric Pressure Measurements in the Eye

An accurate tonometer has been described for measuring the pressure within the human eye, and within various other body cavities[1,2]. It has an exact theory, can be used in any orientation, and is sufficiently fast and gentle so that it does not require anæsthesia for application to the human cornea. The probe of this unit is pressed against the eye and, in essence, if the cornea is momentarily flattened to slightly beyond a pressure sensitive spot on the tip of the flat end of the probe, then the only force sensed will be the intraocular pressure. However, satisfactory results were not obtained on application to sclera. This has now been demonstrated to depend in part on the adjustment of the degree of flatness of the probe end.

The pressure-sensitive spot in the centre of the probe is a rod the end of which is coplanar with the surrounding plate, and which activates a differential transformer[3]. For reasons of cleanliness, the unit is encased in a thin sterilized cover before use. If the output of the instrument as a function of pressure in a test cavity is plotted (Fig. 1), one sees that the result depends on the initial perfection of the plunger setting. Fig. 1 shows the result of application of the tonometer to a condom at the base of a water column, but similar curves are obtained with excised rabbit eyes and with rabbit eyes *in vivo*. If the plunger initially projects beyond the base plate then the cornea and/or the rubber covering will effectively increase the area of the plunger until increasing pressure moulds them down into full contact with the projection. Similarly, the rubber covering can sustain some of the force if the plunger is initially recessed, until it has been fully moulded down into the recess. This leads to curves that are concave downward and upward respectively, as shown. Corneal readings are usually lower than scleral in the former case, and vice versa. This indicates the sclera to be the less elastic. All the curves eventually become parallel when just the actual area of the plunger is effective. It can be seen that, for a 1·5-mm. diameter plunger, a 5μ initial projection is ideal. A tonometer when adjusted this way indicates its plunger to be projecting 5μ when pressed into contact with a flat glass plate. This may apply only to a given method of construction in which irregularity can extend the plunger surface above and below the surrounding plate, thus leaving the average almost coplanar.

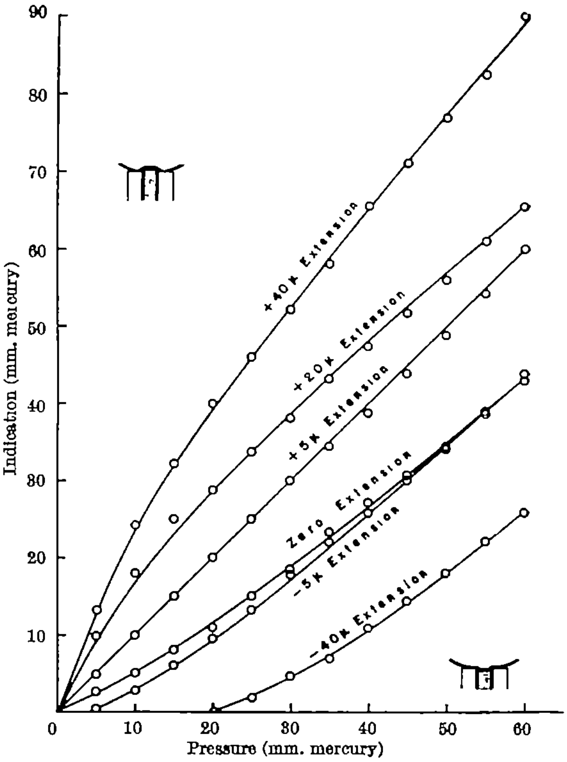

Fig. 1. Response of Mackay–Marg tonometer with initial plunger extension as parameter. Curves concave upward result if the plunger is initially recessed, as shown by the sketch in the lower right corner, and the curve is concave downward if the plunger is initially extended as shown by the sketch in the upper left corner

A unit tested by the water column measurement to be linear through the origin is often found to give the same reading when applied to the cornea or to the sclera in man and rabbits. However, in a significant number of human subjects scleral and corneal readings sometimes do not agree. Tests were made on a dozen subjects using two tonometers (a type manufactured by Biotronics, Financial Center Building, Oakland, California) simultaneously on a single eye, one tonometer being applied to the temporal sclera and the other to the nasal sclera, or one to the cornea and one to the sclera. These readings were often identical, but not always so. This is probably due to the irregular and somewhat compressible and viscoelastic structure of the sclera. We have noticed occasional unreliable readings in similar larger instruments applied to the abdomen due to inhomogeneities (fat lumps) in the wall, presumably for similar reasons. A larger plunger might help here.

Scleral application was desired so that it would be possible to look into the eye while pressing. Since the end of the probe need be only about 5 mm. in diameter. it can instead be applied to the side of the cornea near the limbus and still leave room to observe the eye through an ophthalmoscope. Raising the pressure within the eye to diastolic by pressing will cause the appearance of appreciable pulsations in the blood vessels, and further increase in pressure to systolic will cause the disappearance of pulse due to collapsing of these vessels. This tonometer accurately records the pressure within the eye during such a procedure. It is observed that different regions may start and stop pulsating at somewhat different pressures. A discussion of these pressures has appeared elsewhere, and the interference with the state of normality that measurement produces[3]. A clinical criterion of insufficiency on one side is often taken to be a 25 per cent difference in systolic pressure between the two eyes. The use of a less-exact tonometer and spring-produced pressure is sometimes termed ophthalmodynamometry. A survey of values will be reported separately, but it can be stated here that, of the first half-dozen subjects tested, the diastolic and systolic pressures fell very roughly in the ranges of 50 and 100 mm. of mercury. In all such observations it is found that pressure does not distort the cornea sufficiently to make ophthalmoscopic observation impossible past the de-centred tonometer. Alternatively, one can record the pressure at which the subject's vision disappears. One can also apply the tonometer centered on the cornea with steadily increasing pressure, and in the record the points of maximum oscillation and cessation of oscillation can be taken as the diastolic and systolic pressures, respectively. However, it is not clear to which vessels these readings apply. A suction method[4] can be used to force contact between the present tonometer and the eye to reduce the effect of extraocular vessels, if visual inspection is not being used.

The pressures in the blood vessels are usually considerably larger than the intraocular pressures observed in tonometry. Because of the eventual parallelism of the curves in Fig. 1, pressure indication errors due to lack of coplanarity tend to be constant in the absolute rather than the percentage sense, and thus adjustment is less critical than for tonometry. It has been found that with the Mackay–Marg tonometer when anæsthetics are not used a somewhat greater variability in pressure occurs than if anæsthetics are used. The operator must select a relaxed instant so that the pressure, which will be accurately measured, is not artificially raised by squinting, etc. The presence of corneal bending forces puts a dip in the recorded pressure vs. time curve that is an excellent indicator of the proper degree of corneal flattening, and hence shows where on the graph the reading should be made. With the best possible centering of the probe on a rabbit eye, the pressure corresponding to this trough will be raised by less than 0·5 mm. mercury due to the observation process. Accidental de-centering of the probe by 1·5 mm. will almost cause the loss of the characteristic tonogram form, and the actual pressure in that case will be raised by 4–5 mm. mercury. (All pressures in these experiments were measured with a low compliance capacitance manometer coupled to the eye through a hollow needle.) Holding the probe at an improper angle as it approaches the eye exercises a smaller effect. For angles up to 10° away from the normal to the surface of the eye the pressure within the eye will be raised by less than 1 mm. mercury at the trough point where the reading is to be taken. The effect of an error in application is probably less with a human eye, though the clip in a rabbit tonogram is often less pronounced than in man, thus implying their corneas to be the less stiff. All known errors raise the indication so high pressures are not missed.

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Assay of Thyrotrophic Hormone in Blood

Although many methods for the assay of thyrotrophic hormone have been described, few have been sensitive enough to detect the amounts of hormone normally circulating in the blood, and all have serious shortcomings from the point of view of statistical validity. The method described by Bottari and Donovan[1] involving the measurement of release of iodine-131 from incubated thyroid fragments, while satisfactory from the point of view of sensitivity, suffers from a design which does not lend itself easily to statistical validation. Furthermore, some variation was experienced in the sensitivity of the assay animals, linearity within the stated range of sensitivity was not always obtained, and consequently some erratic results were occasionally encountered.

In the light of further experience, the following modifications have been found to increase the accuracy and the reliability of the assay:

(1) The use of in-bred guinea pigs from a source known to be suitable for assay.

(2) Pre-treatment of the assay animals with either 0·1 per cent propylthiouracil/day for about three weeks, or with tapazole 10 mgm./day for 7–10 days.

(3) The adoption of a standard two-point assay design for each assay with analysis of variance and factorial analysis as described by Bliss[2].

(4) Incubation of the tissue fragments with iodine-131 for 8–10 hr., as against 16–18 hr.

(5) The use of a smaller log-dose interval, usually 0·699, for the two doses, within the limits of 5–150

Table 1

No.	λ	S^2	*F*-ratio slope 7·71 for $P < 0.05$	No.	λ	S^2	*F*-ratio slope 7·71 for $P < 0.05$
1	0·078	10·11	50·01	11	0·144	8·03	21·19
2	0·059	6·04	124·95	12	0·188	19·09	13·44
3	0·195	11·97	10·69	13	0·129	9·83	26·12
4	0·101	3·49	48·18	14	0·033	4·05	403·29
5	0·057	1·31	126·43	15	0·091	2·56	52·74
6	0·144	11·59	21·54	16	0·086	23·46	57·19
7	0·054	2·04	218·40	17	0·258	103·71	6·62
8	0·109	23·04	36·98	18	0·073	3·97	81·9
9	0·204	36·34	10·87	19	0·121	2·39	29·9
10	0·101	4·32	42·45				

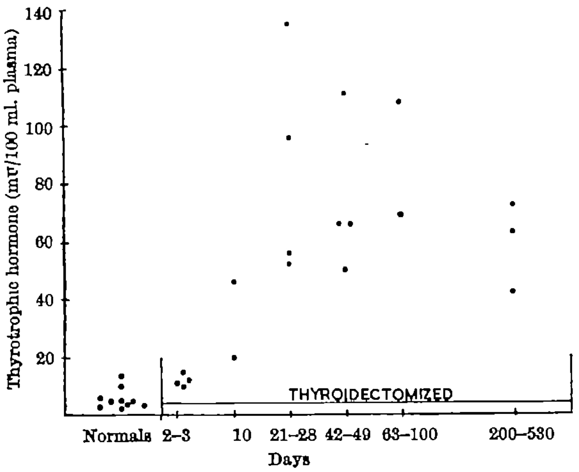

Fig. 1

International milliunits of thyrotrophic hormone per 100 ml.

The mean λ was found to be 0·115 ± 0·002 in a series of consecutive assays. The *F* ratio for the slope was highly significant in all valid assays, *P* varying between < 0·05 and < 0·001 (Table 1). There was no significant deviation from parallelism between International Standard thyrotrophin on one hand, and the thyrotrophic hormone content of human plasma, that of normal rat plasma, and that obtained from the thyroidectomized rabbit. In a series of 30 consecutive assays undertaken on specimens of blood obtained from normal and thyroidectomized rabbits, all tested blindly, only two assays proved to be statistically invalid, and hence had to be repeated. The results were quite consistent with what might be expected on a theoretical basis, namely, that the thyrotrophic hormone content of the blood of the rabbit rises gradually after thyroidectomy (Fig. 1).

It is suggested that this new method (Bottari, Donovan and El Kabir, to be published) is suitable for the assay of thyrotrophic hormone in blood.

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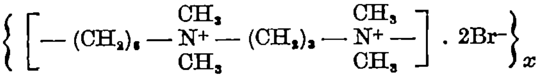
PHARMACOLOGY

Anti-Heparin Agents as Inhibitors of Plasma Kinin Formation

Plasma kinin formation[1] can be induced by treating plasma in a variety of ways, excluding the addition of proteolytic enzymes. In our present work the procedures which we have applied to 'pre-active' human plasma or serum include exposure to a large glass surface[2], addition of ε-amino-caproic acid or disodium edetate[3], dilution[4], re-suspension of the 33 per cent saturated ammonium sulphate precipitate, or addition of the purified kinin-forming material isolated from human plasma (Armstrong, D., and Mills, G. L., unpublished work).

Having observed that heparin[1] and dextran sulphate (Armstrong, D., and Stewart, J. W., unpublished work) accelerate plasma kinin formation, we decided to determine the actions of heparin antagonist drugs on kinin production. We found that both protamine sulphate, and also the non-protein polymer hexadimethrine bromide[5–7] ('Polybrene', Abbott Laboratories, Illinois):



$$\left\{\left[-(CH_2)_6-\overset{CH_3}{\underset{CH_3}{N^+}}-(CH_2)_3-\overset{CH_3}{\underset{CH_3}{N^+}}-\right].\,2Br^-\right\}_x$$

mol. wt. average 6,000 effectively antagonized kinin formation induced by all the aforementioned methods. Toluidine blue, on the other hand, was relatively inactive. Protamine sulphate and hexadimethrine bromide antagonized not only the accelerating action of heparin on kinin formation but also acted as potent inhibitors in the absence of heparin, that is, when production was being induced in citrated plasma, or in serum. They did not interfere with the uterine response to formed plasma kinin, and did not increase the activity of kininase in plasma. Since we have found no evidence of action on substrate, kininogen (in pre-active plasma or serum), their action is apparently on the kinin-forming enzyme itself.

Effective concentrations of protamine sulphate and hexadimethrine bromide are in the range 10^{-6}–10^{-8} gm./ml., the latter being about 50 times the more active of the two compounds. Antagonism of plasma kinin formation induced by glass is an exception, in that higher concentrations of antagonist are required, for example, 10^{-4}–10^{-3} gm./ml., due to the fact that concentration of antagonist in solution is reduced by adsorption on to the glass.

We suggest that the actions of these positively charged macromolecular inhibitors, protamine sulphate and hexadimethrine bromide, resemble those of the naturally occurring inhibitor of kinin formation present in normal human plasma and that the actions of the negatively charged heparin and dextran sulphate molecules resemble those of glass in sequestering off this natural inhibitor from the kinin-forming system.

These agents should assist *in vivo* studies to determine the physiological role of the plasma kinin-forming system.

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Changes in the Blood of the Rat induced by the Monocarboxylic Acid of Cyanocobalamin obtained by Fermentation (anti-Vitamin B_{12})

Monocarboxylic acid derivatives of vitamin B_{12} obtained by mild acid hydrolysis are competitive B_{12} antagonists in *Escherichia coli*. The three isomeric monocarboxylic acids so obtained differ from one another in both their microbiological and hæmatological activities[1-3].

By fermenting a strain of *Propionibacterium shermanii* under suitable working conditions, one of us[3] has succeeded in preparing the microbiologically most active monocarboxylic acid isomer in homogeneous form and relatively large amounts.

The following results have now been obtained in hæmatological tests carried out on animals with this homogeneous monocarboxylic acid.

In normal female Wistar rats weighing 120–150 gm. three consecutive 0·1 mgm./kgm. daily doses injected intraperitoneally induced a 50–70 per cent transient reduction in the number of granulocytes. Granulocytopænia reached its peak 4–8 days following injection. The granulocyte count gradually regained normal level by the 12th–14th day. There was no significant change in the number of lymphocytes and red blood cells (Fig. 1).

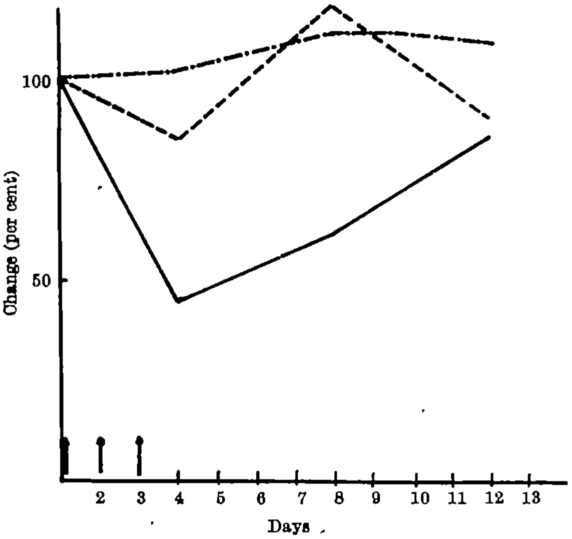

Fig. 1. Effect of monocarboxylic acid derivative of vitamin B_{12} on granulocytic number in normal rats. ——, White blood cell count in animals treated with monocarboxylic acid; —●—, red blood cell count in similar animals; – – –, white blood cell count in controls

Doses increased to 0·5–1 mgm./kgm. failed to produce appreciably more severe granulocytopænia. Administered in doses of 0·1–1·0 mgm./kgm. in combination with our monocarboxylic acid, vitamin B_{12} did not prevent deterioration in the granulocytic count, but methionine did antagonize this effect of the acid.

After their complete blood pictures had been prepared, anæmia was produced in 36 rats of 170–200 gm. weight by withdrawing 3 ml. of blood through the veins of the tail. The animals were then divided into three groups: the first was given 4 consecutive daily 0·1 mgm./kgm. doses of our homogeneous monocarboxylic acid; the second was treated with as much vitamin B_{12}; and the third with sodium chloride only to serve as control. The red and white blood cell counts were estimated daily.

On the evidence of the results presented in Fig. 2, whereas vitamin B_{12} intensified, the monocarboxylic acid slackened, regeneration of red cells.

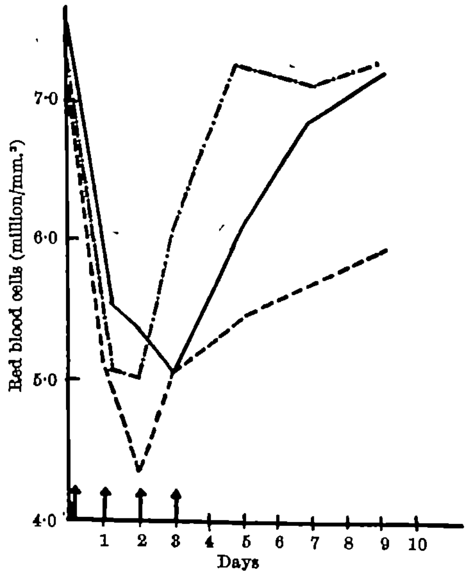

Fig. 2. Effect of monocarboxylic acid derivative of vitamin B_{12} on posthæmorrhagic regeneration of erythrocytes in rats. – – –, Monocarboxylic acid; —●—, vitamin B_{12}; ——, control

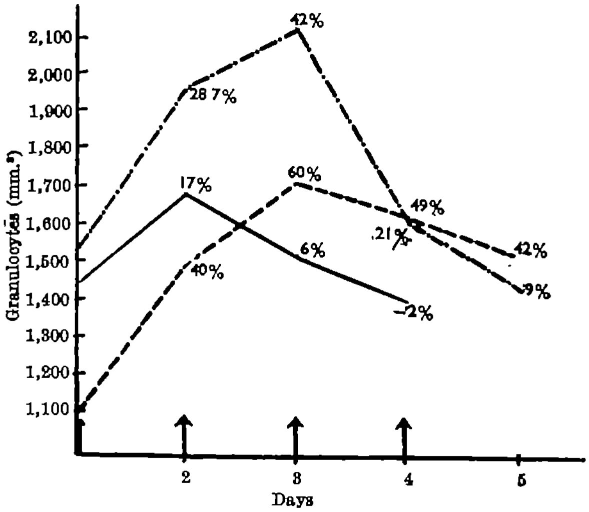

Fig. 3. Effect of monocarboxylic acid derivative of vitamin B_{12} on posthæmorrhagic granulocytosis in rats. ——, Monocarboxylic acid; —●—, vitamin B_{12}; – – –, control

Fig. 3 shows the changes in the white cell count. The monocarboxylic acid markedly inhibited the posthæmorrhagic leucocytosis known to be due principally to a rise in the number of granulocytes. Our qualitative blood examinations likewise confirm the possibility of inhibiting a granulocyte crisis by treatment with monocarboxylic acid.

Summarizing its hæmatological effects on rats, it appears safe to claim that the homogeneous monocarboxylic acid of vitamin B_{12} obtained by fermentation: (1) slackens post-anæmic regenerative red blood cell formation; (2) causes a transient reduction in the number of granulocytes; (3) inhibits post-hæmorrhagic granulocytosis.

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PATHOLOGY

5-Nucleotidase Activity in Experimental Cervicovaginal Carcinoma of the Mouse

In the course of a histochemical examination of the distribution of various enzymes in normal and neoplastic cervicovaginal epithelium of the mouse it was found consistently that high 5-nucleotidase activity is typical for malignant squamous cells. This histochemical technique can therefore be considered to have definite diagnostic value.

Squamous cell carcinoma was induced in the cervix and vagina of 34 adult virgin *C3H/N* mice by biweekly visual painting of the portio with 1 per cent benzpyrene in acetone. The animals were killed at intervals so that the various histological steps of epithelial carcinogenesis are represented. A squamous cell carcinoma induced by the same technique was examined in the 79th, 80th and 81st transplantation generations. (We thank Dr. I. Koprowska (Philadelphia), who kindly provided us with the transplantable tumour.) Normal cervicovaginal tissues were provided by 10 intact adult *C3H/N* mice in different phases of the œstrus cycle as well as by 4 ovariectomized animals. The genital tracts, frozen with carbon dioxide, were cut serially at 10μ in a cryostat and treated in order to demonstrate tissue activity of 5-nucleotidase[1]. The 5-nucleotidase activity of the cervicovaginal epithelium is low except in keratinized cells (stratum granulosum and corneum), which display medium staining intensity. Mucified cells (pro-œstrus) are inactive. Cyclic variations in enzyme activity were observed, that is, a gradual increase in the staining intensity of the basal and spinous cells from di-œstrus toward œstrus (maximum activity) followed by a decrease during the katabolic phases of the cycle (metœstrus). The epithelium of castrated mice is devoid of enzyme activity. Subepithelial connective tissue is strongly active for 5-nucleotidase and deeply situated stroma shows somewhat less enzyme activity. Cyclic variations in enzyme activity parallel to those in the overlying epithelium were found in the deep connective tissue only. Polynuclear leucocytes stain intensely whereas macrophages show no activity. Smooth muscle cells (tunica muscularis vaginæ, myometrium and vessel walls) are characterized by high 5-nucleotidase activity, though cyclic variations in intensity did not occur.

The 5-nucleotidase pattern of hyperkeratotic squamous epithelium is identical to that of œstrus epithelium. Dysplastic cells are devoid of enzyme activity except for the luminal cornified cell layers. No enzyme activity was found in intra-epithelial carcinoma. In invasive squamous cell carcinoma (early stromal invasion, micro- and macro-carcinoma) a high activity of 5-nucleotidase was noted in all component cells but the mucified elements (mucoepidermoid carcinoma). Necrotic portions of the tumour corneus material as well as polynuclear leucocytes show more or less enzyme activity. The pattern of reactivity of transplanted squamous cell carcinoma is not different from that of the primary lesion.

The 5-nucleotidase activity in malignant squamous cells (early stroma invasion, micro- and macrocarcinoma) is definitely increased when compared with that of normal and pathological non-invasive (hyperkeratosis, dysplasia and intra-epithelial carcinoma) epithelium. The enzyme pattern of squamous epithelium of the genital tract of the mouse is not unlike that of the human uterine cervix. In the latter epithelium an increase of the 5-nucleotidase activity was noted during pregnancy so that in man, as well as in the mouse, the intensity of the 5-nucleotidase activity seems to be linked with œstrogen. We have established that human intra-epithelial carcinoma of the uterine cervix is inactive for 5-nucleotidase and that squamous cell carcinomas of the same origin showed little to no activity. Only in anaplastic carcinoma of the human cervix could a distinct activity of 5-nucleotidase in the tumour cells be demonstrated. Thus there exists a difference between the 5-nucleotidase pattern of cervical carcinoma of man and mice. In the latter species the histochemical distribution of the enzyme activity can be used for practical purposes, that is, the diagnosis of invasive neoplastic growth, the identification of benign and malignant keratinized cells as well as for the evaluation of the degree of differentiation of benign and neoplastic squamous cells.

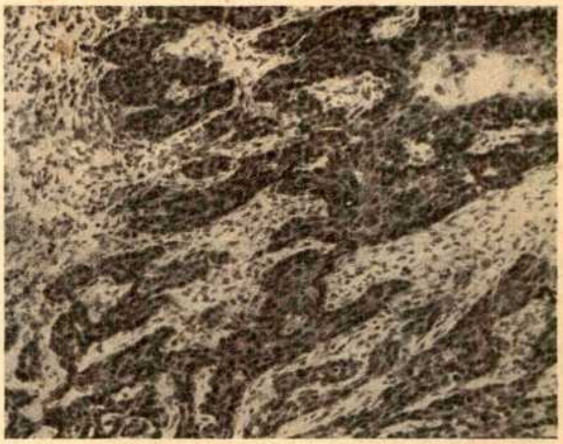

Fig. 1. Transplanted cervix carcinoma (81st generation) (hæmatoxylin–eosin, × c. 105)

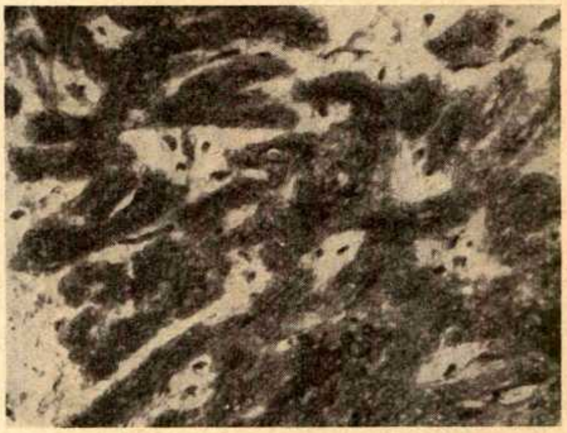

Fig. 2. Activity of 5-nucleotidase in the same tumour. In the negative stroma also activity of 5-nucleotidase can be seen in the capillaries. (× c. 105)

The consistently high 5-nucleotidase activity of malignant squamous cells is quite remarkable, and stands in definite contrast with the activity of most of the enzyme systems studied. Moreover this phenomenon is stable when chemically induced cervicovaginal carcinoma is transplanted for a number (79 +) of generations.

The significance of the high 5-nucleotidase activity in neoplastic cervical cells of the mouse is unexplained.

A high activity of 5-nucleotidase was found in bronchial epithelial cells of mice infected with influenza. In the same cells the influenza virus could be demonstrated by means of a fluorescent antibody technique[2].

The presence of virus-like particles in some of the chemically induced tumours of the mouse cervix has been reported[3].

Therefore it is tempting to relate the increased enzyme activity to the presence of virus-like particles. Another possibility is that the 5-nucleotidase is connected with the syntheses which characterize the process of cornification as squamous cells undergoing keratinization show marked enzyme activity.

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Reaction of the Reticular Tissue of Mice with Autoimmune Hæmolytic Anæmia to 2-Aminofluorene

A strain of mice suffering from hæmolytic anæmia of the autoimmune type has been established by one of us (M. B.). This strain designated *NZB/Bl* is now in the 52nd generation of brother–sister mating. Serological signs of the disease, such as agglutinating antibodies[1] and positive Coombs's tests[2], appear in a few animals from the 4th month of life onward, the incidence rising to virtually 100 per cent in animals older than 9 months.

It seemed of interest to test the neoplastic potentialities of the cells of the reticular tissue of *NZB* mice. 2-aminofluorene was chosen as carcinogen because this agent, in contrast to X-rays, carcinogenic hydrocarbons and œstrogens, has no special affinity for cells of the lymphatic system[3]. Apart from *NZB* animals mice of 2 other inbred strains (*NZC* and *NZO*) were used. All 3 strains originated from the same mixed colony and all were known to have a low incidence of spontaneous tumours of the lymphatic system. The mice were painted 3 times weekly with a 4 per cent solution of 2-aminofluorene in acetone for periods of 6–7 months.

As shown in Table 1 only in the *NZB* mice treatment with 2-aminofluorene induced a significant increase in neoplastic lesions of the reticular tissue. In 11 of the 14 affected animals the thymus was involved, but in 3 the process was limited to abdominal organs. One leukæmia, 1 reticulum cell sarcoma and 1 thymoma were transplanted. The grafts grew progressively, causing death of the recipients.

Table 1. INCIDENCE OF LYMPHOMAS* IN MICE TREATED WITH 2-AMINOFLUORINE AND IN CONTROLS

Strain	*NZB*	*NZC*	*NZO*
Total no. of controls	137; 76♂, 61♀	160; 44♂, 116♀	274; 130♂, 144♀
Controls with lymphomas	5; 4♂, 1♀	3; —, 3♀	3; 2♂, 1♀
Total no. of treated mice	72; 35♂, 37♀	73; 45♂, 28♀	76; 36♂, 40♀
Treated mice with lymphomas	14; 7♂, 7♀	1; —, 1♀	2; —, 2♀

* Thymomas, lymphatic leukæmias and reticulum cell sarcomas.

Recent investigations[4] have established that the hæmolytic anæmia of *NZB* mice is an inheritable condition. In offspring of reciprocal crosses of *NZB* with *NZC* or *NZO* mice the occurrence of hæmolytic anæmia indistinguishable from that seen in *NZB* mice has been observed.

The lymphatic elements of *NZB* mice differ from those of the 2 other strains not only by their ability to form auto-antibodies against red blood corpuscles but also by increased susceptibility to 2-aminofluorene, that is, by a higher liability to undergo neoplastic transformation. Whether this is due to a primary instability of these elements or to hyperplastic processes occurring in many lymphatic organs concomitant with the development of the hæmolytic anæmia remains to be investigated.

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HISTOCHEMISTRY

Histochemical Localization of β-Glucuronidase in Healing Wounds of the Axolotl

β-Glucuronidase, the enzyme related to cell proliferation and the formation of connective-tissue ground-substance, has been demonstrated in the basal layers of the oral mucous membrane and the epidermis, the blood vessels of the normal corium, in healing wounds of the skin of the back, the tongue and the palate of the rat[1–3], and in healing wounds of the dorsal skin and the tongue of the common iguana[4].

In continuation of this investigation I have examined the activity of this enzyme in healing wounds of the dorsal skin and the tongue of the axolotl *Amblystoma mexicanum*, the animals being killed after 5, 8, 16, 23 and 36 days. After excising the wound with an extensive zone of normal neighbouring tissue and fixation in formalin/chloral hydrate, the enzymatic activity was determined by the method of Fishman and Baker[5] on frozen sections with different incubation times. In the superficial necrotic band of the connective tissue in the 5-day specimens a negative reaction was observed, whereas immediately beneath this negative zone there was an intensely positive zone due to the presence of inflammatory exudate, and particularly leucocytes.

As healing progressed, the fibroblastic proliferation zone reacted moderately but consistently. The histiocytes and giant cells appeared to react more intensely. In the 23-day specimens the epidermis, which had proliferated over the margins of the wound, exhibited a significant increase in enzymatic activity.

In the light of these results I conclude that epithelial proliferation is closely related to β-glucuronidase activity. In connective tissue this enzyme is associated with tissue formation and macrophage function. As these results correspond with those obtained in the rat[1] and in the common iguana[4], more extensive significance must no doubt be attached to the β-glucuronidase.

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HISTOLOGY

Preservation of Tissue Sections in Coons's Fluorescent Antibody Technique

In using Coons's fluorescent antibody technique[1,2] for the localization of antigens in tissues, difficulty may be encountered in preserving the sections cut from fresh-frozen material. Although it is customary to dry the sections on to a coverslip in a current of air before staining, dehydration of the sections during storage for several days at the low temperature of the cryostat can cause excessive distortion of the tissue and loss of immunological reactivity of the antigen.

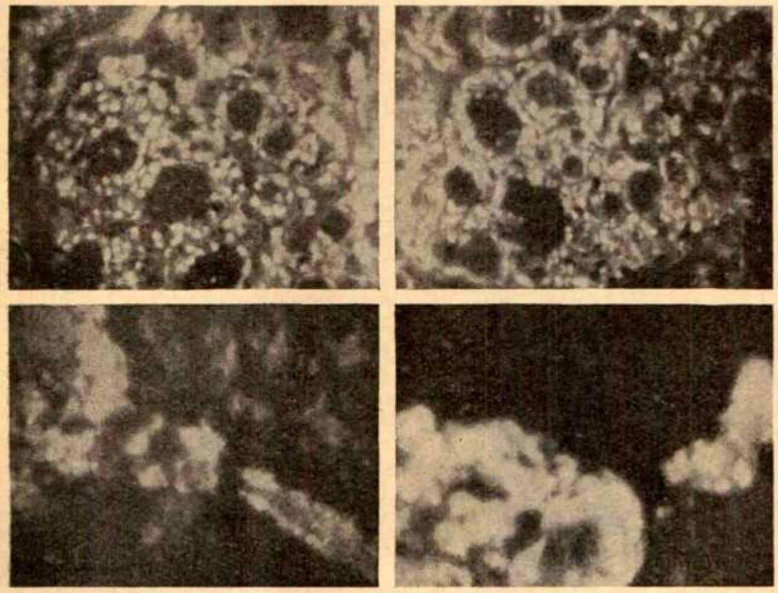

Fig. 1. Top left, The sandwich technique using rabbit anti-human γ-globulin labelled with fluorescein isothiocyanate to demonstrate anti-nuclear factor in the serum of a patient with disseminated lupus erythematosis. The section is of thyroid and was used within 2 hr. of being cut

Top right, Sandwich technique using the same fluorescent labelled anti-human γ-globulin and patient's serum as in top left, but the section of thyroid tissue has been preserved by polyethylene glycol for 2 weeks

Bottom left, Glomerulus of a human kidney treated with a fluorescent labelled anti-β-lipoprotein antiserum to demonstrate the distribution of β-lipoprotein. The kidney section was used within 2 hr. of being cut

Bottom right, glomerulus of a human kidney treated with the same fluorescent anti-β-lipoprotein antiserum. The section was preserved by polyethylene glycol for 4 days prior to use

Any material used to preserve tissue sections for immunological analysis must fulfil certain criteria: it must not interfere with the antigen–antibody reaction by denaturation of protein, it must itself be non-reactive in the antigen–antibody system, and it must be easy to apply.

It was found that polyethylene glycol ('Carbowax', Carbide and Carbon Chemical Co., New York), mol. wt. 285–315 with a freezing range −15° to 8° C., fulfilled the criteria for use as a preserving medium. The polyethylene glycols of higher molecular weight and their methoxy-derivatives have been used for some time as embedding media in pathology[3,4], and the physical properties of suitable mixtures are well recognized[5–7]. The polyethylene glycols of lower molecular weight appeared particularly suitable for our purposes since they are readily soluble in saline and this, together with their small size, renders them easily removed from tissue sections prior to the application of the fluorescent labelled antibody.

These polyethylene glycols of low molecular weight, in distinction to the polyethylene glycols of higher molecular weight, are liquid at room temperature with a freezing point near 0° C. The compounds of higher molecular weight are therefore unsuitable for the technique.

The method used was to take the section mounted on a coverslip and dip it immediately into polyethylene glycol 300 for 5 sec. The coverslip and its section, now coated with polyethylene glycol, were replaced immediately in a cooled rack in the freezing cabinet at − 20° C.; within seconds the polyethylene glycol was frozen solid.

When it was required to use a section treated in this way the coverslip was removed from the cryostat, warmed on the finger and washed in a pool of physiological saline three times for 5 min. each time. The saline should be pooled on the coverslip rather than allowed to flow on the coverslip so that the section does not lift. This procedure was carried out at room temperature. The section could then be treated in the usual manner.

It was found that sections could be preserved without distortion for at least up to four weeks and did not lose their antigen–antibody specificity. The distribution of antigen was apparently unaltered by the procedure. Sections untreated by this method could only be preserved up to 6 hr. before distortion due to dehydration made them unusable (Fig. 1).

Where it is known that the antigens are soluble in saline, the sections may be momentarily dipped in a suitable fixative, for example, ethyl alcohol, methyl alcohol, acetone, prior to coating with polyethylene glycol. In this way the saline used to remove the polyethylene glycol removes a minimal amount of antigen from the tissue.

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BIOLOGY

Phases of the Hair-Growth Cycle

MORPHOLOGICAL considerations led Dry[1] to distinguish three stages in the hair-growth cycle; namely, anagen, catagen and telogen. Anagen referred to the period of active growth, including development of the follicle and subsequent synthesis of the hair. The rapid growth in anagen abruptly ceases and the follicle is largely destroyed in a brief phase, termed by Dry as catagen. The follicle remnant and associated hair are in a phase of apparent inactivity known as telogen in Dry's system. This classification of the hair-growth cycle has been extended[2] by subdividing anagen into six phases on the bases of detailed histological observations.

These 'structural' classifications include in one phase (anagen) developmental and functional stages of the hair follicle, and yet distinguish degeneration of the follicle as a discrete phase (catagen). From a physiological point of view, this is clearly unreasonable. To classify the hair-growth cycle in a histologically and physiologically acceptable manner, it is suggested that the term anagen refer only to the phase of follicle development and that the functional phase, between anagen and catagen, be known as metagen. Specifically, metagen would commence with the keratinization of the hair shaft and continue until the synthesis of the hair ceases at catagen.

On structural grounds, telogen has frequently been described as dormant[1-8]. If this were so, it would be permissible to consider the hair-growth cycle as consisting of periods of activity followed by periods of inactivity. Such an interpretation of the growth cycle is, however, misleading, since telogen is not a phase of 'rest'. Metabolically telogen may be regarded as a phase of recovery from the destructive metamorphosis of the hair follicle in catagen. It is the metabolic activity in telogen, however subdued, that results in the initiation of the histological events associated with the phase of anagen. Indeed, the propensity of the follicle to grow hair is a reflexion of metabolic activity during telogen.

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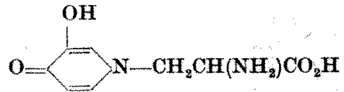
Inhibition of Growth of Hair by Mimosine

Loss of hair in animals following ingestion of seeds and foliage of *Leucaena glauca* was first reported in 1897[1]. Sudden loss of hair in native women has been ascribed to consumption of *Leucaena glauca* seeds[2]. The toxic principle contained in this plant is a water-soluble amino-acid termed leucenol, and is identical with mimosine obtained from *Mimosa pudica*[3]. Mimosine seems to be the preferred name for the compound, both by historical precedent[4] and to avoid confusion with leucinol, an alcoholic derivative of leucine. Mimosine is found primarily in the seeds of *Leucaena glauca*, lesser amounts being present in the foliage and stems[5]. Its chemical structure is:



In this investigation, both purified mimosine and ground seeds of *Leucaena glauca* caused inhibition of growth of hair and loss of hair in mice. Mimosine was extracted from whole ground seeds with boiling water[6]. Its absorption spectrum measured in a Beckman *DU* spectrophotometer was identical with that of a sample obtained from a commercial source (Fluka AG, Chemische Fabrik, Buchs SG). Purity was confirmed by unidimensional paper chromatography (methanol/water/pyridine, 80 : 20 : 4) and column chromatography (150 × 0·9 cm. sulphonated styrene—8 per cent divinyl benzene, Spinco 150*A*, 50° C. at *p*H 4·25).

Mimosine or whole ground seed was mixed with ground commercial mouse pellets and formed into cakes by the addition of agar. Groups of mice were fed with cakes containing 5 per cent and 10 per cent whole ground seed and 0·5 per cent and 1·0 per cent mimosine. Control animals received cakes prepared without seed or mimosine. A large area of the body of each animal was plucked free of hair to induce a new anagen cycle in the hair follicles of these areas.

All animals eating the control diet re-grew thick hair coats in the epilated areas within 8–10 days. The animals fed 5 per cent ground seed and 0·5 per cent mimosine also re-grew hair normally. No hair re-growth was observed in animals receiving 10 per cent ground seed or 1·0 per cent mimosine (Table 1). The 1·0 per cent mimosine diet was continued for 9 weeks with no evidence of new hair in the plucked areas. During the ninth week, loss of hair on the face and head of these mice was noted.

Damage to the roots of hairs in human beings is caused by a variety of cytotoxic therapeutic agents[7]. This damage is due to an inhibition of growing (anagen) hairs. Resting (telogen) hairs are not affected. Gross evidence of loss of hair depends on the degree of damage to the growing hair root. If complete atrophy occurs, the hair is shed from its follicle. Damage to growing hair roots reflects the high metabolic and mitotic activities of these tissues. Compounds toxic for rapidly growing tissues usually cause a proportional degree of damage to hair[7].

The loss of resting hairs is a gradual, continuous physiological process. Normally these hairs are replaced by new growing hairs. If growth of new hair is inhibited there can be no replacement. As a result, the amount of hair remaining is slowly reduced and is often overlooked until it becomes severe. It is this type of loss of hair which occurred on the face and head of mice fed for a long period on 1·0 per cent mimosine.

Table 1. EFFECTS ON GROWTH OF HAIR BY FEEDING *Leucaena glauca* SEEDS OR MIMOSINE IN MICE

Diet	Hair regrowth in plucked area at 8–10 days	Hair loss in facial area at 8–9 weeks
Control	+	0
5 per cent seed	+	0
10 per cent seed	0	+
0·5 per cent mimosine	+	0
1·0 per cent mimosine	0	+

Previous statements that mimosine is incapable of causing loss of hair in some animals[8] or that loss occurs only in young animals[5] failed to take into account the growth-cycle of animal hair. Unless the hairs are in the anagen phase of growth during drug administration, no damage to hair will occur. Many animals exhibit long periods when most hairs are in the telogen phase, and a new growth-cycle must be induced (for example, by plucking) to assess the inhibitory effects of toxic agents. Massive loss of hair in very young rats[5] occurs because most of their hair is in the growing phase.

Mimosine has been shown to act as a tyrosine analogue, capable of inhibition of tyrosine decarboxylase and competitive inhibition of tyrosinase[9]. Thus, the toxic action of mimosine on anagen hairs may be due to inhibition of tyrosine-utilizing enzymes, or perhaps incorporation of mimosine into biologically vital proteins in place of tyrosine.

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Swimming-Rate of Bull Sperm

THE velocity of bull sperm has been estimated by a number of different workers in the past few years. Recent reviews have both summarized their results and indicated the diversity of techniques, dilutions, diluents and other conditions which have been used[1,2].

In spite of this diversity, and although many of the factors which have been varied are known to affect the swimming-rate of sperm, results have been sufficiently similar for a value of 100 μ/sec. to be regarded as a good approximation to the 'normal' velocity. This value has been used in order to estimate the time which would be taken by sperm traversing the female tract by their own motility[3].

We have estimated the rate of swimming of bull sperm in a variety of different media, including mucus from the uterine cervix of the cow. We used the simple technique of measuring with a stop-watch the time required for the sperm to swim the length of a calibrated eye-piece micrometer scale. The diluted semen was enclosed in a sealed chamber to prevent evaporation, so that it was possible to record a satisfactory number of rates at 37° C. before the preparation began to deteriorate. Under these conditions, the mean rate of 705 sperm, derived from a number of different bulls, at a dilution of one in 128 in physiological saline, was 131·9 μ/sec. ± 1·25 (*S.E.*). The mean rate for 530 of these sperm the speed of which was estimated within 10 min. of warming to 37° C. was 131·9 ± 1·5 μ/sec., and for the 175 which were timed during the subsequent 45 min. it was 131·7 ± 2·2 μ/sec.

It is therefore possible, by means of this simple technique, to arrive at a value comparable with those obtained by more elaborate methods. Swimming-rates were also found to be similar when the semen was diluted to the same extent in a variety of other media such as Rothschild's medium[4], Baker's medium[5], isotonic saline buffered with phosphate, and seminal fluid.

The swimming-rate in cervical mucus, however, was considerably lower than in any other medium investigated. Twenty-five experiments were carried out with ten samples of mucus from eight cows and twelve samples of semen. The mean swimming-rates in these experiments varied from 22·0 to 80·7 μ/sec., and the mean of all the means was 56·2 μ/sec.

The mucus used in all these experiments had been stored for some time at −10° C. The possibility that freezing and/or storage might have produced deleterious changes in the mucus was checked by carrying out a further nine experiments with six different samples of mucus fresh from the cows, and six samples of semen. The range of mean swimming-rates in these nine experiments was 37·5–70·5, and the mean of the means 55·6 μ/sec.

It appears, therefore, that the swimming-rate of bull sperm in cervical mucus is only about a half of that which is usually adopted for calculations of the rate of movement in the female tract. It is still possible, however, that progress along a canal filled with mucus might be more rapid than along a comparable channel of saline. Because of the phenomenon of orientation in mucus[6], the sperm might travel in a straight line from one end of the cervix to the other, if the mucus were orientated in strands along it, whereas in saline the movement has the nature of a diffusion process.

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Electron Microscopic Appearance of Close Relationships between Adult Guinea Pig Fibroblasts in Tissue Culture

1-CM. squares of full-thickness skin were aseptically excised from adult male guinea pigs. 10 days later explants of granulation tissue laid down in the wounds were cultured on plasma clot by the flying coverslip method. 36–48 hr. later, cultures were fixed intact in buffered osmium tetroxide or potassium permanganate, dehydrated with ethanol, and embedded in 'Araldite'. Parts of the fibroblast outgrowth were sectioned and examined in a Siemen's 'Elmiskop I' electron microscope.

Plasma membranes of adjacent cells ordinarily maintained a 100–200 Å. separation, but in places a more intimate relationship occurred. After osmium fixation (Fig. 1, top) it appeared as a quintilinear region ~ 160 Å. across. In permanganate fixed material (Fig. 1, bottom) the transverse dimension was similar, and the central dense line was twice as wide (about 40 Å.) as the two lateral dense lines.

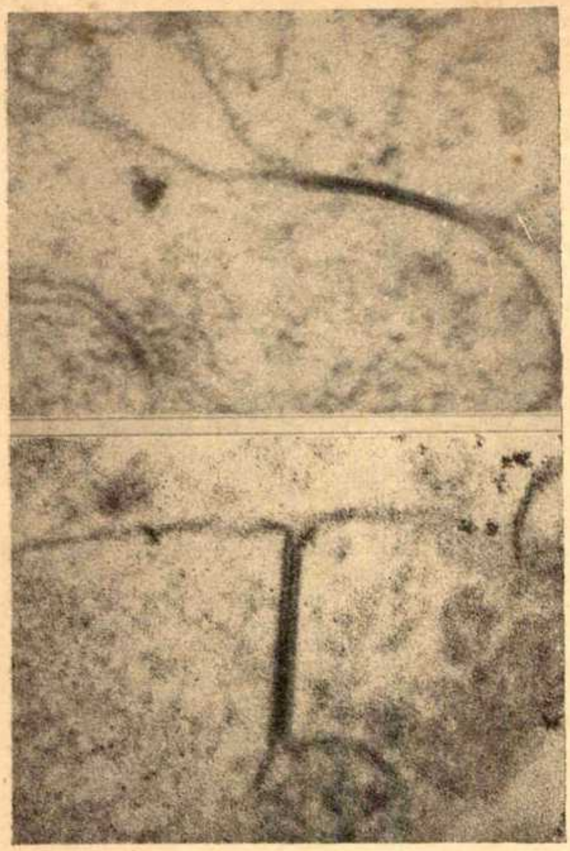

Fig. 1. Top: Osmium fixation (× 105,000); bottom, permanganate fixation (× 150,000)

Similar appearances have been reported for Schwann cell membranes by Robertson[1], who termed them "external compound membranes", and by Gray[2], who used the term "closed contacts", between glial processes and capillaries. Quintilinear regions of comparable dimensions have also been found in human cervical epithelium[3] and epidermis[4].

The functional significance of quintilinear close relationships between the plasma membranes of fibroblasts is as yet obscure, but they may be of importance for intercellular adhesion. It is of interest that Gray and Whittaker[5] found that 'closed contacts' of neuroglia remained intact after treatment of the tissue with isotonic and hypotonic sucrose.

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Ability of the Trophoblast to destroy Cancer Tissue

THIS communication presents the results of some preliminary experiments which demonstrate that in the mouse the trophoblast can actively invade and destroy the tissue of a mammary cancer.

In 1902, Beard[1] first directed attention to the similarity between the destructive capacity of the trophoblast and that of cancer. Since that time, this feature of trophoblast has been the subject of many papers.

In many mammals, in the early stages of pregnancy, trophoblast, which develops from the implanting egg, infiltrates and erodes part of the uterine lining. When, as a result of this action, the conceptus is firmly attached to, or embedded in, the maternal tissue, the destructive capacity of the trophoblast normally diminishes and is finally lost. In some pathological conditions, however, its destructive phase proceeds unchecked, becoming truly malignant as the chorioepithelioma (for a comprehensive account of this subject see ref. 2).

It has been previously demonstrated[3,4] that the mouse egg, transplanted to the anterior chamber of the eye, gives rise to trophoblast which infiltrates and destroys the tissues of the eye. Also, Fawcett[5] showed that the trophoblast, arising from a mouse egg transplanted beneath the kidney capsule, invaded the kidney cortex.

I have also investigated the invasive behaviour of mouse trophoblast in a wide range of extra-uterine sites, and have shown: (*a*) that the sex of the host or the fact that the donor and host were of different strains did not affect the development or activity of the trophoblast[6]; (*b*) that mouse trophoblast, developing from a mouse egg transplanted beneath the kidney capsule of a rat, actively destroyed the kidney tissues[7]. This capacity of the trophoblast to withstand the immunological reaction of the host was partially explained by the recent findings of Hulka, Hsu and Beiser[8]. They showed how human trophoblast was able to absorb from the maternal circulation antitrophoblast antibodies; (*c*) that trophoblast can destroy some tissues (for example, spleen) exclusively by phagocytosis, while others (for example, testes) are destroyed by a cytolytic substance liberated by the trophoblast[9]. *In utero* trophoblast seems to use both methods of destruction; (*d*) the growth and invasive capacity of trophoblast is greatly accelerated by warmth and suppressed by cold[9]; (*e*) that trophoblast preferentially destroys tissues that have a high blood content[9]; (*f*) that in all the extra-uterine sites tested, active invasion by trophoblast persists for the same length of time as it does *in utero*.

A natural sequal to this series of investigations was to see whether the trophoblast was able to destroy cancer tissue. Transplantation of cancer cells to normal tissues has been extensively investigated; but no reference in the literature can be found to the transplantation, *in vivo*, of tissues in the opposite direction. However, normal cells (fibroblasts) have been introduced to cultures of cancer cells *in vitro*. The results vary. In some cases the cancer cells retarded the growth of the fibroblasts, and finally overgrew and destroyed them. Using other types of cancers the fibroblasts were stimulated to more rapid proliferation[10].

Runner[3] introduced, into the anterior chamber of the eye, mouse blastocysts together with small clusters of transplantable tumour cells. Of the three types of tumour cells used, two types (a spindle cell sarcoma and a carcinoma derived from the mammary gland epithelium) grew, and in these cases the blastocysts did not develop. The third type (a spindle cell sarcoma) was unable to grow in the eyes in which the blastocysts were developing. Runner does not mention any reaction between the trophoblast and the cancer cells.

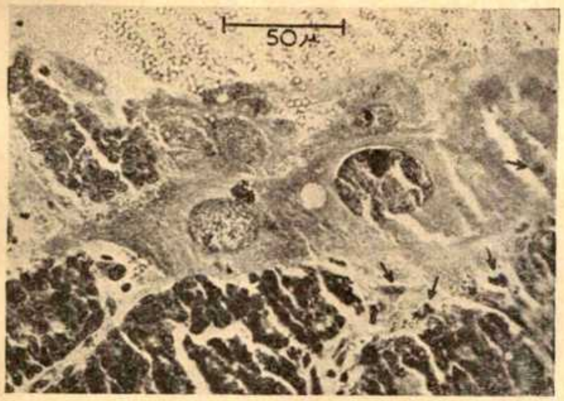

Fig. 1. Trophoblast containing one large and several smaller nuclei, invading the tumour tissue (bottom). The arrows indicate some of the individual cells of the tumour which have been ingested, while to the upper left a large cluster of tumour cells has been isolated by the infiltrating cytoplasm of the trophoblast. At the top can be seen part of the cavity aready eroded by the trophoblast

Of special interest are the results of some experiments performed in 1935 by Murphy[11]. He injected "extracts" of rabbit placentæ into the peritoneum of mice bearing mammary cancer. This produced marked regression of 26·7 per cent of the tumours, and complete absorption of a further 21·3 per cent. Normally less than 1 per cent of mammary cancers spontaneously regress. Murphy does not state what the placental extract was. It is surprising that such apparently important results seem never to have been followed up, nor indeed have they been critically discussed.

In the present series of experiments the tumours to which trophoblasts were transplanted were spontaneous mammary carcinomas occurring in the $C+$ strain of mouse. Each tumour was in a separate animal. The donor mice from which trophoblast was obtained were of a heterogeneous laboratory stock. Ectoplacental cones (the proliferative centre of the trophoblast measuring about 1/3 mm. in length) were extracted from the 7½-day-old conceptuses. One cone was introduced just below the surface of each of six tumours.

Five days later the host mice were killed and the tumours examined. The place to which the ectoplacental cone was transplanted was marked, in four of the six mice, by a prominent hæmorrhagic patch about 2–3 mm. in diameter.

Histological examination revealed that in these four tumours the ectoplacental cone had given rise to trophoblast giant cells, the nuclei of which were about 40μ in diameter (Fig. 1). The trophoblast had, in places, penetrated deeply into the tumour tissue, and most of it was distributed as a margin to a cavity in the tumour that the trophoblast had already eroded. This eroded cavity was not confused with the naturally occurring blood-filled cysts of the advanced mammary carcinoma. The actual process of destruction appeared to be by phagocytosis. The cytoplasm of the trophoblast, as it infiltrated, engulfed the tumour cells. These were afterwards broken down by intra-cellular digestion. The cavity eroded by the trophoblast contained extravasated blood and cellular debris.

The results of the preliminary experiments are encouraging and suggest a need for further investigation especially of the following aspects: the ability of the trophoblast to destroy hetero-specific mammary cancer, the fate of trophoblast transplanted to cancers other than the mammary carcinoma, whether trophoblast will destroy cancer tissue more readily than it will the contiguous non-malignant tissue, whether trophoblast cells in suspension can be introduced to the tumour via the blood stream.

These experiments are now in hand, and the results will be published at a later date.

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Visceral and Eviscerated Carcass Fat of Adult Male Albino Mice

I have performed a series of experiments to see the effect of various doses of corticosterone, cortisone, and hydrocortisone on the visceral and eviscerated carcass fat[1].

The experimental animals were Holtzman male albino mice. As controls they were all given daily 0·05 ml. of Merck diluent (*CMC*), which was the vehicle of the glucocorticoids, for a period of 12 days. This treatment did not seem to have any influence on body-weight. When the animals were killed, their ages ranged from 89 to 96 days in the various groups. After the animals were killed, the stomach and intestines of each were completely evacuated and the contents examined for cestode parasites. This was deemed necessary because, in some previous experiments, mice from a number of animal houses were found to be highly infested with *Hymenolepis nana*.

The viscera of each animal were removed, weighed individually and mixed with 10 times their weight of anhydrous sodium sulphate. The mixture was homogenized in a mortar, and 5·5 gm. of the mass, corresponding to 0·5 gm. of the tissues, were weighed and transferred to a screw-cap vial. Thirty ml. of chloroform were added to the latter, the cap covered with aluminium foil and tightly screwed on. The vial was then shaken in a mechanical shaker for 24 hr. after which the mixture was filtered. Ten additional ml. of chloroform were added to the residue, and the resulting filtrate was combined with the previous one. The chloroform of the total filtrate was removed in a 'Rotovap' and the residue dried in a 60°–65° C. tissue-drying oven overnight and weighed.

This extraction procedure has been used previously by a number of investigators. Payne[2] found that it showed good correlation with the Soxhlet intermittent type of fat extraction on duplicate samples of mouse and rat livers of various fat concentrations.

The determination of the eviscerated carcass fat was carried out with essentially the same procedure except that the anhydrous sodium sulphate added was eight times the weight of the carcass, since the water content of the latter was less than that of the viscera. Afterwards, the mixture was passed through

Table 1. VISCERAL, EVISCERATED CARCASS, AND ENTIRE BODY FAT OF ADULT ALBINO MALE MICE

Exp.	No. of mice*	Fat (per cent) Eviscerated carcass	Visceral	Entire body	Total visceral fat / Total eviscerated carcass fat
(I)	4	9·55 ± 0·584	12·72 ± 0·486	10·35 0·486	0·453 ± 0·018
(II)	6	0·54 ± 0·634	13·97 ± 0·973	10·69 ± 0·730	0·506 ± 0·017
(III)	5	10·80 ± 1·820	15·80 ± 2·565	12·17 ± 2·055	0·539 ± 0·033
(IV)	6	12·16 ± 0·936	18·26 ± 1·54	13·78 ± 0·998	0·544 ± 0·052

* The average body-weights (gm.) in the four groups were: 40·0 ± 1·78; 39·9 ± 1·00; 34·9 ± 0·66; and 38·9 ± 0·35, respectively.

a Hobart meat grinder several times, then mixed in a Hobart mixer and afterwards transferred to a mortar and homogenized. An aliquot corresponding to 0·5 gm. carcass was taken and treated in the same manner as the visceral aliquot.

The results are given in Table 1. The last column indicates the ratio of the total visceral fat to the total eviscerated carcass fat.

The results of the analysis of the visceral and eviscerated carcass fat of the first three groups indicate rather close similarity. However, the animals of the fourth group show a considerably higher fat content, both in the viscera and body wall. It is pertinent to point out here that the animals in group I were killed in December, in groups II and III in January, while those in the fourth group in May.

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Humoral Nature of Osteogenic Activity of Transitional Epithelium

THE osteogenic activity of transitional epithelium was discovered in the course of ligation of the renal artery[1] and bladder mucosa transplantation[2]. In both cases bone is induced in connective tissue along the line of contact with invading cells of hyperplastic transitional epithelium[3]. The question arises whether direct physical contact is required for induction, or whether transitional epithelium contains some diffusible substance with osteogenic activity which may act as an inductor well away from epithelium. All efforts to induce osteogenesis with cell-free extracts and homogenates of bladder mucosa have until now been unsuccessful. The same applies to the products of transitional epithelium secretion which occurs under the experimental conditions. In such experiments only extramedullar mielogenesis was induced[4].

In the experiment described here, homografting of the transitional epithelium within diffusion chambers was performed. The results obtained indicate that transitional epithelium actually contains some chemical substance which can diffuse outside the epithelial cell and stimulate osteogenesis without cell contact.

Diffusion chambers[5] consisted of two 'Millipore' filters (type HA = $0·75\mu \pm 0·02\mu$, 150μ thick). 5×10^4 cells in Hanks's solution were placed in each chamber. The cells were obtained by treating minced urinary bladder mucosa with trypsin or from whole bladders inverted so that the mucosa was exposed to the trypsin. Suspensions consisted of epithelial cells with small amounts of connective tissue cells. The chambers were implanted subcutaneously in adult guinea pigs. 30–35 days after implantation, the chambers with surrounding connective tissue were removed, fixed in 80 per cent alcohol and embedded in paraffin. Serial sections were stained with periodic acid–Schiff hæmatoxylin and Gomori's stain for alkaline phosphatase.

Transitional epithelium was found in 12 of the 20 intact chambers removed. In 8 of these 12 chambers bone formation was present on the host side of the filters. In no case were epithelial cells present outside the chambers, neither was osteogenesis observed around the chamber without growing transitional epithelium inside the chamber (46 chambers with different contents which were fixed on the 25th–52nd day).

Inside the chamber large membranes of growing epithelium were present (Fig. 1 *A*). They represented well-differentiated transitional epithelium with three layers of cells each having different histochemical characteristics typical for that layer of transitional epithelium[3] (Fig. 1 *A*, *B*).

The differentiation of epithelium and histogenetic factors acting in the chamber will be discussed elsewhere.

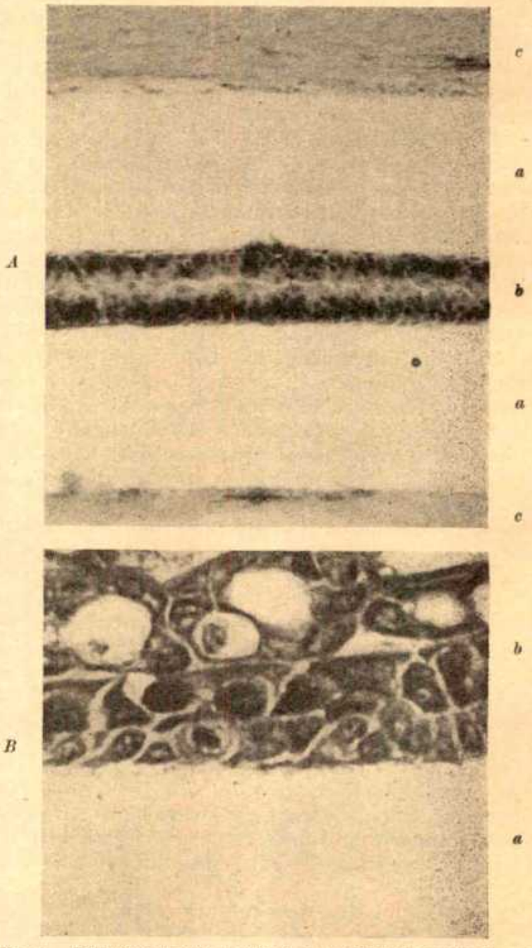

Fig. 1. Diffusion chamber with homografted transitional epithelium 30 days; alcohol. *A*, Gomori reaction (× *c*. 10); *B*, periodic acid – Schiff hæmatoxylin (× *c*. 10). *a*, Filter; *b*, epithelium inside the chamber; *c*, host connective tissue

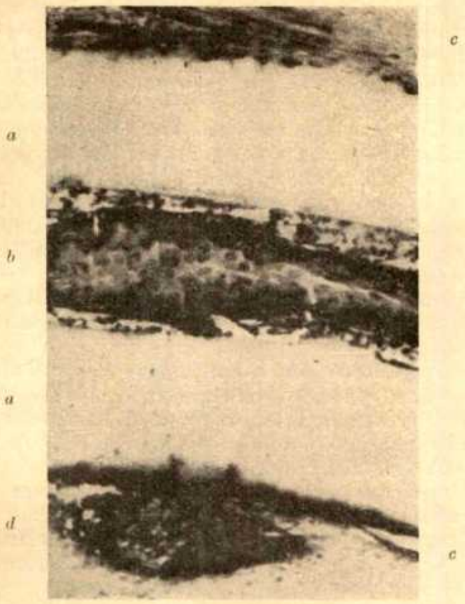

Fig. 2. Diffusion chamber with homografted transitional epithelium 35 days; alcohol. Gomori reaction (× *c*. 10). *d*, Bone tissue outside the chamber

In some cases inside the chamber not only epithelium layers but also connective tissue were growing without the two mixing. Osteogenesis inside the chamber was not observed. On the 30th day after implantation proliferation of host connective tissue around the chamber was obscure and the Gomori reaction was negative (Fig. 1 *A*). In this tissue distinct phosphatase-positive areas of typical osteogenesis could be recognized (Fig. 2). They were composed of osteoblasts and periodic acid–Schiff-positive bone trabeculæ (Fig. 3). Some of them were lying in close contact with the host surface of the filter (Fig. 2); the others were some distance from it (about 80μ) (Fig. 3). Osteogenesis was evoked on the host side of the 'Millipore' filter opposite the transitional epithelium occupying the inner surface of the filter. This transitional epithelium had a high content of glycogen.

The details of histogenesis of bone induction with the characteristics of connective tissue outside and inside the chambers as well as alkaline phosphatase and polysaccharide distribution in the filter itself will be described elsewhere. The results of other investigators and our own results show that the implantation of the diffusion chamber itself does not induce osteogenesis in the surrounding tissue. On the other hand, even the single epithelial cells can be easily recognized in the connective tissue, especially by using periodic acid–Schiff and Gomori reactions. This is why the absence of the epithelium outside the chamber and the intact character of the filters prove that the inductive influence originates only in the epithelium, lying inside the chamber.

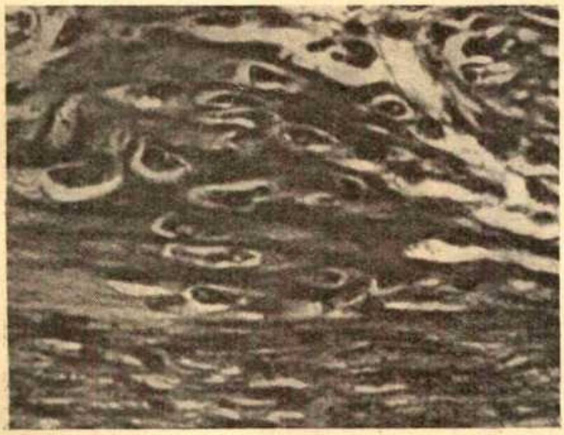

Fig. 3. Bone tissue induced in the host connective tissue outside the chamber 30 days; alcohol. Periodic acid – Schiff hæmatoxylin. (× *c*. 20)

Thus, the transitional epithelium induces osteogenesis by means of a cell-free substance which can diffuse through the filters in the same way as substances from the spinal cord[6] and osteogenic tissue[7] do.

It can be assumed that the transitional epithelium of normal urinary bladder produces and secretes the inducing substance in the same way as it did in the diffusion chamber. My results show that when epithelium is separated from vessels, it produces in the underlying connective tissue a concentration of inducing substance which is sufficient for osteogenesis at a distance of at least 150μ from the epithelium. Questions arise not only concerning the chemical nature of the inductor, but also its physiological significance (in particular, whether it is hormonal in nature[3]). The reasons why connective tissue growing inside the chamber as well as the urinary tract mucosa do not ossify are also of interest. It may depend on the tolerance to inducing influences of the connective tissue of the urinary tract. It may be, however, that in normal conditions the inductor is liberated predominantly in the vessels, or, on the contrary, excessive concentration of the inductor may not be effective. The investigation of these possibilities is now in progress.

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Uptake of Water by the Lizard, *Moloch horridus*

Moloch horridus is an agamid lizard living in the desert regions of Western and South Australia. It weighs as much as 50 gm. and has been described by Buxton[1] as "a repulsive animal with tubercles and spines, it has the power of absorbing water through the skin after showers of rain". This ability of absorbing water through the skin is characteristic of many Amphibia and is correlated with a ready loss of water by the same route[2]. Reptiles living in arid conditions would find a water-permeable skin uneconomical for water conservation so that this mode of taking up water may be questioned for *Moloch*.

The following experiments were therefore made to investigate the uptake of water. This was estimated by changes in weight after the animal had been lying with its belly in water, the results being expressed as

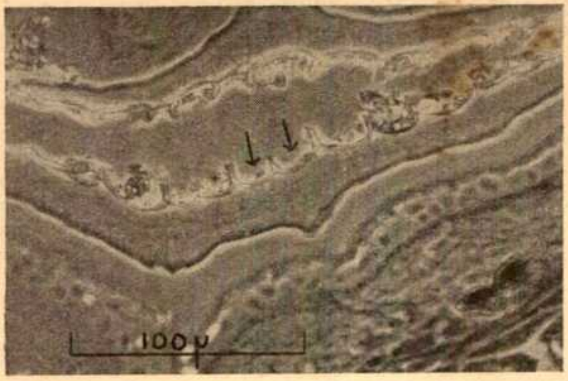

Fig. 1. Section through a skin fold showing the keratin ridges. Phase contrast photograph of a stained preparation. The arrows point to the channels in the keratin layer

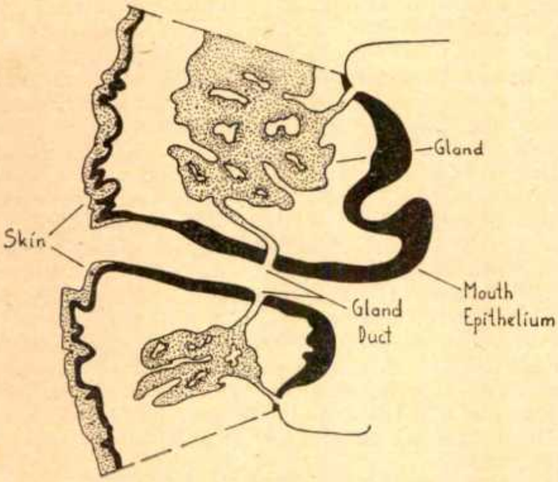

Fig. 2. Diagrammatic vertical section of the upper and lower lips reconstructed from stained serial sections

mean percentage gain in weight ± the standard error for six animals. Adequately hydrated animals, that is animals that had been allowed free access to water, when placed in water gained 2·68 (± 0·1) per cent in weight in the first 5 min. and did not gain thereafter. If, however, the animals had been made deficient in water by the intraperitoneal injection of 2 ml./100 gm. body-weight of 3 per cent sodium chloride 24 hr. before the experiment, then the gain in weight continued after the first 5 min. even though the animal did not drink in the usual manner. Thus after 95 min. the gain in body-weight was 5·6 ± 0·4 per cent and after 155 min. 6·5 ± 0·7 per cent. While the weight was increasing the jaws were noticed to be opening and closing and the significance of this was investigated by holding the jaws closed with adhesive tape. Then water-deficient animals, when lying in water, gained in weight an amount similar to the adequately hydrated animals (2·67 ± 0·1 per cent) in 5 min. and did not gain weight thereafter. If, however, the tape was removed, then the jaws started to move and a further gain in weight ensued.

During the first 5 min. the skin, like blotting paper, appears to absorb water and indeed an advancing water front can be seen moving over the skin. When the water reaches the mouth the water-deficient animals move their jaws and take in water. As confirmation, an animal deficient in water was allowed to lie in water containing a colloidal dye (Evans blue). Later the dye was found in the stomach despite the fact that drinking in the ordinary way was never seen.

The movement of water over the skin seems to be by capillarity along fine open channels in the outer keratinized layer of the skin. These channels are about 20μ across and are separated from one another by sharp keratin ridges (Fig. 1). The lips are well supplied with mucous glands some of the ducts of which open on to the opposable surfaces of the lips (Fig. 2). Possibly water from the skin on reaching the lip edges mixes with the hygroscopic mucus, is passed into the mouth and swallowed.

Ditmars[3] described what appeared to be absorption through the skin in another genus of agamid lizards, the *Uromastyx*. These lizards live in the desert regions of North Africa and South-West Asia and it seems likely that a mechanism may operate in these animals which is similar to that in *Moloch*.

The ecological significance of this mechanism may be that animals with an impervious skin suitable to the desert can still use it to collect water, from dew or sporadic showers, which is then swallowed.

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Function of the Inferior Vena Cava Valve of the Harbour Seal

THE anatomical descriptions of the venous system of the seal have impressively described the large hepatic venous sinuses and the existence of a sleeve-like valve surrounding the inferior vena cava[1]. These hepatic venous sinuses are capable of holding a large volume of blood[1]. It has been tempting in the past to assume that the purpose of this anatomical system was to store a large volume of blood in the hepatic venous sinuses for use during periods of diving. This would require that the inferior vena cava valve impede flow of blood from the abdominal cavity during diving.

Five young female harbour seals (*Phoca vitulina*) were studied to ascertain the time of constriction of the inferior vena cava valve. They were maintained in live cars floated in the bay, fed fresh herring, and remained healthy throughout the period of investigation. The seals were trained to dive using a teeter-board tilted into an aquarium as previously described[2].

Advantage was taken of two anatomical features of the seal. Its jugular vein is rudimentary in nature and returns little blood from the cranial vault[1]. The major portion of the venous blood from the cranial cavity returns via the extradural vein (Fig. 1) and enters into the veins in the abdominal cavity by way of the renal venous plexus. A plastic cannula was threaded into the extradural vein through a thin-walled needle inserted through an area anæsthetized with 'Meticaine'. The cannula was then advanced to the level of the neck of the seal. Albumin labelled with iodine-131 and Evans blue were used as indicators and were either administered superior to the inferior vena cava valve by way of the fore flipper vein, or administered caudally to the vena cava valve by way of the renal venous plexus or the hind flipper veins.

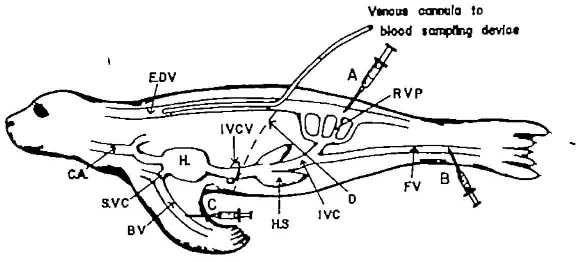

Fig. 1. Schematic diagram of vascular system and study technique. *B.V.*, Brachial vein; *C.A.*, carotid artery; *D.*, diaphragm; *E.D.V.*, extradural vein; *F.V.*, femoral vein; *H.*, heart; *H.S.*, hepatic sinuses; *I.V.C.*, inferior vena cava; *I.V.C.V.*, inferior vena cava valve; *R.V.P.*, renal venous plexus; *S.V.C.*, superior vena cava

A and *B* are injection sites below the level of the *I.V.C.V.*; *C* is an injection site above the level of the *I.V.C.V.*

Blood was sampled from the extradural vein catheter at 5-sec. intervals, and the appearance of the indicators was determined when the seal was out of water, submerged, and tilted without water in the aquarium.

Indicator administered into the fore flipper appeared in extradural vein blood in 20–25 sec., whether the animal was out of water or submerged. Albumin labelled with iodine-131 administered caudally to the inferior vena cava valve appeared in the extradural vein blood samples within 25 sec. of the time of injection when the animal was submerged, but the appearance time was delayed when the animal was out of water (Fig. 2).

The assumption that the inferior vena cava valve contracts when the animal dives does not fit practically the anatomical arrangement for the return of blood from the head to the venous system below the diaphragm. Harrison reported that the inferior vena cava valve is innervated by the phrenic nerve, and that the valve could be made to constrict by phrenic stimulation[3]. These findings by Harrison would be more compatible with function of the inferior vena cava valve during the time that the diaphragm is contracting.

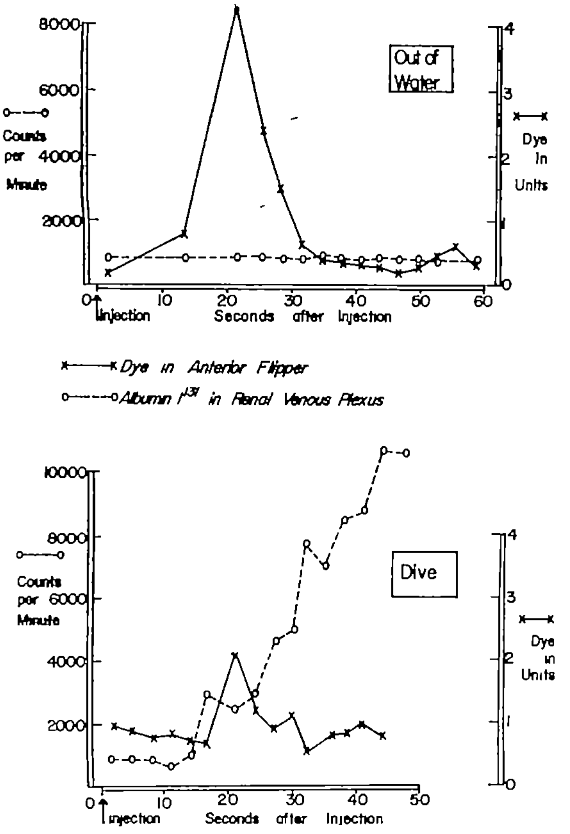

Fig. 2. Comparison of appearance of Evans blue and iodine-131 in the extradural vein in the same seal out of water and during diving. The high background at the time of injection of iodine-131 was due to previous determination of blood volume

The finding that indicator administered caudally to the inferior vena cava valve was delayed in appearance at the extradural vein is consistent with impairment of blood flow from the abdomen to the thorax when the seal is out of water. This finding is more consistent with the anatomical configuration of the venous system of the seal.

The seal breathes against a relative negative pressure when it swims with head above water due to the additional force of the water on the body of the animal. In such a situation, whether it be breathing against a continuous negative pressure, or standing in water up to the axillæ, the human being exhibits a brisk water diuresis[4,5]. It is believed that diuresis in man is due to displacement of blood into the thorax with diuresis resulting from atrial stretch receptor stimuli[4].

It would be a disadvantage to the seal to have blood displaced into the thorax when it surfaces to breathe. It is suggested that the function of the inferior vena cava valve of the seal is to restrict flow of blood from the abdomen to the thorax and prevent pulmonary engorgement with blood when the animal surfaces to breathe. The finding that continuous negative pressure breathing does not alter the rate of urine flow in the seal[6] would agree with this hypothesis.

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Natural and Acquired Immunity Factors inhibiting Penetration of some Hexacanth Embryos through the Intestinal Barrier

EVIDENCE that immunity to tæniid infestations in the intermediate host may be encountered first when embryos attempt to penetrate the small intestinal wall soon after hatching was recently reviewed by Froyd and Round[1]. They were able by oral dosing with eggs of *Taenia saginata* to infect young calves, but not older animals, when the majority of the latter were already infected. They were able, however, to

Table 1. Average No. of Cysticerci of *T. ovis* in Sheep resulting from Injection and Challenge

Dose-rate of eggs used in injections	No. of sheep per group	Cysticerci associated with route of administration				Cysticerci unassociated with site of injection (oral challenge with 6,500 eggs)		
		Intramuscular	Subcutaneous	Intravenous*	Oral	Intramuscular	Subcutaneous	Controls 6 sheep
10,000	2 each	15·0	2 0	10·5	690 0	1 0	2 5	
20,000	2 each	18 0	4 5	51·5	184 0	3 0	11·5	
30,000	2 each	22 0	12 0	92·5	361 5	6 5	4·5	
Total	24	18 3	6 2	51·5	411 8	3·5	6 2	63·0

* Includes unidentified cystic lesions in lungs.

Table 2. Average Number of Cysticerci of *T. pisiformis* in Rabbits resulting from Injection and Challenge

Dose-rate of eggs used in injections	No. of rabbits per group	Cysticerci associated with route or site of injection				Cysticerci unassociated with site of injection (oral challenge with 2,300 eggs)		
		Intramuscular	Subcutaneous	Intravenous*	Oral	Intramuscular	Subcutaneous	Controls 6 rabbits
10,000	2 each	0	0	14·5	4·5	3 0	2·5	
20,000	2 each	0	0	4·0	15·5	1 5	1 0	
30,000	2 each	0	0	0	15·0	0	—	
Total	22	0	0	6·2	11·7	1 0	1 8	113 2

* Includes unidentified cystic lesions in lungs.

establish infections in 13 of 26 older animals by subcutaneous and intramuscular injection of artificially batched and activated embryos. They suggested that the intestinal phase of immunity was thus successfully by-passed.

In preliminary experiments in this laboratory on resistance of the intermediate host to the hexacanth embryo, it has been found possible to infect previously unexposed sheep with *T. ovis*, *T. hydatigena* and *T. pisiformis* and rabbits only with the last-named parasite by intravenous injection of embryos hatched and activated by Silverman's[2] technique.

T. pisiformis has not been recorded in natural infections in sheep, and the establishment of this parasite by intravenous injection of activated embryos suggests that the intestinal wall of sheep may act as a natural barrier against penetration of embryos of this parasite when ingested by this host.

Table 1 shows that infections of *T. ovis* can also be established by intramuscular and subcutaneous injection of activated embryos. The number of cysts established increased with the dose of embryos given; it was greater by intravenous than by intramuscular or subcutaneous injection. However, the number 'taking' by these routes was always less than that by oral ingestion of eggs.

Twelve sheep injected intramuscularly or subcutaneously were afterwards challenged orally with 6,500 eggs. The average number of cysticerci which developed in sites unassociated with the site of injection was 6·2, whereas the average number was 63·0 in six control sheep given the same oral dose of eggs. There was some evidence that some of the cysticerci found in the vaccinated animals may not have been due to the challenge dose, but may have been carried from the site of injection, since two cysticerci were found in lymph nodes draining the injection site and in only two instances were lesions found in the head muscles, heart or diaphragm, whereas one or more of these sites in each control animal were infected.

Unlike *T. ovis* in sheep, cysticerci of *T. pisiformis* do not naturally develop in muscles, and rabbits injected intramuscularly or subcutaneously with hatched and activated embryos of *T. pisiformis* developed no cysticerci at the site of injection (Table 2).

Subsequent oral challenge with eggs of *T. pisiformis* in rabbits which had previously been injected with hatched embryos showed, as observed in the trial of *T. ovis* in vaccinated sheep, that few lesions developed from this challenge. An average of only 1·7 lesions was found in the liver of nine vaccinated rabbits, whereas the average number of lesions in the six controls was 113·2. Moreover, none of the few embryos which became established in the former reached maturity, whereas an average of eight viable cysts was found in the controls.

The results of these trials support the theory that immunity against the cystic stage of tapeworms has two parts: an early response, which may act at the intestinal level and be the more important, and a late response which acts at the site of predilection.

These results also suggest that both natural (species) and acquired (experience) immunity phenomena may occur at the intestinal level and that antibodies produced either against somatic antigens or, more likely, against the metabolic products of embryos undergoing a phase of intense activity during metamorphy are protective.

Whether or not embryos of different species produce common antigens is not yet known. But it appears likely that injection of homologous hexacanth embryos in one form or another may provide some degree of protection for the mammalian intermediate host against subsequent natural infections.

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Relation between Chemical Composition and Nutritive Value of Uganda Grasses

In the various regression equations which have been published the relation between the percentage of crude fibre and the digestibility of organic matter was used[1-3]. Walker and Hepburn[3] used the percentages of crude fibre and crude protein to calculate the digestibility of crude protein, while Watson and Ferguson[4] calculated protein equivalent by using crude protein only. Glover, Duthie and French[5] also used percentage crude protein only, although this was later modified by Glover and French[6] for cases where there was an abnormal ratio of crude fibre to crude protein by proposing an equation using both crude fibre and crude protein. Watson and Ferguson[4] used the percentages of crude fibre and crude protein in their equation for the calculation of starch equivalent, while Mitchell[7] and Hallsworth[8] used crude fibre only.

Digestibility trials with zebu steers fed on various hays prepared from local grasses and on fresh grass have been carried out here in order to examine the relation between chemical composition of grasses, digestibility coefficients and starch equivalents.

The results of techniques and of some digestibility trials with zebu cattle have been published[8-12]. Ten digestibility trials, 7 of which were with *Chloris gayana*, *Cynodon dactylon*, *Panicum maximum* and *Themeda triandra* hays and 3 with freshly cut *Pennisetum purpureum*, have been completed, and the results show the following relation between chemical composition and nutritive values, which can be expressed by the following regression equations.

$$(1) \qquad Y = 139{\cdot}67 - 1{\cdot}0101\,x \quad r = 0{\cdot}9659 \quad P < 0{\cdot}001$$

where Y = digestibility coefficient of organic matter and x = percentage crude fibre + nitrogen-free extract or 100 − (percentage crude protein + percentage petroleum ether extract + percentage total ash).

$$(2) \qquad Y = 9{\cdot}588 + 4{\cdot}284\,x \quad r = 0{\cdot}9521 \quad P < 0{\cdot}001$$

where Y = digestibility coefficients of crude protein and x = percentage crude protein.

$$(3) \qquad Y = 119{\cdot}97 - 1{\cdot}0918\,x \quad r = 0{\cdot}9761 \quad P < 0{\cdot}001$$

where Y = starch equivalent and x = percentage crude fibre + nitrogen-free extract, or 100 − (percentage crude protein + percentage petroleum ether extract + percentage total ash). The protein content of fodders used varied between 4 and 17 per cent.

If these results are confirmed by work with a larger variety of roughages in subsequent digestibility trials it should mean that the cumbersome determinations of crude fibre can be eliminated, and to find out the full nutritional value it would be necessary to carry out analysis of crude protein, fat and total ash only.

It should be noted that in the organic matter regression equation the slope is very near 45° and for all practical purposes the factor 1·0101 is almost unity giving a very simple regression equation $Y = 140 - x$. A closer regression equation may be possible for proteins if hays and fresh fodder are treated separately. This should be confirmed by further trials.

We thank Mr. K. W. Harker for help in calculations.

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Trapping the Airspora by Inhalation

Aerobiologists have developed various sampling techniques for investigating the concentration of airborne pollen and spores. The methods have been summarized by P. H. Gregory[1]. The gravity sedimentation methods on slides and Petri dishes have been superseded mainly by methods of sampling by impaction and allowing a quantitative estimation of the airspora for a given quantity of air. The two main principles of sampling by impaction are to move an object through air on a rotating device, that is, Rotorod sampler[2,3], or to draw the air by pumps, fans or aspirators through filters or on to slides. So far as I know none of the impaction methods trap spores by inspiration although such devices might give the nearest estimations of the number of particles inhaled. In these experiments air is drawn through the filter of a respiratory mask.

Volumetric investigations with the respirator sampler were carried out by adapting the 'Microfilter' dust respirator (Siebe, Gorman and Co., Ltd.). It comprises a moulded plastic face-piece and filter holder. A soft sponge rubber face pad and four elastic bands ensure tight fitting. The respirator is fitted with two valves, a non-return inlet and an outlet. Two perforated metal plates and a screwed plastic cover hold the filter in position. None of these features is changed for the experiment, but the filter pad of Merino wool and the muslin diaphragms are replaced by 'Fibroceta' (British Celanese), a very light wadding made from acetate fibres. The wide air spaces allow good air filtration and the impaction of the airborne particles on the fibres. This synthetic wool can be dissolved in acetone, thus avoiding the difficulties of washing off the impacted particles. Spores and pollen retain their shape in acetone.

In the present experiments a physiotherapist was breathing regularly through this respirator mask at a rate of 20 l./min. She was placed in a closed chamber where air was kept in motion by a high-speed fan. She inhaled for 30 min. During this period lycopodium spores were sprayed every 2 min. from an insufflator (total of 1 gm.). The insufflator was placed horizontally at a distance of 20 cm. from the filter holder and vertically 60 cm. above the filter holder. After 30 min. the filter was removed outside the chamber and dissolved in 200 ml. of acetone, leaving the spores and a fine granular material which could be easily distinguished from the spores when under the microscope. The acetone suspension was centrifuged and the precipitate suspended in 2 ml. of water. The number of spores contained in this suspension were counted in a hæmocytometer in a field of 1 sq. mm., depth of 0·1 mm. The number of spores per cubic metre of air was 133,300.

On advice of Dr. P. H. Gregory the efficiency of the respirator mask was tested by running a Rotorod sampler during the period of inhalation. The Rotorod sampler was placed at the same distance from the insufflator as the filter holder. The number of spores per cubic metre was 50,300. The lower figure of spores sampled by the Rotorod sampler may be due not to less efficiency but to overloading with spores in this experiment.

The new method of trapping the airspora by inhalation may prove to be a reliable and efficient technique, valuable in investigations of respiratory allergy.

I thank Dr. P. H. Gregory for discussion, loan of the Rotorod sampler and for estimating the deposition of lycopodium spores on the Rotorod sampler.

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ENTOMOLOGY

Regulation of Water Economy in *Rhodnius prolixus*

IN a previous report[1], the existence in insects (*Anisotarsus, Blaptica*) of a neurosecretory regulating circuit was postulated in order to explain the regulation of water economy. Later experiments with *Schistocerca cancellata*[2] did not confirm Ramsay's hypothesis, as proposed for *Aedes aegypti* (control by means of the peristalsis of the hindgut[3]), but could be interpreted on the hypothesis that regulation occurs by a direct modification of the excretory activity of the Malpighian tubes, although other hypotheses were not excluded.

Table 1

	Water excreted as per cent of the water in ingested blood	Final volume of the lymph (mm.^{3})
Decapitated	15·4±3	45·2
Controls	51·7±5·4	19

$n = 30$ $P < 0{\cdot}01$

The following results were obtained recently with *Rhodnius prolixus*: (1) Fifth-stage larvæ, decapitated immediately (10–20 min.) after feeding, retained in their hæmolymph after 24 hr. a greater amount of the water contained in the ingested blood (73 per cent) than the non-decapitated controls (42 per cent). Normal elimination of turbid excrements rich in sediments occurred in both decapitated and control larvæ. (2) Similarly decapitated larvæ and controls were parabiotically attached at their heads. No reciprocal actions were observed. Transfusions also gave similar results. (3) The retention of water in the hæmolymph obtained by decapitation may be duplicated by plugging the anus. (4) If cannulated animals are used to avoid possible mechanical retention not due to the Malpighian tubes, the values given in Table 1 were obtained, 16–24 hr. after feeding (and decapitation).

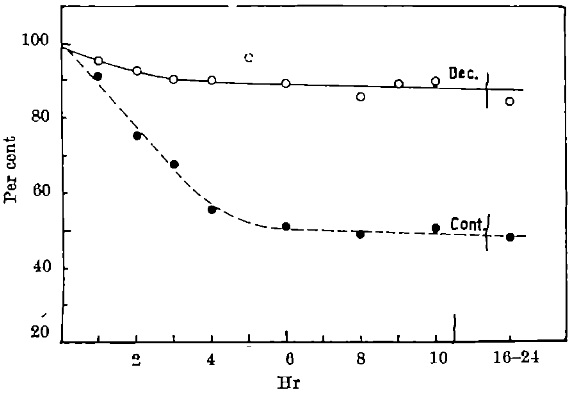

Fig. 1. ○, Decapitated; ●, controls

In Fig. 1, urine excretion (as percentage loss of weight of all the animals with respect to the amount of water contained in the ingested blood) is plotted against time.

Except for the inconclusive results given by parabioses and transfusions, the results reported here suggest that the disturbed water economy observed in the decapitated bugs is due to an abnormal functioning of the Malpighian tubes rather than to a simple modification of the peristaltic movements of the alimentary canal or to mechanical blocking of the anal spincter.

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Identification of Geraniol as the Active Component in the Nassanoff Pheromone of the Honey Bee

HONEY bees (*Apis mellifera* L.) attract each other by a variety of biological stimuli. The olfactory stimulus produced by the worker bees has received attention during a number of investigations ever since Sladen[1] suggested that the attractant odour originated from a special organ discovered by Nassanoff in 1883. This scent organ is located on the anterior part of the seventh abdominal tergum of the worker bee.

Sladen[1] observed bees at the hive entrance with their abdomens elevated, exposing the scent organs. The bees fanned air over the scent organs, thus dispersing the odour. Von Frisch and Rösch[2] found that foraging bees exposed their scent organs at flowers if these yielded an abundant supply of food. Because of the alluring effect which this secretion of the honey bee has on members of the same species it has been included in a group of biologically active substances termed 'pheromones'[3].

A hive-specific body odour is usually superimposed on the scent from the Nassanoff organ. However, Renner[4] obtained the Nassanoff pheromone free of interfering body odours by wiping the exposed scent organ with small bits of filter paper. He showed that the Nassanoff pheromone was not specific for race and colony.

Chemical identification of the active principle of the Nassanoff pheromone could have significant influence on modern agricultural practices dealing with insect pollination of fruit and seed crops.

The pheromone was absorbed on bits of Whatman No. 1 paper following Renner's wiping procedure. The scent obtained on the wipes from individual bees varied both in quality and quantity. Some wipes had a dry odour different from the sweet fruity odour characteristic for the normal wipes. Controls were prepared by wiping body areas other than the scent organ.

In two-choice olfactometer experiments more bees were attracted by odoriferous wipes than by controls. Wipes with dry odour attracted fewer bees than those with fruity odour, which indicated that the attractant principle of the Nassanoff pheromone was associated with its fruity odour.

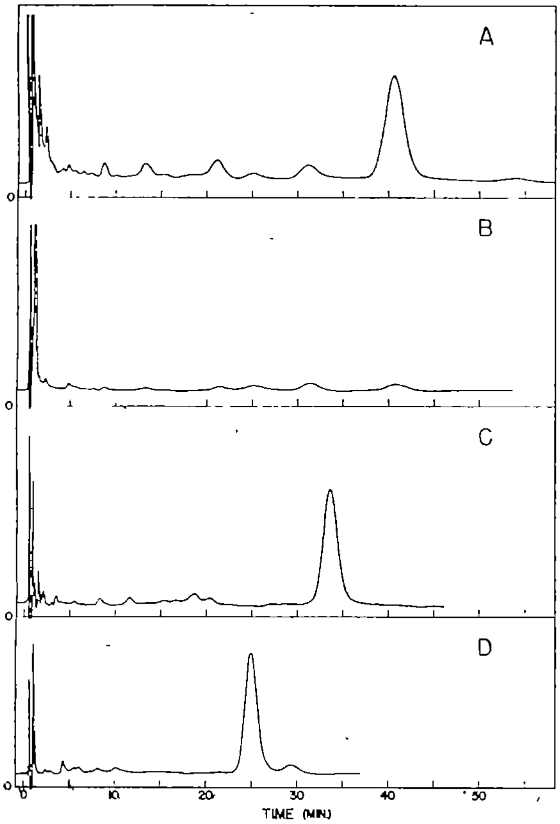

Fig. 1. Gas chromatograms of extracted Nassanoff pheromone on a diethyleneglycol succinate polyester column at 110° C. (*A*), on a 'Carbowax' 1,500 column at 130° C. (*C*), and on a silicone 550 column at 120° C. (*D*). An extract lacking the characteristic odour was also chromatographed on the diethyleneglycol succinate polyester column (*B*)

Thirty wipes which exhibited the characteristic fruity odour were extracted with 20 μl. of carbon disulphide. Centrifugation yielded approximately 12 μl. of a yellowish solution. A 5-μl. portion of this solution was chromatographed in a Perkin–Elmer 154*C* vapour fractometer equipped with a hydrogen flame ionization detector. Using a 4½ ft. × ⅛ in. column containing 10 per cent of diethyleneglycol succinate polyester on 160–170 mesh 'Anakrom *U*' solid support at 110° C., the chromatogram Fig. 1*A* was obtained. Similar treatment of wipes exhibiting the non-characteristic dry odour produced the chromatogram in Fig. 1*B*. The controls gave an almost flat curve (not shown). Thus, it is evident that the characteristic fruity odour must be due to the main component in Fig. 1*A*. Fig. 1*C* is the chromatogram of the characteristic bee material, run at 130° C., using 10 per cent of 'Carbowax' 1,500 as the liquid phase, and Fig. 1*D* is that using 10 per cent of silicone 550 plus 1 per cent of diethyleneglycol succinate polyester to reduce tailing, and run at 120° C.

Using Brown's technique[5], in which the 'affinity fractions' are plotted on triangular co-ordinates, it was determined that the main component of the characteristic bee material was most likely an alcohol. The retention times of this main component on each of the columns were identical with the retention times of geraniol run on the same columns. On all three columns the isomer nerol had shorter retention times than geraniol and was readily separated from it.

The characteristically smelling wipes from 500 bees were extracted and the extracts subjected to gas chromatography using the diethyleneglycol succinate polyester column. Only a small fraction of the effluent from the column was led to the detector. The main component was trapped and transferred in carbon disulphide solution to a 0·1 mm. ultra-micro cavity cell. The infra-red spectrum (Fig. 2*A*) was obtained on a Perkin–Elmer model 21 spectrophotometer using a lens-type beam condenser. This spectrum was identical with a spectrum of geraniol (Fig. 2*B*) obtained in the same way, and different from nerol. The broad peak at 3,300 $cm.^{-1}$ in Fig. 2*B* results from use of a higher concentration of geraniol. Identical shifts of peaks in the infra-red spectra were observed after bromination of both the bee component and pure geraniol.

A comparison of the gas chromatographic peak areas indicated that the wiping procedure yielded, on the average, about 0·25 μgm. of geraniol per bee.

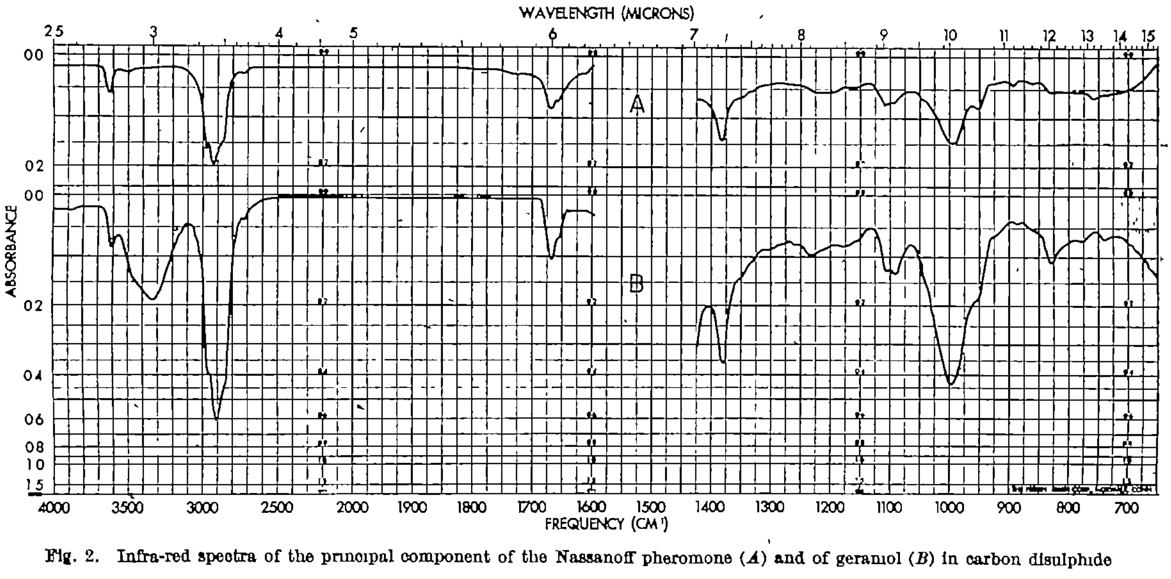

Fig. 2. Infra-red spectra of the principal component of the Nassanoff pheromone (*A*) and of geraniol (*B*) in carbon disulphide

Pyrophosphate esters of geraniol have been identified as intermediates in the synthesis of squalene from acetate in the rat liver enzyme system[6]. However, this is the first reported occurrence of free geraniol in animal secretions.

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An Aphid Flight Chamber

In studying the settling responses of winged aphids and their reactions to different host conditions it is desirable to use insects which have flown freely. Kennedy[1] mentioned a chamber for giving single aphids flight exercise, and the same author[2] has recently described a modified apparatus for maintaining a swarm of flying aphids for experiments on optomotor attraction.

In the chamber described here a large number of aphids can be allowed to take off, fly freely and alight naturally on plants.

The chamber (Fig. 1) is made of hardboard bolted to an angle iron frame, the inside walls, roof and floor being coated with blackboard paint. The floor is raised off the ground so that the observer can sit in the hatch. A 12-in. aerofoil fan blows air into the chamber through a cloth screen on the bottom of a box set in the roof. The cloth screen is of blue terylene with 100 threads of about 105μ diameter per inch. The box has two sloping glass sides; above each are hung two 400-W. high-pressure mercury fluorescent lamps (not shown in Fig. 1) which shine into the chamber through the glass and cloth. Air speeds measured within the chamber are 2 ft./sec. 3 in. below the cloth, 1·5 ft./sec. 12 in. below and 0·5 ft./sec. 24 in. below.

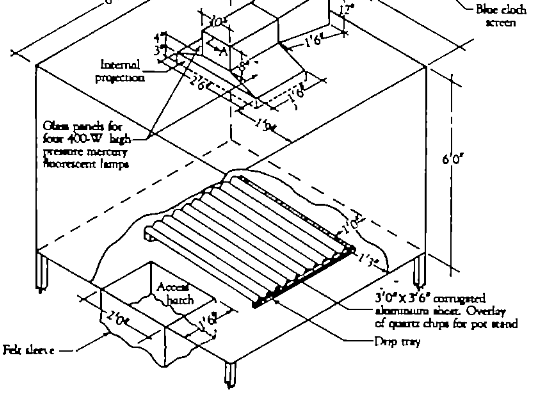
Section at A A
Guide vanes
A
12"
12" aerofoil fan with speed control
Blue cloth screen
6'0"
6'0"
12"
10"
1'6"
4"
3"
A
Internal projection
8"
2'6"
1'6"
1'9"
Glass panels for four 400-W high-pressure mercury fluorescent lamps
6'0"
1'0"
1'3"
Access hatch
2'0"
1'6"
3'0" X 3'6" corrugated aluminium sheet. Overlay of quartz chips for pot stand
Drip tray
Felt sleeve

Fig. 1

Myzus persicae last-instar nymphs are put on tobacco seedlings in 1-in. pots on the stand on the floor of the chamber; about 150 pots can be accommodated. After the last moult and on completion of the teneral period, the aphids have taken off and flown for up to 85 min., alighting again on the plants and settling down to reproduce. Most first flights have lasted between 40 and 70 min. The aphids fly at varying heights between one and four feet from the floor of the chamber. The mean height at which they fly seems to depend on their size and for very large ones the airflow has to be increased slightly. Generally all aphids reared under the same conditions fly at the same height and it is not necessary to adjust the fan speed during the course of an experiment.

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GENETICS

Variation of Bristle Number in Relation to Speed of Development in *Drosophila melanogaster*

The bristle number of the fourth and the fifth abdominal segments of *D. melanogaster* is interesting for two reasons:

(1) The character has been examined extensively in quantitative genetics[1-3].

(2) Bristles are connected with bipolar cells of the nervous peripheral system[2,4] and as such they parallel morphogenetic variations of the tangoreceptor organ, in so far that variations in the area studied reflect variations on the whole body surface.

Some authors[2,3] have attributed the fluctuation of bristles to a low number of genes while a residual variation is imputed to the so-called 'genetic background'. The residual variation requires further definition. Since it is considered to reflect a variation in the development of the nervous system, it could possibly be linked with such a fundamental trait as duration of development.

In order to verify an eventual relation between bristle number and development time a hundred pairs from the stock Canton Special, hatched within 10 hr., were collected. Considering the results of Durrant[5], eggs were procured from flies of a given age, namely, 5 days after pupal hatching. These flies were allowed to lay on the same medium for 14 hr. and discarded afterwards. The eggs were maintained at 25° C. and the pupal hatchings regularly recorded. The correlation coefficients between bristle number and duration of development were found to be $-0{\cdot}281$ ($P < 0{\cdot}01$) for the males and $-0{\cdot}344$ ($P < 0{\cdot}01$) for the females. The difference between these coefficients is not significant. In fact, this relation was not retained because the use of speed of development, that is, the reciprocal of duration of development, leads to a better fit (Fig. 1). Using time of development, the ratios of the variance due to regression on the residual variance are 27·9 (males) and 44·8 (females). These variance ratios increase to 34·1 and 47·4, that is, the fitting of the regression increases in

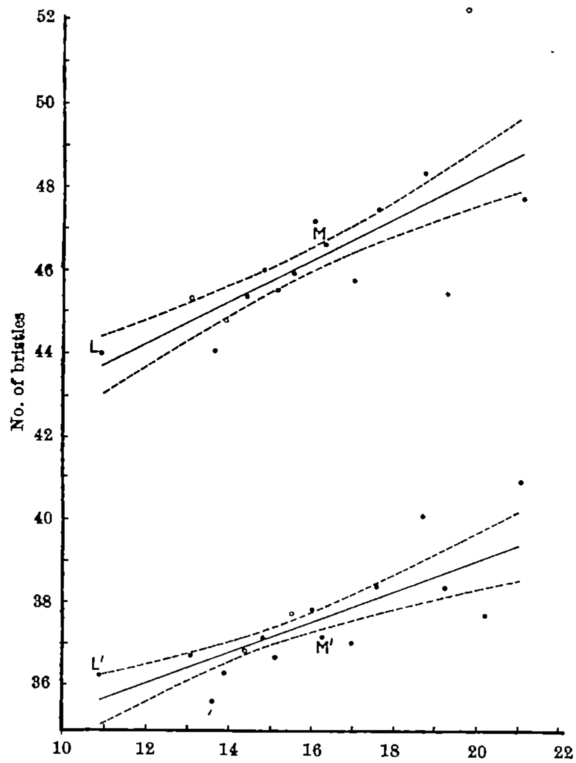

Fig. 1. Relationship between bristle number and developmental speed. Heavy lines, females; thin lines, males. Continuous lines, observed regression line; broken lines, range within which the true line is located with a probability of 95 per cent

this proportion when development speed is used instead of development time. Similarly, the correlation coefficients are slightly augmented to + 0·308 and + 0·353. The 95 per cent probability range of the regressions[6] is quite narrow and points to a very strict dependence of bristle number on speed of development. The question then arises as to whether the variation of bristles due to lower or higher developmental rates is transmissible. It is known that speed development in *D. melanogaster* has a hereditary component[7], and, further, that numbers of bristles are generally resistant to environmental modification[4]. However, on the basis of my experiments, the genetic causation of the correlative behaviour of numbers of bristle and speed of development cannot be proved.

In any event, the facts disclosed show a strong correlation of speed of development with number of bristles and therefore with the development of the tangoreceptor system. This correlation might be genetic and morphogenetic or it might be due to a parallel modification of both traits under the influence of staling culture medium. Whatever explanation is valid, the facts show sufficiently that in genetic studies of number of bristles more emphasis should be placed on sampling techniques with respect to speed of development.

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Hybrid Vigour and Inheritance of Height in Rice

PREVIOUSLY[1], while investigating the inheritance of photoperiodic reaction in rice, in this laboratory it was observed that the F_1 plants of the crosses between Aus (summer) and Aman (winter) varieties of rice showed hybrid vigour in respect of plant height, tiller number and grain yield. Earlier workers[2] also recorded hybrid vigour in the F_1 generation of some rice crosses. However, it is interesting to note that in the present investigation this vigour in one of the growth characters, that is, height, was found to persist in the F_2 generation (Table 1). Mean height of the F_2 plants in every case was found to exceed the mean height of the taller parent. The F_2 frequency distribution in each cross indicated mostly a unimodal curve with transgression beyond the maximum limit reached by parents. It was also observed that the number of the F_2 plants exceeding the maximum limit of the parents was proportionately more in crosses which involved parents with almost the same maximum limit in height, that is, in Patnai-23 × Dhairal and Bhasamanik × Charnock crosses. While in other crosses involving parents with comparable maximum height, as in Patnai-23 × Charnock or in Bhasamanik × Dhairal crosses, the proportion of transgressing segregation in F_2 was less. All these indicate a polygenic inheritance of this character. The proportion of the F_2 plants exceeding the maximum limit of the parents under different crosses

Table 1. FREQUENCY DISTRIBUTION OF HEIGHT AND ITS MEAN VALUE IN THE DIFFERENT SETS OF F_2 POPULATIONS AND THEIR PARENTS

Varieties		Class values and No. of plants occurring in each class												Total plant	Mean value (cm)
		79 5	89·5	99 5	109 5	119 5	129 5	139 5	149 5	159·5	169·5	179·5	189 5		
Parent varieties															
1 Patnai-23 (Aman)		0	3	17	82	71	25	2	0	0	0	0	0	200	114 83
2 Bhasamanik (Aman)		26	89	74	10	1	0	0	0	0	0	0	0	200	92 95
3 Charnock (Aus)		14	65	85	34	2	0	0	0	0	0	0	0	200	96·48
4 Dhairal (Aus)		1	5	52	69	51	21	1	0	0	0	0	0	200	109·53
F_2 population															
♂ Parent	♀ Parent														
1 Patnai-23	× Charnock (23*)	2	1	6	14	44	91	95	54	8	0	0	0	315	135·99
2 Patnai-23	× Charnock (24*)	1	1	6	25	44	65	65	34	4	0	0	0	245	130·30
3 Patnai-23	× Charnock (25*a**)	0	1	6	21	33	35	11	2	0	0	0	0	109	128·34
4 Patnai-23	× Charnock (25*b**)	1	0	2	4	12	42	72	32	6	1	0	0	172	136 796
5 Charnock	× Bhasamanik (32*)	1	0	3	11	20	55	60	35	5	0	0	0	190	130·58
6 Patnai-23	× Dhairal (14*)	0	0	5	4	10	25	50	81	72	38	10	1	296	147 46
7 Dhairal	× Patnai-23 (19*)	0	0	1	2	12	37	79	73	32	11	2	0	249	143 25
8 Dhairal	× Patnai-23 (21*)	0	0	1	2	7	38	104	119	87	25	6	0	384	148 39
9 Dhairal	× Bhasamanik (7*)	1	2	3	13	40	81	100	70	42	5	3	1	361	139·12
10 Bhasamanik	× Dhairal (10*)	2	0	1	11	22	46	89	48	20	5	1	0	245	138 16

* Cross-number. All heights are given in cm The date of sowing was June 14, 1958.

suggests that the cumulative effect of the genetic complex influencing the plant height is different in different varieties. Several workers observed the quantitative nature of this character, while monogenic, digenic and trigenic segregations were also reported in some crosses[2].

Due to the influence of hybrid vigour and gene complexes on the expression of the plant heights, the inheritance of this character in the present investigation has become very complicated. Further investigations are required to throw more light on this aspect.

I thank Prof. P. K. Sen, Department of Agriculture, University of Calcutta, for his help.

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Department of Agriculture,
University of Calcutta.

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[1] Sen, P. K., and Mitra, G. N., *Nature*, **182**, 119 (1958).

[2] Ramiah, K., *Rice Breeding and Genetics* (Ind. Coun. Agric. Res. Pub., 1953).

VIROLOGY

A Rapid Method for Microanalytical Determination of the Amount of Tobacco Mosaic Virus in Plant Tissues

THE development of the kinetic studies of virus biosynthesis requires an accurate and rapid method for determining the amount of virus nucleoprotein contained in small samples of tissue. Bancroft and Curtis[1] described a simple method which utilized trichloroacetic acid (TCA) as the substance for precipitating and extracting virus nucleic acid. Although this method is rather rapid, it results in the destruction of virus nucleoprotein and demands careful attention to the final concentration of TCA, because TCA itself has a high absorbance at 260 mμ which may cause gross errors in the measurement of the small amount of viruses. The disadvantages of the method were overcome by using ammonium sulphate instead of TCA: the accuracy and rapidity of the method were also increased.

One to ten disks of tobacco leaves infected with the virus (9 mm. diam.) were homogenized with 6 ml. of distilled water. 5 ml. of the homogenate and 2 ml. of chloroform were mixed and shaken for 5 min., cooled at 4° C. for 15 min. and then centrifuged at 3,000 r.p.m. for 15 min. 4 ml. of the resultant supernatant were transferred into a centrifuging tube, to which 1·33 ml. saturated ammonium sulphate was added (0·25 saturation) at room temperature. The mixture was thoroughly stirred until the precipitate produced had almost disappeared, and then allowed to stand for 10 min., followed by 15 min. centrifugation at 3,000 r.p.m. The resultant precipitate was dissolved in 4 ml. distilled water, stood for 10 min., and centrifuged at 3,000 r.p.m. for 15 min. The optical density at 260 mμ of the supernatant was measured using a spectrophotometer.

The absorption spectrum of the supernatant is shown in Fig. 1. The curve was found to be approximately the same as that of Reddi[2]. It is clear from Fig. 2 that measurement of tobacco mosaic virus by this method is not disturbed by the presence of normal protein of host tissues. The infected leaf tissues were homogenized with 20 times their wet weight of distilled water and the homogenate of healthy tissues was added. The virus concentration of these homogenates was determined by the present method. No. 6 on the abscissa in Fig. 2 shows the presence of infected homogenate only and No. 0 shows that of normal ones only. Nos. 1–5 represent the mixed homogenates containing 1/6–5/6 of infected homogenate to total volume respectively. It was found that the optical densities of the nucleoprotein increased in a straight line according to the increase of the amount of infected homogenate in spite of the presence of healthy homogenate.

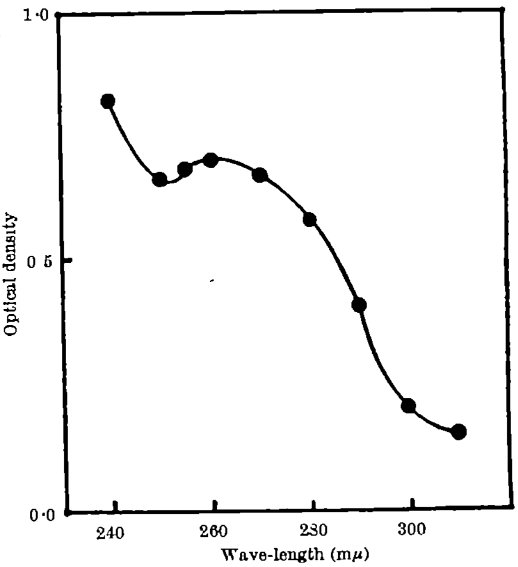

Fig. 1. Absorption spectrum of tobacco mosaic virus

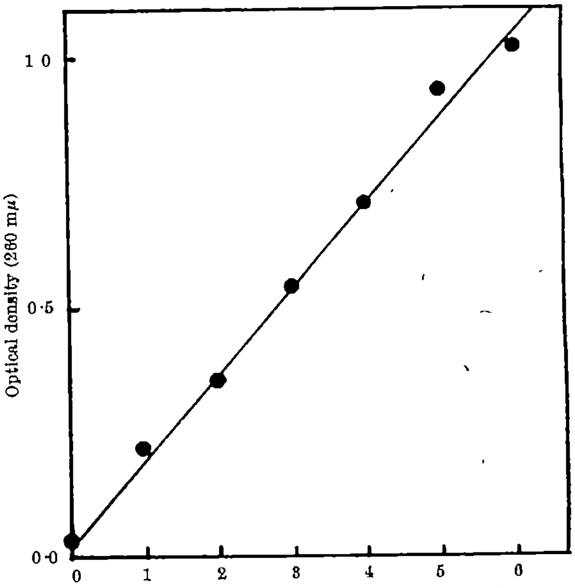

Fig. 2. Optical densities of the nucleoprotein obtained from infected leaf homogenates in the presence of healthy leaf homogenates

This method will be applied to the rapid determination of this virus in the very small quantity of samples.

TAKESHI TANIGUCHI

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Nagoya University,
Anjyo, Japan.

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[1] Bancroft, J. B., and Curtis, R. W., *Phytopath.*, **47**, 79 (1957).

[2] Reddi, K. K., *Biochim. Biophys. Acta*, **24**, 238 (1957).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 21

Institution of the Rubber Industry (at the Institution of Electrical Engineers, Savoy Place, London, W.C 2), at 4 p.m.—Prof G. Stafford Whitby: "The Biogenesis of Rubber—How the Tree Makes Rubber" (Seventeenth Foundation Lecture).

University of London (at the Wright–Fleming Institute of Microbiology, St Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Dr. A. Isaacs: "The Antiviral Action of Interferon"*. (Third of four Almroth Wright Lectures).

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. H. Cole· "Fisheries". (Last of three Cantor Lectures on "The Development of Natural Resources".)

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. I. A. Menzies: "The Electrodeposition of the Less Common Metals".

Society for Visiting Scientists (at the English Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p m.—Discussion Meeting on "The Turkey X Disease". Chairman· Prof. Alexander Haddow, F.R.S Speakers· Dr. P. C. Spensley, Dr. J. M. Barnes and Mr. J. G. Carnochan.

Tuesday, May 22

University of London (at Queen Elizabeth College, 61–67 Campden Hill Road, London, W.8), at 4.15 p.m.—Dr. P. N. Hobson: "Aspects of the Role of Micro-organisms in Digestion".*

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Annual General Meeting. 7 p.m.—Presidential Address.

Wednesday, May 23

British Institution of Radio Engineers, Education Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, London. W.C.1), at 2 p.m.—Discussion on "Laboratory Work in Advanced Radio and Electronic Engineering Education—Its Aims and Methods".

Eugenics Society (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. J. M. Thoday: "The Causes and Functions of Genetic Variety".*

University of London (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p m.—Prof. Yigael Yadin (Hebrew University of Jerusalem): "New Light on Bar Kochba: The Recent Expedition to the Dead Sea Area".*

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting.

Thursday, May 24

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m —Prof. J. S. Mitchell: "Some Problems in the Study of Radio-Sensitizers and Radioactive Drugs".*

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. T. M. Harris, F R S.· Illustrated talk on a Fossil Plant from New Zealand (Anniversary Meeting).

Royal Society (at Burlington House, Piccadilly, London, W. 1), at 4 30 p.m.—Prof. E. N. da C Andrade, F.R.S., and Mr. W. J. D. Jones: "The Influence of Grain Size and Surface Conditions on the Creep of Polycrystalline Metal under Tension"; Mr. E. H. Sondheimer. "The Boltzmann Equation for Anisotropic Metals".

Friday, May 25

University of London (at the Royal Holloway College, Englefield Green, Surrey), at 8.15 p.m.—Dr. Audrey Butt· "Medicine in Primitive Tribes".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p m.—Prof. B. J. Mason: "The Physics of Snow, Rain, Hail and Lightning".

Friday, May 25—Saturday, May 26

British Society for Immunology (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1)—Spring Meeting.

Monday, May 28

University College, Department of Biochemistry (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. R. K. Morton (University of Adelaide)· "The Flavohæmoprotein Cytochrome b_2—a New Type of Dehydrogenase".*

University of London (at the Wright–Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m —Prof. E. S. Lennox (New York): "Antibody Synthesis Analysed at the Cellular Level".* (Last of a series of four Almroth Wright Lectures)

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Owen Lattimore: "The Geography of Ghengis Khan" (The Dickson Asia Lecture)

Monday, May 28—Saturday, June 2

Fourth International Instruments, Electronics and Automation Exhibition (at Olympia, London).*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Fellow (with a first degree in biochemistry, chemistry or botany, and research in biology) in Plant Biochemistry in the Department of Botany, to participate in research on the mechanism of photosynthesis—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 25).

Assistant Lecturer or Lecturer (with qualifications in any branch of pure mathematics, numerical analysis or statistics) in the Department of Mathematics—The Registrar, University College of South Wales and Monmouthshire, Cathay's Park, Cardiff (May 26).

Lecturer (Grade II, preferably at least 30 years of age) in the Department of Physics, The Registrar, The University, Bristol (May 28).

Lecturer in the Department of Mechanical Engineering—The Secretary, University College, Gower Street, London, W.C.1 (May 28).

Lecturer (Grade II) in Physiology—The Registrar and Secretary, The University, Bristol (May 30).

Lecturer in Psychology at Rhodes University, Grahamstown, South Africa; and a Lecturer in Psychology at Rhodes University, Port Elizabeth, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, May 31).

Research Demonstrator (graduate in botany) in the Department of Botany, to engage in research, preferably in plant physiology and biochemistry, plant pathology or ecology—The Registrar, University College, Singleton Park, Swansea (June 2).

Junior Lecturer (with interests in the fields of either physical or organic chemistry) in Chemistry—The Registrar, Trinity College, Dublin, Republic of Ireland (June 4).

Lecturer or Junior Lecturer in Biochemistry—The Registrar, Trinity College, Dublin, Republic of Ireland (June 4).

Assistant Lecturer (man or woman with a special interest in petrology) in Geology—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 8).

Lecturer or Assistant Lecturer (Probationary) (man or woman graduate, preferably with experience in experimental high energy or nuclear physics) in Physics—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 8).

Leverhulme Lecturer (medically or non-medically qualified) in Parasitology—The Registrar, The University, Liverpool, quoting Ref. CV/78/T (June 8).

Lecturer or Assistant Lecturer (with special qualifications in some aspects of the systematics or comparative morphology of vascular plants) in Botany—The Secretary and Registrar, The University, Southampton (June 9).

Temporary Botanist or Agriculturist, to assist in work on diseases of potato tubers—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 15).

Assistant in Organic, Physical or Inorganic Chemistry at Queen's College, Dundee—The Secretary, University of St. Andrews, c/o Queen's College, Dundee (June 16).

Lecturer (with experience in the field of rheology or some other section of industrial physics) in Applied Physics at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 18).

Lecturer (with an honours degree in physiology, pharmacology or pharmacy) in Pharmacology; Demonstrators (2) in Pharmaceutics; a Demonstrator in Pharmacognosy; and a Demonstrator in Pharmaceutical Chemistry—The Registrar, The University, Nottingham.

Postdoctoral Biochemist or Protein Chemist, for fractionation and investigation of coagulation factors in plasma—Dr. G. B. D. Scott, Department of Morbid Anatomy, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

Scientific Information Officer (Abstractor) (with a degree in agriculture or natural science, and able to translate from German)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

Senior Laboratory Technician I in Hæmatology at the Connaught Hospital, Walthamstow, London, E 17—The Secretary, Forest Group H.M.C., Langthorne Road, Leytonstone, London, E.11.

Student Technician in the Department of Pathology, Connaught Hospital, Walthamstow, London, E.17—The Secretary, Forest Group H.M.C., Langthorne Road, London, E 11.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Nuclear Energy in Britain during the last War. By Sir George Thomson. (The Cherwell-Simon Lecture delivered in Oxford on 18 October 1960.) Pp. ii+16. (Oxford: Clarendon Press; London: Oxford University Press, 1962) 3*s*. 6*d*. net. [192

The Engineer Buyers Guide, 1962. Pp 1008. (London: *The Engineer*, 1962.) 10*s*. [192

General Register Office The Registrar General's Statistical Review of England and Wales for the year 1960. Part 2 · Tables, Population. Pp. xi+180. (London: H.M. Stationery Office, 1962.) 12*s*. 6*d*. net. [192

Bulletin of the British Museum (Natural History) Entomology. Vol 11, No. 4: Miss L. E Cheesman's Expeditions to New Guinea. Trichoptera. By D. E. Kimmins. Pp. 97–187. 30*s*. Vol. 11, No. 5 · A Key to the Species of *Actornithophilus* Ferris with Notes and Descriptions of New Species. By Theresa Clay. Pp. 189–244 + plates 4–11. 30*s*. (London: British Museum (Natural History), 1962) [192

The British Hydromechanics Research Association. Fourteenth Annual Report, October 1960 to September 1961. Pp 44. (Harlow: The British Hydromechanics Research Association, 1962.) [222

The Succession of Life Through Geological Time. By Kenneth P. Oakley and Helen M. Muir-Wood. Fifth edition. Pp vii+94 (12 plates) (London: British Museum (Natural History), 1962.) 5s. [222

Hopkin and Williams, Ltd. Organic Chemical Reagents. Monograph No. 43: Stable Dithiol Derivatives. By Dr. R. E. D. Clark. Pp. 8. (Chadwell Heath: Hopkin and Williams, Ltd., 1962.) [222

The British Association/Granada Guildhall Lectures, 1961. The Language of Animals. By Sir James Gray. Why Scientists Talk. By Prof. Hermann Bondi. The Gap—and the Bridge. By Sir John Wolfenden. (Communication in the Modern World.) Pp. 80. (London: University of London Press, 1962.) 4s. 6d. [222

Science Teaching Techniques, No. 9. (The Budgerigar as a Focus of Interest for Scientific Studies in City Schools An Astronomy Course for Secondary Schools. An Approach to the Problem of Identification in Ecology. Fats, Figures and Faces. Soldering for the Science Teacher. A Pre-Nursing Syllabus.) Pp. 64+2 plates. (London: John Murray (Publishers), Ltd., 1962. Published for the Association of Teachers in Colleges and Departments of Education; The Association of Women Science Teachers: The London Association of Science Teachers; and The Science Masters' Association.) 4s. [222

Hand-List of Coniferae, Cyadaceae and Genetaceae in the Royal Botanic Gardens, Kew; and The National Pinetum, Bedgebury. Fifth edition. Pp. iii+41. (London: H.M. Stationery Office, 1961.) 5s. net. [222

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Bowater Paper Corporation, Ltd. Bowaters Today. Pp. 50. (London: Bowater Paper Corporation, Ltd., 1962.) [222

Low Temperature Research Station, Cambridge. Annual Report, 1960. Pp. 40. (Cambridge: Low Temperature Research Station, 1962.) [232

Dublin Institute for Advanced Studies: Observatory of the School of Cosmic Physics. Dunsink Observatory Publications, Vol. 1, No. 3: Cosmic Ray Flares: a Description and Discussion of the Ten Great Solar Flares which have Generated Cosmic Rays Recorded at Ground Level. By M. A. Ellison, Susan M P McKenna and J. H. Reid. Pp. 51–88. (Dublin: Dublin Institute for Advanced Studies, 1961.) [232

Other Countries

Universidad Nacional Autonoma de Mexico: Departmento de Fisiologia de la Facultad de Medicina. Programas para el Curso de Fisologia de la Carrera de Medicine para los Anos de 1957, 1958, y 1959. Pp. 84. (Mexico, D.F.: Universidad Nacional Autonoma de Mexico, 1961.) [222

III Informe de Preologia. Sección de Filosofia de la Ciencia: Instituto Luis Vives de Filosofia. Rusemen del Trabajo sobre Preologia realizado en equipo durante el Curso 1959/60 con la colaboracion de los Sres. Camarero, Ceberio, Cordon, Pérez Sadaba, Ribes, Rosinol, Sànchez Ferlosio, Sànchez Martines, Vela de Almazán y otros. Pp. 30. (Madrid: Consejo Superior de Investigaciones Cientificas, Serrano, 127, 1961.) [222

Auckland War Memorial Museum. New Zealand Medicinal Plants. By S. G. Brooker and R. C. Cooper. Pp. 46+12 plates. (Auckland: Auckland Institute and Museum, 1961.) [222

Smithsonian Institution: The United States National Museum. Annual Report for the year ended June 30, 1961. Pp. viii+188. (Washington, D.C.: Government Printing Office, 1961.) [222

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United States Department of Health, Education and Welfare. Division of General Medical Sciences, Center for Aging Research. Public Health Service Publication No. 884: Second Conference on Gerontology and Geriatrics, Moscow, 1960. (Abstracts of Papers.) A Translation from the Russian. Pp. viii+81. (Washington, D.C.: Government Printing Office, 1961.) 35 cents. [222

Fisheries Research Board of Canada. Bulletin No 130: The Swordfish (*Xiphias gladius* L.), Its Life-History and Economic Importance in the Northwest Atlantic. By S. N. Tibbo, L. R. Day and W. F. Doucet. Pp. vi+47. (Ottawa: Queen's Printer, 1961.) 75 cents. [222

Annals of the New York Academy of Sciences, Vol. 96, Article 1: Some Biological Aspects of Schizophrenic Behavior. By D. V. Siva Sankar and 105 other authors. Pp. 1–490. (New York: New York Academy of Sciences, 1962.) [222

National Institute of Genetics, Japan. Annual Report, No. 11, 1960. Pp. vi+111. (Misima, Sizuoka-ken: National Institute of Genetics, 1961.) [222

Smithsonian Institution: Bureau of American Ethnology. Bulletin 179: River Basin Surveys Papers, Nos. 21–24. Edited by Frank H H. Roberts, Jr. (Inter-Agency Archæological Salvage Program.) Pp. xviii+337+56 plates. (Washington, D.C.: Government Printing Office, 1961.) 2.25 dollars. [222

Canada: Department of Mines and Technical Surveys. Map 1039A: Alberta and Northeastern British Columbia showing Oil and Gas Fields. Eighth edition. Geological Maps. Index Sheet 31, 40: Ontario. Index Sheet 31: Quebec–Ontario. Index Sheet 32: Quebec–Ontario. Index Sheet 41: Ontario. Index Sheet 42: Ontario. Index Sheet 43: Ontario—District of Keewatin. Index Sheet 44: District of Keewatin—Ontario. (Ottawa: Director, Geological Survey of Canada, 1960 and 1962.) [222

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United States Department of Agriculture: Agricultural Research Service. (In co-operation with the Texas Agricultural Experiment Station.) Technical Bulletin No. 1255: Airborne Movement of the Pink Bollworm and other Arthropods. By Perry A. Glick and L. W. Noble. Pp. ii+20. (Washington, D.C.: Government Printing Office, 1961.) 15 cents [222

Certain Aspects of the Research on Dirichlet Series. By Prof. B. N. Prasad. (Presidential Address—27th Conference of the Indian Mathematical Society, Ahmedabad, December, 1961.) Pp. 23. (Lakshmi Niwas, George Town, Allahabad: Prof. B. N. Prasad, 1961.) [222

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University of Wisconsin: Department of Geology. Geophysical and Polar Research Center. Research Report No. 2: Reconnaissance Residual and Regional Magnetic Maps of the Arctic Ocean Basin—a Preliminary Report. By Ned A. Ostenso, Richard J. Wold, and G. P. Woollard. Pp. 4+2 tables+6 figures. (Madison, Wisc.: The University, 1962.) [222

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 72: Mineralogy and Paragenesis of Lead-Zinc-Copper Ores of the Bathurst-Newcastle District, New Brunswick. By S. Roy. Pp. xii+20+11 plates. 1 dollar. Bulletin No. 80: Contributions to Canadian Palaeontology. Part 1: On the Ordovician Corals *Palaeophyllum rugosum* Billings and *Nyctopora billingsi* Nicholson. By D. Hill. Part 2: Notes on Some Ordovician Corals. By G. Winston Sinclair. Pp. ix+18+7 plates. 1.25 dollars. Paper 61–14: Ground-Water Resources of Moncton Map-Area, New Brunswick. By P. A. Carr. Pp. ii+7. 50 cents. (Ottawa: Queen's Printer, 1961) [222

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Institut Royal Météorologique de Belgique. Observations Ionospheriques—Station de Dourbes. Septembre, 1961. Pp. 26. Octobre, 1961, Pp. 26. Publications, Série A, No. 24: Annuaire Climatologique pour 1960. Pp. 94. (Bruxelles: Institut Royal Météorologique de Belgique, 1961.) [222

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United States Department of the Interior: Geological Survey. Bulletin 1116–B: Geophysical Abstracts, 185, April–June, 1961. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. iii+171–350. 40 cents Water-Supply Paper 1496–B: Copper-Spark Method for Spectrochemical Determination of Strontium in Water. By M. W. Skougstad. Pp. iii+10–31. Water-Supply Paper 1527: Floods in the Skagit River Basin, Washington. By James E. Stewart and G. Lawrence Bodhaine. Pp. v+66+4 plates. Water-Supply Paper 1535–F: Solutes in Small Streams Draining Single Rock Types, Sangre de Cristo Range, New Mexico. By John P. Miller Pp. iii+23. Water-Supply Paper 1619–A: Hydrology of a Spring in a Glacial Terrain near Ashland, Ohio. By Stanley E. Norris. Pp. iii+17. Water-Supply Paper 1705: Surface Water of the United States, 1960. Part 3–A: Ohio River Basin Except Cumberland and Tennessee River Basins. Prepared under the direction of E. L. Hendricks. Pp. xii+583. Water-Supply Paper 1709: Surface Water Supply of the United States, 1960. Part 6–A: Missouri River Basin above Sioux City, Iowa. Prepared under the direction of E. L. Hendricks. Pp. x+449. (Washington, D.C.: Government Printing Office, 1961.) [232

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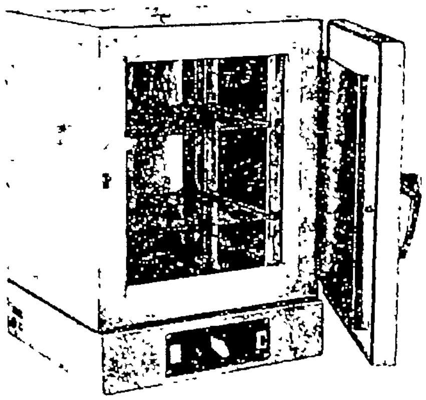

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DEATH OF A LABORATORY

By F. J. BOLTON

I. Even the acid-splashed benches have gone now, beside the walls
Stained blue and yellow where a flask blew up, nothing stands
But rusting iron supports. Higher, a blackboard falls
Drunken, half covering the door. In chalk of red and green, your hands
Assured and firm once drew upon its surface formulæ
As intricate as scaffolding, a patterned honeycomb extending
Unleashed across the plastered wall itself, where still I read
The molecular grouping of your own particular compound's ending.
A winter wind, focusing through the shattered panels of the window frame,
Spills on the floor mosaics of coloured glass, corks from a battered bin,
An ooze of heating oil, a snake of rubber tubing, steel tripods blacked by flame.
To-morrow week, they say, the demolition squads move in.

II. The Research Department was born three years before the War.
Then, with contracts for textile chemicals flowing in, a dream
Of twenty years before was firmly crystallized in walls
Of stone and brick and concrete. The fertile stream,
Dammed up during the lean and angry 'Thirties, burst out
In a torrent of wild purposefulness, of fierce mad
Projects and irrepressible enthusiasms, a bout
Of totally unrealizable ambitions. All these were stilled on that sad
Night when four bombs withered our fragile building to a shell.
But, miraculously, the body breathed in sleep, slowly grew strong again
And when, in the late 'Forties, the intellectual well
Was primed once more, our thoughts seemed clear and spare
As trees do after winter rain.

III. "A team is no stronger than its staunchest link" I said
Tritely "and to have a chance, an inventive mind
Is the one essential for original research." Led
Forward by this instinct, restricted by the blind
Parsimony of my directors, I searched among the very young
Outsiders, the unknowns, the untried, hoping there to find
The hundred-to-one chance, the hidden pearl, the one
Who would ask for nothing further than to work and lead
His team, younger men even than himself; above all,
A celibate, with disciplined tastes, committed and keyed
To labour for the future only, to blossom or fall
By the fruit of his own completely personal inspirations, abjuring
All luxury. Six months I took, that year before the War, and why
I chose you from the press, cajoling, luring
You from certain academic peace into this flaming trial
Of industrial warfare, I cannot rationally say; sometimes
I suppose it may have been your hand-writing, lean like yourself
And tall, your most appealing air
Of unsophisticated youth, clear laugh, rough uninhibited dress,
Your frankness, charm, the brush of flaxen hair
Falling continually across your brown eyes in the hour of stress.
But, from whatever hidden source my inspiration rose,
In this one choice of all my life, unconsciously, sheer genius shows.

IV. I remember, when the mottled structure was patched and tied together,
What dreams we harboured of relentless growth, of crystal glass
Palaces, plastic and steel, laboratory into laboratory, ever
Endlessly expanding within a telescopic future no war

Could possibly hinder. What naïve laughter we enjoyed
In planning an unorthodox shape of bench, a floor
Proof to the most violent solvents; colours soft and hard
On wall and ceiling, cerulean blue and primrose yellow.
I remember how, together, we built up our staff
Raw from college or school, some your own
Selected students, some lean and ambitious, some mellow,
At peace with the world, even at twenty, all
Enthusiastic, all disillusioned of ease, forewarned
To expect nothing but toil and trouble, to fail
Or flourish by their own silver store of talent, hard-won,
With no promise but that of fellowship, admission to a band
Of young, unconventional, non-conforming and open-minded men.
A Scotsman we had, a Yorkshireman, an Australian,
Two Londoners, an Indian, a Russian Jew, but, to us, then
With our passion for the team and not the man,
The company's and not the individual's glory, none, none was alien.

V. The sun was hanging in the sky, orange, over the Welsh mountains when you came
That night into my office. It was late, I should have been at home, instead
I stayed to read reports, to doze and watch the dying flame
Leaping and fingering through the Midland haze, the leaden
Clouds from the uncountable, the infinite number of furnace-stacks
Blocking out Nature. Half understanding, I heard you say
"I wish you'd come up into the lab. Something very strange has happened in my test-tube rack
In a set of colour tests I laid aside a week ago last Tuesday".
"Something very strange", indeed strange, stranger than we knew
Or could imagine. From that inspired observation grew
A textile industry, huge factories, a library of volumes extending still,
Miles, miles of micro-film; lectures, millions of words to fill
Vast halls and college theatres; a door
Opening on endless ocean vistas; truly a gem-like find, of the order
Of penicillin, or nylon, or polythene. A new
Original reaction, a chapter in the text-books named after you;
The joy of uncovering, in the jig-saw puzzle of life,
One perfect link, pristine and virgin-true, that no strife,
No revolution, or no Dark Age ever could undo.

VI. A glow of pleasure pulses through me as I remember how, for you,
This brought a glorious rebirth; a special Chair,
Newly created in the University; your own school of research, the air
Of academic tranquillity regained with honour kept entire;
The Royal Society; and talk now of a Nobel Prize.
But, for me, the typhoon forced its entry,
The locusts swarmed across my sky, an army of black-coated,
Bowler-hatted-brief-case-umbrella-carrying financial gentry
From Mayfair, Leeds, Liverpool and San Francisco;
The accountants, the fixers, the take-over-bidders,
The Organization men, the Yes-men, the Never-never men,
All swamping, rocking my helpless, rudderless, vessel.
The shareholders, stupefied with greed, unseeing, rid
Themselves, for a paltry two million pounds, of all their problems of development,
Selling their patent-rights, their factory and staff to a sprawling Corporation
Stitching, encapsuling each limb of every single Western nation.
And for the staff, an opening would be found "where practicable",
If your profile fitted in that continental crossword puzzle,
Yes, you might be permitted to toil on, to claim, at sixty-four, your pension rights.
But, for the original, the eccentric, the individual or the rebel
No place was vacant. To me, with grave concern, they said "Your sight
Is not as clear as once it was. Your age
Entitles you to contemplate a life of quiet

Enjoyment. Take while you can a voyage
Round the world and then retire, relying on the wealth
Accruing from your Compensation. After all, you must be passing rich.
So go now, full of achievement, and cultivate your health".

VII. As the spirit bloweth where it listeth, so can we never know
Where the germ of a conception will be born, beside a dock,
Perhaps, in Stepney, where a chemist, watching his sugar crystals go
Cascading down a conveyor while he checks his stock,
Finds fortune in an unexplained fluorescent glow
Appearing miraculously in a turning belt below.
The one, the heaven-sent, the spark-of-the-morning invention
Does not invariably strike on the benches of the strong battalions,
Along the polished corridors where the meticulous salary-scales flow,
Where the modulated pension scheme lives, breathing on the firm foundation
Of a Lombard Street bank grounded on hardest mountain rock.
It came to you here, under-paid, despised, unknown,
To you here, hidden in an industrial slum, here,
Whom the university professors, the leaders of industry, grown
To magnificence on fat years of boom, had for all those years
Written off their trim maps of what had been and what was yet to come.

VIII. And yet, this invention was not a single isolated deed
Growing within your mind from barren soil, was not
An individualistic exercise, fruit of long contemplation, seed
Of withdrawal. Born, in hard truth, from the most intense, most hot
Controversy, discussions, winding, weaving between us all
For months and years; from hard reading and thinking, toil
And sweat; from fellowship, from friendships that no call
Of personal ambition nor jealousy nor pride nor pique could spoil
In all those months and years; and these bonds live,
The binding mortar of facing a hopeless task together,
Fighting great odds together, opposing those who despised
Us and our folly, those who felt fear, who wondered whether
Such flights were not beyond our tiny power, strive as we might;
Still, to this day, the love and togetherness survive.

IX. This building, deserted now, lost, of no interest, of no financial value,
Has history in its veins; back, back, two hundred years ago
At the beginning of the Industrial Age, when men first knew
Ways to make vitriol or caustic lye, a slow
And dangerous business, breaking men's lives, grew
Here. Blinded, with lungs corroded by chlorine, blood toxic with lead,
Men still built on, rank pressing on rank until
At last, when we took over, the structure had become a rambling shed,
Used the last sixty years for storing carboys, an overspill
For empty drums, enamel pans and parts of copper still-heads.

X. Here the natural order is reversed, steel and concrete grow
To fields and woods; where once the Bunsen burners glowed
Scattering the sunlight, primroses and daffodils
Will greet the eye, gilding the parkland
That is to lie here. Where science's sharp chill
Winds once blew through corridor and dark-room, pails of sand
Are changed to rose-tubs, tracery of stainless steel and glass
Tubes, condensers and columns to tracery of leaves
And beech-wood, twigs and buds; to soft grasses
Silvered under the light the May moon weaves.
And, as the prophet or the painter or the poet stains
For ever his native ground, leaving, even on the evanescent rain,
An invisible mirror-image that no doom, no war can drain
To nothingness; so here, will your discovery clear
And crystal bright, linger for ever in the autumn air.

SCIENCE AS A PROFESSION

EXCEPT in Roy Lewis and Angus Maude's *Professional People* the position and problems of the professions in the society of to-day have received little discussion since Sir Alexander Carr-Saunders and P. A. Wilson published their major study a generation ago. The recent Cantor lectures of the Royal Society of Arts scarcely fill this gap, nor indeed do they attempt to do so, for they are ostensibly limited to the influence of the professions on economic life. Mr. A. H. Ensor, in the third lecture, discussing the commercial professions, gives banking much more extended treatment than it received from either Lewis and Maude or from Carr-Saunders and Wilson, though rather at the expense of accountancy, and his lecture has little relevance to the professional problems of which the scientist or technologist in general is often acutely aware to-day.

Mr. A. M. Holbein, in dealing with the industrial and technological professions, is much more fundamental in his approach, particularly in dealing with engineering, where he traces briefly the development of the professions and defines a professional engineer in terms approved by the Commonwealth Engineering Conference in 1954 and earlier at a conference of representatives from the engineering societies of Western Europe and the United States. The definition refers to competence in virtue of fundamental education and training to apply the scientific method of outlook to the analysis and solution of engineering problems and the emphasis is on technical competence, though admittedly not narrowly conceived. It does not refer to standards of professional qualifications or conduct, nor to the idea that professional status is essentially an implied contract to serve society over and beyond all specific duty to client or employer in consideration of the privileges and protection society extends to the profession.

The weakness of the definition appears more particularly when Mr. Holbein attempts to indicate the range and scope of what he terms the industrial and technological professions, though the difficulty may well arise out of the particular approach he adopts. He does not, as with engineering, in which incidentally he makes the sound comment in passing that the influence of the engineering profession on economic and public life might have been more profound had the great engineering institutions been less rigid in their attitude to one another, trace the development of particular occupations up to the point at which a reasoned argument could be developed for professional status. On the contrary, he appears to assume that almost any industrial grouping will afford specific professional groupings, and the result is a hotchpotch of 'professional' occupations which confuses the lines of those which are firmly and clearly based on such a discipline as that of chemistry or physics. Apart from the engineer, Mr. Holbein scarcely displays one profession that would have been admitted unchallenged in Carr-Saunders and Wilson's survey, and although admittedly the chemist and the physicist are covered by Sir Patrick Linstead's lecture the picture Mr. Holbein gives is in consequence unconvincing and perhaps unfortunate.

Nor is this all. While he rightly distinguishes between the professional man or woman as an individual and the professional institutions, particularly in considering the professional influence on economic life, it might have been wiser to take the influence of the institution first. In 1933 Sir Alexander Carr-Saunders and Mr. P. A. Wilson wrote: "If men found in vocational associations their permanent anchorage and shelter, they could move out from those secure positions to shape organizations into instruments for the fulfilment of their purposes. . . . So long as men are unable effectively to shape the instruments through which they serve, they will feel helpless; and if they feel helpless in regard to matters affecting their own vocation and its daily exercise, they will feel still more helpless in regard to matters beyond their immediate ken".

The passage of thirty years has testified to the wisdom and prescience of those words. The function of professional institutions in the economic life of to-day has steadily increased in importance as the scale of professional and economic organization has grown and the complexity of professional relations has multiplied. More than ever to-day professional men need their permanent anchorage and shelter; even more important is it that such anchorage and shelter should be seen to depend clearly on professional standards and conduct. The professional man may have to look first to his professional institution in which he qualified, but the practice of his profession in a particular way may involve other professional considerations—to many scientists teaching presents a pertinent example.

That may be one reason for deploring the excessive multiplication of professional distinctions; it is a weighty reason for emphasizing the contractual aspect of professional life rather than technical qualification, its social at least as much as its technical responsibility. It is over these that Mr. Holbein seems to pass too lightly, though he clearly recognizes them, and refers to the problems of professional education involved, including that of training the supporting echelons of technicians and craftsmen. He does not advocate any lowering of standards, though he suggests that the boundaries of the engineering institutions in Britain should be interpreted more liberally, and he just touches on the difficult problem of the place of the professional man in government.

Mr. Holbein scarcely does more than indicate the existence of this problem. On the mechanism of advice he seems to look to a super-organization of professional bodies, competent to present the collective view of the professions, and on the participation of the individual technologist he only refers to the bearing of the general element in his education. The technical or scientific element in any problem, however, is specific, not general, and the mechanism of advice must accordingly provide for the specific.

Much more fundamental thinking is required on these issues which Carr-Saunders and Wilson explicitly raised but they lie rather outside the influence of the professions on economic life, though, so far as the individual is concerned, the very growth and multiplication of the professions enhances the importance of the professional ideal, independent of its particular technical or occupational characteristics. It is that ideal with its sense of public responsibility and conception of personal service that will inspire the kind of service required and counteract the aberrations that have sometimes injured the public interest.

On the scale of the professional influence, Mr. Holbein puts the number of qualified engineers in industry and public corporations in 1960 as more than 64,000. To this must be added those in independent practice. In the meantime, it should be noted that of 66,000 on the medical register, some 47,000 are employed in the National Health Service. Sir Patrick Linstead, quoting the last report on the demand for scientific manpower, gives a total of 72,000 scientists and more than 100,000 technologists in 1959, a total as large as that of men in the Church, the Bar, Medicine, the administrative grade of the Civil Service and officers in the Fighting Services put together. Even excluding technologists, there are now more professional scientists at work in Britain than doctors or officers in the Armed Services, and it should also be remembered that the Administrative Class of the Civil Service and the Armed Services employ a small but increasing number of those qualified also as professional scientists. Furthermore, as Sir Patrick Linstead notes, the practice of science is now among the largest of the professions and also one that has grown up almost entirely during the past half-century.

Sir Patrick recognizes that the scientific professions, though so numerous, do not constitute a sphere comparable to the Bar or medicine, in which practice is restricted by law, nor does he pursue this issue of registration or even discuss the characteristics of a profession. Indeed, much as Mr. Holbein, he is concerned with the effect of science, rather than of the professions as such, on economic life. His lecture has more in common with Heath and Hetherington, *Industrial Research and Development in the United Kingdom*, or with the excellent monograph published by the Royal Institute of Chemistry, *What Industry Owes to Chemical Science*, than with Carr-Saunders and Wilson's work or that of Lewis and Maude.

This is disappointing for, competent as, of course, is Sir Patrick's discussion of the influence of the scientist, or of science, on the protection and maintenance of life, on production, on communication and computation, on education, or on innovation, it scarcely distinguishes the real professional issues, and these are important. The community should, indeed, appreciate the conditions of former innovation and invention and be ready to ensure the existence of such conditions. That, however, is rather an aspect of the influence of society on the inventor or the professional man whereas what one might have expected in a series of Cantor Lectures under this general title is consideration of the influence on the economic life of the country of the professional, rather than the scientific and technical, aspect of the life of this considerable body of trained men and women, the significance of their professional outlook and standards of conduct, the character of their professional institutions, and their appropriateness for serving professional and public needs to-day, and perhaps a glance at the complex welter of problems which arise out of the interrelations of professional institutions themselves, questions of qualification and training, and of relations between professional bodies themselves and those which meet the needs of the supporting technicians and craftsmen on whose services the professional scientist and technologist depends in increasing measure if his services are to be used with full efficiency.

Whatever their intrinsic merits, these Cantor Lectures disappoint the hopes justifiably raised by their title. To the discussion of the place of the professions in the modern world they contribute little, if anything. The large issues which have grown in importance and complexity since Carr-Saunders and Wilson wrote are ignored; others which were only emerging when Lewis and Maude wrote ten years ago are no less passed by; even the significant fact that probably at least 10 per cent of the population is now a professional worker passes without comment. Nor is the observation that already in 1952 the influence of the professions was possibly waning pursued nor are the real dangers to professional standards in some educational developments and the expansion of state employment examined critically.

Yet within the sphere of the professions and their influence, and, conversely, of the influence of society on them, are poised major problems of our day: the vital issues of power and influence, of the relation of policy to knowledge; of professional skill and wisdom in the formation and execution of public policy; of professional education and technical training, quantitatively as well as qualitatively; of the safeguarding of public standards in a score of fields, as well as questions of professional conduct and responsibility in varied and changing circumstances. Is it too much to hope that recognition of an opportunity missed may inspire the Royal Society of Arts or one or more of our great professional institutions to initiate the public discussion of some or more of these issues, starting where Carr-Saunders and Wilson or Lewis and Maude left off, and displaying the immense contribution which the professional idea, faithfully and imaginatively served, can bring to the solution of some of the most urgent problems of our day ? For if the professional institutions do not themselves meet the new challenge not simply to professional competence and standards, to professional integrity and independence and the spirit of public service, if they do not face the complex issues of administration that are involved in the relation of professional man and client in the context of nationalization and concentration in many forms, the issue of leadership is liable to go by default.

COMPARISON OF EDUCATION AND EMPLOYMENT IN THE U.S.S.R. AND THE UNITED STATES

Education and Professional Employment in the U.S.S.R.

By Nicholas de Witt. (Prepared for the National Science Foundation by the Office of Scientific Personnel, National Academy of Sciences—National Research Council.) Pp. xxxix + 856. (Washington, D.C.: National Science Foundation, 1961. Available from U.S. Government Printing Office.) 5.50 dollars.

THIS exhaustive study is much more than an enlarged and revised edition of the author's previous book, *Soviet Professional Manpower* (1955). Like its predecessor, this formidable survey is based on a tremendous amount of data derived from Soviet publications, carefully assembled, processed, tabulated, analysed, cross-checked and supplemented by travellers' reports and comparisons with the training and employment of professionals in the United States. Numerous tables, charts and graphs illustrate the organization of general and vocational training and the employment of specialists in the various fields of the national economy. The voluminous appendixes contain a great wealth of statistical data on enrolment, graduations, rates of failure and attrition, numbers and types of schools and specialized institutes, percentages of Party members among university and other staffs, of women and members of national minorities in different occupations as well as lists of school text-books, subjects taught, analyses of school and university time-tables, comparisons of medical and engineering curricula in the U.S.S.R. and United States and estimates of respective standards of attainment.

The far-reaching educational reform of 1958, which aims at introducing productive labour into secondary schools and favours the 'sandwich course' at the expense of full-time higher education, is fully discussed. Mr. de Witt argues that it was brought about by the unfavourable demographic trends and the changing labour requirements of the Seven-Year Plan. The demographic trends are the result of Soviet losses in the Second World War, which Mr. de Witt estimates at forty millions and which are known to have depleted the rural even more than the urban population. The number of school-children in the four junior grades fell from more than twenty millions in 1939 to twelve millions in 1953 and was still well below the pre-war figure in 1959. In other words, and bearing in mind the overall deficit of 21 million males, the number of 18-year olds joining the Soviet labour force in the 1960's can just about cover losses due to death and retirement; the expansion of the labour force envisaged by the Seven-Year Plan can be achieved only by employing more women and teenagers in the younger groups. The latter are being drawn into the productive process, on a full- or part-time basis, by the implementation of the new Education Act. Furthermore, the condition of two years spent in employment now required of most university entrants will help spread the full impact of juvenile labour shortage over the lean years. Many young people will probably remain in their jobs, since wages are more remunerative than students' grants, and provide skilled manual labour which seems to be particularly needed. The road to higher education remains, however, open by way of extension-correspondence courses, which in 1960–61 catered already for 1·2 million students, that is, more than half the student population. These courses have been hitherto a cheap and unsatisfactory substitute for full-time education, though they made possible the post-war re-training of teachers on a large scale. However, improving teaching techniques and radio and television facilities may eventually bring knowledge on a mass scale to scattered out-of-the-way townships and remote collective farms.

The chapter on advanced degrees and scientific research attempts no survey of Soviet science but provides much information on the personnel, remuneration and organizational set-up of higher education and research institutions. The loosely integrated research activities of the All-Union and republican academies of sciences, establishments of higher education and industrial research institutes (now under local national economic councils) are to be streamlined. On April 10, 1961, a Committee for the Co-ordination of Research and Development was set up under a deputy chairman of the Council of Ministers; it includes the President of the U.S.S.R. Academy of Sciences and the chairmen of several State committees (for automation, chemistry, inventions and discoveries, aviation engineering, armament engineering, radio-electronics, atomic energy, shipbuilding, etc.). Basic scientific research will be carried out by the Academy of Sciences while research in the applied sciences will be carried out in other research institutions.

A detailed survey of the employment of professionals and semi-professionals reflects the bureaucratic structure of Soviet society. Despite the vast numbers of graduates trained, particularly in recent years, many top managers in industry and trading organizations are still people without formal qualifications. Altogether, the output of graduates in the United States has been considerably greater in the past three decades than in the U.S.S.R. (6 millions and 4 millions respectively between 1928 and 1959), but 1·8 as many engineers have been trained in the U.S.S.R. as the United States, 2·4 as many specialists in agriculture and 2·4 as many physicians. On the other hand, in the field of social sciences, humanities and liberal arts the number of Soviet graduates is but a fraction of the figure for the United States. Both countries employ a similar percentage of their graduates in industry, transport and construction and also in Government administration, but whereas 21 per cent of the graduate force in the United States is in trade and distribution, only 1·1 per cent of their counterparts in the U.S.S.R. are in these occupations; however, the U.S.S.R. employs 43 per cent of its graduates in the field of education and the United States only 21 per cent.

The factual information contained in the book under review is quite remarkable, but the author has not and could not include in his survey the 'imponderables' that constitute a child's cultural environment. Therefore, the conclusion of his postscript implying that the U.S.S.R. trains competent but narrow-minded specialists while the system in the United States produces men and women with a broad general education fails to take into account the impact of the cultural heritage transmitted, for example, through leisure reading; in the U.S.S.R. it includes Russian and foreign classics selling in millions of copies.

E. Koutaissoff

LAND USE IN GHANA

Agriculture and Land Use in Ghana

Edited by J. Brian Wills. Pp. xviii + 504 + 59 plates. (London : Oxford University Press, 1962. Published for the Ghana Ministry of Food and Agriculture.) 63*s*. net.

THIS book is a collection of papers from 26 different contributors, the majority of whom no longer work in Ghana. These papers have been written over a period of nine years so that some of the earlier ones have had to be revised. To publish at all during a time when staff in the departments concerned was involved in the hand-over from European to African is an achievement in itself.

The book is divided into two parts, the first part dealing with the factors which influence agriculture and land use and the second part with crop husbandry, animal husbandry and forestry. In the first part there are papers on climate, geology, soils, vegetation, water supplies, flora and fauna, communications and land tenure, ending with a description of the pattern of land use in Ghana. The papers differ in length and approach. Weather and climate, soils, soil fertility and land tenure receive particularly detailed treatment, while soil erosion and conservation, mammalian fauna and communications are dealt with more briefly. Half the second part is devoted to cocoa, reflecting the importance of this crop in Ghana and the existence of the West African Cacao Research Institute staffed by people accustomed to writing scientific papers. Cocoa disease receives more attention than cocoa cultivation, reflecting the efforts made in maintaining the cocoa yield against damage by virus diseases and by capsids. A footnote to p. 252 states that the annual production of cocoa reached a new maximum of 316,000 tons in 1959–60, too late for a paper on the factors which brought about this record and a fitting conclusion to the section on cocoa. The other half of the second part deals with crops other than cocoa and with the applied aspects of forestry, tsetse flies, animal husbandry and crop storage. Other crops occupy only 40 pages and there is a lot more that could have been recorded here. Much is buried in files and some more has not been written before an officer left the country. The lime industry in the Central Region and the plant breeding work on annual crops are examples of topics which might have received more attention, but the editor may have had compilation difficulties.

The Minister of Food and Agriculture (February 1960) commissioned the book for officials, teachers, lecturers and research workers. They will find it a valuable work of reference with good diagrams, numerous tables of data, clear maps, excellent bibliographies and index. The classified bibliography is notably comprehensive. However, the editor admits that gaps exist and a fair notice must say what they are. The book deals almost entirely with environmental factors affecting land use. There are only two short papers on the human factor—the Ghanaian farmer. Agricultural surveys are included in the bibliography but they are not brought together in one paper describing how the farmer deals with his environment. Techniques of extension work are scarcely mentioned and little is said about the impact on the farmer of such new methods as mechanization, chemical control of pests and diseases, use of fertilizers and new crop varieties. Marketing likewise appears in the bibliography, but scarcely at all in the text. Education connected with agriculture and land use is omitted entirely. It is perhaps no longer possible to describe the whole field of land use in one book and this book is not a small one. It brings together in one volume a great amount of useful reference material which might otherwise have been dispersed and hard to find.

B. J. Silk

THE STRUCTURES OF SOLIDS

Structure Reports for 1954

Vol. 18. Edited by A. J. C. Wilson, W. B. Pearson, J. M. Bijvoet and J. Donohue. Pp. viii + 845. (Utrecht: M.V. A. Oosthoek's Uitgevers Mij, 1961. Published for the International Union of Crystallography.) 120 Dutch fl.; 240*s*.; 33.50 dollars.

THE International Union of Crystallography is a small Union, but it is extremely lively. Among its activities it has a very successful and useful publication policy, laid down at the time of its formation in 1946 and carried through by relatively few editors and their colleagues on editorial commissions. All of them are actively working in the field of crystallography. Its publications cater not only for the needs of research workers in structural crystallography but also for many of those of their colleagues in chemistry, physics and other major disciplines. There is the monthly periodical, *Acta Crystallographica*, which contains original papers mostly in English, but some also in French, German or Russian. There are the three volumes of the *International Tables for X-ray Crystallography* (the third of which will appear this summer) and there are also Volumes 8–18 of the *Structure Reports*, successors to the seven volumes of *Strukturberichte* (1927–39).

Year by year these *Reports* present an ordered account of material of structural interest extracted from original papers published in a wide variety of journals, including, to give some of the more exotic examples, the *Berichte Deutsche Keramische Gesellschaft*, the *Ukraïnskiĭ Khimicheskiĭ Zhurnal* and the *Travaux de Société Pharmacie de Montpellier*. As stated in the introduction, these are not abstracts in the ordinary sense, since occasionally the *Report* may be longer than the paper itself, the "abstractor" having added comments or an additional table derived from the data actually given by the author. Editorial matter thus added is distinguished by being enclosed in square brackets. Other quite long papers are described in a single line, not because they are not interesting, but because their main interest lies outside the field of structural crystallography.

One of the sensations felt on first seeing this volume is that of astonishment that within one year (for nearly all the reports in Volume 18 refer to the year 1954) so much new material should be available. Results on more than 1,900 substances by as many authors are presented here. The metals and alloys appear in alphabetical order of names, aluminium to aluminium zirconium, antimony to antimony tellurium uranium and antimony tin, and so on to more than four pages (illustrated by six diagrams) on zirconium and the thermal expansions of its two crystalline forms, as measured by X-ray diffraction. This section occupies 332 pages and is really one complete book, invaluable especially to metallurgists and engineers.

The two following sections, on inorganic and organic compounds, are also volumes in themselves. The classification is of course no longer alphabetical and the arrangement by structure types used in the earliest volumes is no longer possible, but the order followed, which is roughly chemical, is explained, and there are excellent subject-, formula- and author-indexes, including a 'duplicate' formula-index of carbon compounds only. The subject-index includes not only names of substances but also references to properties, such as entropy, epitaxy, expansion, ferrimagnetism, hydrogen bonds; to methods such as electron diffraction and microscopy, neutron diffraction and irradiation, low-angle scattering, microwave spectra; and many references to reviews. It seems strange not to find any mention in the subject-index of interatomic distances, atomic co-ordinates or space groups; but these are in fact to be found for every substance for which they have been measured in the year under review, after the name, formula and unit cell dimensions. A few curious index entries such as Aberdeenshire and U.S.S.R. seem to indicate an excess of zeal somewhere; and one could have wished also for more of the excellent diagrams that illustrate the book and for some method of numbering that did not call most of them Fig. 1.

It is perhaps too much to ask of the editors and abstractors, who are nobly doing this in their spare time, that the present gap of seven years between the year under review and the year of publication should be narrowed. But in spite of the gap, this volume and its predecessors can be most highly recommended as works of reference for all branches of science and technology that deal with solids and their properties.

KATHLEEN LONSDALE

BIOLOGY AND CANCER

Radiation Biology and Cancer

A Collection of papers presented at the Twelfth Annual Symposium on Fundamental Cancer Research, 1958. Pp. vii+493. (London: Peter Owen, Ltd., 1960.) 63*s*.

Genetics and Cancer

A Collection of papers presented at the Thirteenth Annual Symposium of Fundamental Cancer Research. Pp. ix+459. (London: Peter Owen, Ltd., 1961.) 70*s*.

FOR the study of the various problems of cancer much information is required from the fields of biological research. The M. D. Anderson Hospital of the University of Texas holds annually a symposium to discuss the latest results of current research with the view of correlating them with the various aspects of cancer. The proceedings of the last two symposia have been published: the subject of the twelfth was "Radiation Biology", that of the thirteenth "Genetics in Relation to Cancer".

Fourteen papers of the former meeting were devoted to the effects of radiation on normal and malignant cells; on cells cultured *in vitro* and to the mechanism by which recovery from radiation injury may be achieved. Methods have been described which could be used to measure and compare the effects of different types of radiation by utilizing cell lines cultured *in vitro*. Three papers, read in another session, were concerned with mutagenesis and tumour viruses in relation to radiation. Ten contributors in the symposium discussed the induction of neoplasia by ultra-violet and ionizing radiations. The possibility that therapeutic radiation, particularly in childhood, may have carcinogenic effect, has been stressed by several participants. The Bertner-Foundation lecture was delivered by J. Furth (Boston) on the subject of "Radiation Neoplasia and Endocrine System"; and dealt with the tumours of the thyroid, mammary gland, adrenal and ovary.

At the symposium on "Genetics and Cancer" twenty-three papers were presented. The somatic mutation theory of cancer and the relevance of recent findings in the field of microbial genetics, for example, lysogeny, transduction, plasmagenes, etc., have been discussed in relation to neoplastic transformation of cells. One of the contributors tried to reconcile the mutation and virus theory of cancer on the basis that certain genetic elements having properties in common with viruses might be involved in the genesis of cancer. As the neoplastic cell has a changed heredity, several contributions were devoted to the genetic material, the deoxyribonucleic acid of normal and malignant cell. The possibility that various carcinogenic agents may act by altering nucleic acid synthesis or by interfering with genetic replication has been discussed.

The results of extensive investigations of chromosome behaviour in various experimental tumours and tumour cell strains have been surveyed in great detail. These investigations show that chromosome number and structure in malignant cells very frequently deviate from those in normal cells and that the cell population of experimental tumours is highly heterogeneous. The development of drug resistance in some tumours may have been favoured by such a heterogeneity. Four contributors to the symposium dealt with genetic studies of human cancers. The results of statistical analysis of close relatives of patients with cancer of the breast and stomach indicate that susceptibility to these cancers is inherited in certain families and that repeated child-bearing reduces the risk of breast cancer. The value of twin studies and pedigree analysis has been discussed by one of the contributors. The Bertner-Foundation lecture dealt with the importance of host–virus relationship in determining the final expression of a particular disease; it was delivered by R. E. Shope (New York).

The brief survey of the contents of the two volumes shows that the meetings brought together experts in the field of physics, biochemistry, genetics, cytology, microbiology, medicine, statistics, etc.; it can be expected therefore that many readers of the papers presented at these symposia may find the subjects which have been discussed are too specialized and are only indirectly related to cancer. In my own opinion, however, the aim of the organizers to acquaint workers in cancer with recent findings of scientific research, which may eventually have a bearing on the problem of malignant disease, has been achieved.

P. C. KOLLER

THE IMPERIAL STANDARD POUND AND ITS PARLIAMENTARY COPIES IN TERMS OF THE INTERNATIONAL KILOGRAMME

By P. H. BIGG

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Introduction

THE Weights and Measures Act, 1878, adopted as the imperial standard of weight the weight *in vacuo* of a certain platinum–iridium cylinder marked P.S. 1844 1 lb. This cylinder had been provided following the destruction of the troy standard by the fire in the Houses of Parliament in 1834. The Act required that three parliamentary copies, constructed at the same time, should be compared once in every ten years with each other and once in every twenty years with the imperial standard. A fourth copy, immured in the Palace of Westminster, was immune from this requirement. A fifth, platinum–iridium, copy was legalized in 1886.

On the other hand, the use of metric weights was legalized by the Weights and Measures (Metric System) Act, 1897. The basis of reference in the metric system is the British national copy of the kilogramme, No. 18. This standard is one of a series of standard masses, established in 1889 and held by signatories to the Metric Convention, which are copies of the International Prototype Kilogramme. The prototype is in the custody of the Bureau International des Poids et Mesures at Sèvres, near Paris, and is the ultimate reference standard.

Legally, therefore, in Great Britain there are two independent systems of mass, based on the pound and the kilogramme respectively, a situation which in these days of increasingly precise measurement is illogical. The family of kilogrammes is more modern and much more extensive than that of the pounds, and, moreover, it is made of better material, namely, 10 per cent iridio–platinum. The stability is excellent; only a small minority of the copies has exhibited changes exceeding 0·02 mg since 1889. The international prototype was included in comparisons made in 1939 and 1946 and its high stability in relation to other members of the family was closely established. Consequently for science and technology the pound was re-defined in 1959 as equal to a definite proportion of the kilogramme, namely, 0·453 592 37 kg, and for trade this same quantity has been incorporated in Bills placed before Parliament, though so far insufficient parliamentary time has been available for their full discussion. Nevertheless, it has always been important to know the precise relationship between the pound and the kilogramme, and in consequence the two have been compared on a number of occasions. Recently, in connexion with the proposal to re-define the imperial pound it became desirable to establish the current experimental relationship and this work was accordingly undertaken at the National Physical Laboratory. A detailed report is being published elsewhere but it is thought that the present more general report will be of interest.

Comparisons of Pounds and Kilogramme 18

As one kilogramme is rather more than two pounds it was convenient to compare the kilogramme 18 with the imperial pound and its four copies taken in pairs. Since a knowledge of the values of the parliamentary copies as well as of the imperial standard was of interest, the pounds were taken in all ten possible pairs. The difference between 1 kilogramme and 2 pounds being 92·815 g (to the nearest mg) it was most economical of weights to place 100·015 g on the balance pan with the pounds and 7·2 g on the pan with the kilogramme, the total number of makeweights thus being limited to six, apart from those of very small denomination which were necessary to secure the closest possible equality of the loads compared.

The National Physical Laboratory precision balance[1], capable of discrimination to 1 μg (0·001 mg) in the comparison of kilogrammes, was used for the work, and it was found on assessing the consistency of the results of the various weighings that the standard deviation of an observed result (5 degrees of freedom) was 2·0 μg. Having regard to the fact that this variation included contributions due to errors of the small makeweights and the buoyancy allowances, as well as the balance errors, the standard deviation is remarkably small.

In order to deduce from the weighings the values of the pounds it is necessary to apply very accurate values for the various makeweights. The main makeweights were taken from a 500-g set (*PI* grammes) of platinum–iridium weights belonging to the Board of Trade which had been standardized for the work in terms of kilogramme 18. The standard deviation of the values of these weights varied from 0·9 μg for the 100-g weight to 0·3 μg for the 0·005-g weight. Various smaller weights, of aluminium and eureka alloy, were known with a standard deviation within 0·4 μg on the first occasion of standardization and 0·1 μg on the second, when an Oertling deci-micro quartz-fibre torsion balance was used.

Before the kilogramme/pound comparisons took place, the standard of reference, the national kilogramme 18, was carefully steamed in accordance with the internationally accepted process of cleaning described as 'lavage'. Before and after steaming it was compared with two platinum standards, kilogramme *E* and the headweights 500, 200*A*, 200*B*, 100 of *PI* grammes, and was found to have lost 14 μg due to the steaming, presumably due to the loss of an invisible surface film. After the kilogramme/pound comparisons, kilogramme 18 was sent to the Bureau International des Poids et Mesures (BIPM), where it was compared with the prototype kilogrammes 9, 25 and 31 belonging to the Bureau.

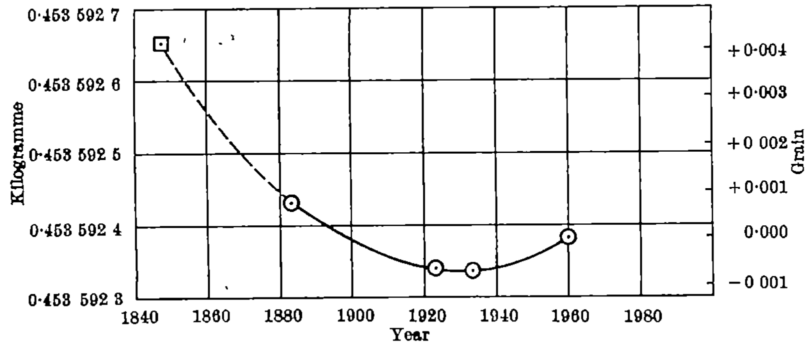

Fig. 1. Values of the imperial standard pound in kilogrammes

On return from the Bureau it was again compared with kilogrammes *E* and *PI* and the results are given in Table 1.

Table 1. Results of Comparisons of Kilogrammes (unit 1 μgm.)

	E–PI 41 900 +	*18–PI* 44 000 +	*18–E* 2 100 +	Residual
Before comparisons with pounds	29·8	107·9	77·8	− 0·8
After comparisons with pounds and re-verification at BIPM	25·8	109·7	(93 6)	(+ 9 7)

Table 2. Values of Kilogramme 18

Year	Mass	Surface condition
1889	1 kg + 70 μg	After steaming
1924	1 kg + 51 μg	No steaming*
1933	1 kg + 58 μg	No steaming
1948	1 kg + 71 μg	After steaming†
1961	1 kg + 59 μg	After steaming

* Kilogramme 18 had been cleaned in January 1923 with a mixture of ether, alcohol and ammonia.

† On this occasion the reference standards of the Bureau had been subjected to steaming for the first time since their establishment.

Table 3. Values of the Pound Standards, 1960

Pound	Value (kg)	Error of copy pound in terms of the imperial standard (grain)
Imperial standard	0·453 592 383	
Copy No. 1	0·453 592 599	+ 0·003 33
Copy No. 2	0·453 592 452	+ 0·001 06
Copy No. 3	0 453 592 297	− 0 001 33
Copy No. 5	0·453 592 138	− 0 003 79

During the final comparison of the second cycle, that between 18 and *E*, foreign matter was observed on one of the balance pans and one-half of the weighing was accordingly repeated; the closing error was nevertheless relatively poor. The other results, however, serve to indicate that during the vital weighings kilogramme 18 remained highly stable and the value certified by the BIPM could with confidence be applied to the kilogramme/pound comparisons.

The certified value for kilogramme 18 is given in Table 2 together with the values previously certified.

It may be noted that the standard deviation of the five values is 9 μg and that of the three values associated with lavage is 7 μg. Further, it was stated by the Bureau, presumably on the grounds of its general experience of such weighings, that the kilogramme cannot be said with certainty to have changed since 1948. The standard deviation 7 μg was adopted by the National Physical Laboratory for association with the 1961 value when assessing the results of the kilogramme/pound comparisons.

Results

Calculation of the values of the pounds in kilogrammes by the method of least squares, using kilogramme 18 = 1 kg + 59 μg, led to the results given in Table 3. The standard deviation of each value, comprising contributions from kilogramme 18 (5·0 μg), the weighings (1·1 μg) and the makeweights (1·4 μg) was 5·3 μg and the uncertainty of the values in Table 3 is accordingly 16 μg (corresponding to 0·000 25 grain) on a 99 per cent confidence basis.

Discussion

The values found for the imperial pound in the present and in previous determinations are plotted in Fig. 1.

It will be seen that all the values lie in a smooth curve and that for the first time the latest value shows an increase in comparison with immediately preceding values. The increase since the last two (closely concordant) evaluations is 42 μg or 0·0006 grain, that is, about 1 part in 11 million, a statistically significant amount. It is difficult to explain the increase except in terms of the accumulation of invisible film on the surface of the pound which is resistant to removal by light brushing, the wear which may be presumed to have occurred being relatively slight in effect. It may be noted that the pounds were submitted to liquid cleaning in 1922, but more recently this has seemed inappropriate because several of them, including the imperial standard, are not integral but had plugs of platinum incorporated in their flanks when they were originally made, presumably to remedy local faults. The weights were conditioned for the weighings merely by dusting with a camel-hair brush.

The differences between the imperial standard and the copy pounds in the present and previous determinations are shown in Fig. 2. The differences for 1960 are estimated to be accurate to approximately 0·000 08 grain (5 μg) on a 99 per cent confidence basis. The curve for Copy No. 1 is exceptionally

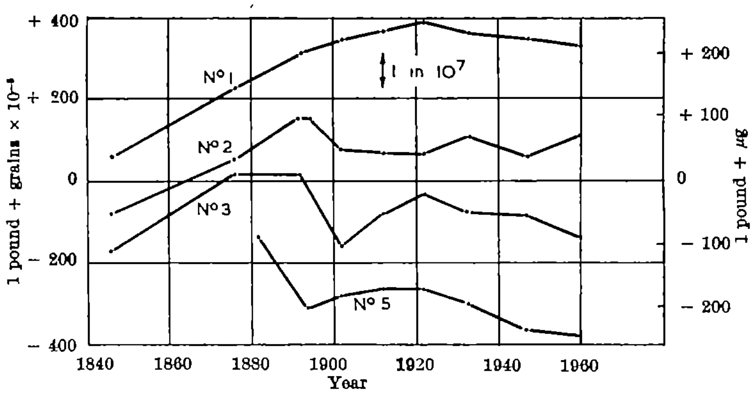

Fig. 2. Values of the parliamentary copies of the pounds

smooth during the whole period of its life, indicating that neither it nor the imperial standard has exhibited spasmodic fluctuations. Since 1922 all the standards with the exception of No. 2 have shown smooth progressions. Copy No. 2 was the subject of difficulty during the 1947 decennial comparisons when definite evidence of a change during the comparisons, amounting to 14 × 10^{-5} grain, was found. The reduced rate of change of copy No. 5 during 1947–60 as compared with 1932–47 has been due to its being used less during the later period. Considering the mean values of the copies for the several years we find the values which are set out in Fig. 3. Whether copy No. 2 is included or not the tendency exists for the copies to have lost relative to the imperial standard since 1922, or alternatively for the standard to have gained relative to the copies. Bearing in mind that by comparison with the kilogramme 18 the value of the imperial standard was almost the same in 1922 and 1932, we compare below the change since that decade as indicated by the pound/kilogramme comparisons and by the pound/copy pound comparisons. The results are as follow:

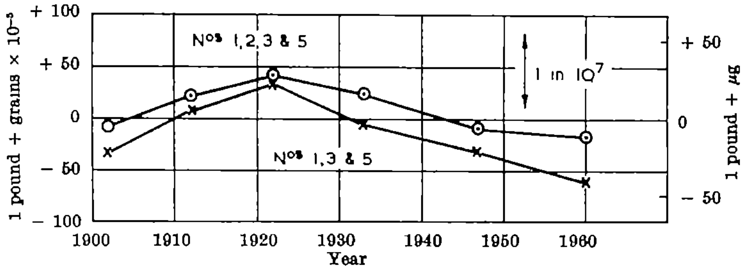

Fig. 3. Means of values of certain parliamentary copies of the pounds

Gain of imperial standard pound according to pound/kilogramme comparisons	42 μg
Gain of imperial standard pound relative to Copies 1, 2, 3 and 5	33 μg
Gain of imperial standard pound relative to Copies 1, 3 and 5	49 μg

Although too much confidence must not be attached to the conclusion, because the copies have been used more than the imperial standard, it nevertheless seems that the evidence from the copies, supporting that from the pound/kilogramme comparisons, is that the imperial standard has gained mass during the past few decades by about 1 part in 10 million.

It may be noted that the latest value of the pound, 0·453 592 383 kg, is very close indeed to the value adopted in July 1959 for science and technology[2] by the national standards laboratories in the English-speaking countries, namely, 0·453 592 37 kg. This closeness of agreement is fortunate, but purely accidental. At that time the United Kingdom pound was thought to be 0·453 592 338 kgm. (1932 value) and the U.S. pound was legally defined as 0·453 592 4277 kgm. As the pound is divisible into 7,000 grains it was advisable to choose an equivalent which is readily divisible by 7, and in consultation with the United States the choice fell on 0·453 592 37 kg, this being approximately midway between the United Kingdom and United States pounds. At the time it was thought that the kilogramme/pound curve, which of recent years was horizontal, would continue so, providing evidence of a stable relationship between the two units. That the 1960 value has not fulfilled this expectation but has demonstrated a decided upward turn to the curve shows how valuable was the decision to adopt once and for all, for precise measurements in science and technology, a constant relationship between the pound and the kilogramme. It is to be hoped that parliamentary time will soon be found to legalize this relationship.

The work described here was carried out as part of the programme of the National Physical Laboratory for the maintenance of standards, and this article is published by permission of the Director. Miss D. Oxley carried out all the weighings and Mr. F. H. Burch was in immediate charge of the work. Acknowledgement is made to the Standard Weights and Measures Department of the Board of Trade for co-operation in transporting the pound standards between its normal storage places and the National Physical Laboratory.

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[1] Gould, F. A., *Proc. Phys. Soc.*, B, **62**, 817 (1949).

[2] *Nature*, 183, 80 (1959).

ORGANIZATIONAL PROBLEMS OF SCIENTIFIC RESEARCH IN THE UNIVERSITIES*

By Prof. P. M. S. BLACKETT, F.R.S.

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WHEN an institution increases markedly in size, changes must be made in organizational methods. This is true of a government department, an industrial firm, a university and a university science department. I will mainly confine myself here to the postgraduate research and teaching in a university science department, though I shall also refer to undergraduate teaching.

Naturally I am drawing widely on my own personal experience as a university teacher and research worker, so my remarks may be unduly conditioned by my particular experiences. An even greater risk is that I may be taking too much the view of the man-at-the-top, for I have been head of a department, or rather three different departments, for just thirty years, and so have exercised great, but rather undefined, powers. I vividly recalled something which happened at the Princeton bi-centennial celebrations in 1946. That great British industrial scientist, Dr. C. K. Mees, creator of the Kodak Research Laboratories in the United States, gave a brilliant lecture on the right way to run an industrial research laboratory which was, of course, the way his laboratory was run. Then a young scientist from Mees's laboratory got up and spent half an hour telling us with great clarity what it was really like—as seen from the working researcher's point of view.

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* Substance of an address given at the opening of the new Physics Building at King's College, Newcastle upon Tyne (University of Durham), on March 16 (see also *Nature* of April 14, p. 134).

Much of what I have to say is generally accepted doctrine, but other parts, I think, are not. Anyway, it has been useful to me to formulate my views on these important matters, so I hope it will also be to those who hear or read them.

I am very convinced of the extreme value of associating research and teaching. In fact, a large fraction of the most creative scientists of Britain are engaged in both, and it is essential to retain this. Moreover, the welcome increase of postgraduate specialized training provides a valuable part of the work of the teaching staff and one in very close and valuable relation with their researches.

Now it is clear that since before the War a numerical revolution has taken place in the production of trained scientific and engineering manpower in Britain. In 1938 the annual output was 5,000: in 1960 it was 17,000, and the present target is given as 30,000 by the early 1970's: this implies a rate of increase of 7 per cent a year over the next decade. A large part of this rapid expansion is taking place in existing university departments, which are therefore growing rapidly in size, generally at a rate of 5–10 per cent a year. Compared with 1938, an average physics, mathematics or chemistry department has nearly four times as many undergraduates and a rather greater increase of staff—thus giving a welcome improvement in the staff/student ratio. On the other hand, the number of senior posts, particularly professorships, has not increased as fast. Taking all university subjects, the ratio of professors to total staff was 1 : 5 in 1938 and only 1 : 8 to-day. In pure science the ratio is now only 1 : 9 or 10.

There cannot be much doubt that this failure of the number of senior posts to keep pace with the increase of the other staff, so reducing the chances of promotion and the exercise of individual initiative, has been a major cause of the loss of many outstanding scientists to the United States and to not a little general disgruntlement among university staffs [see also *Nature*, 193, 911, 922; 1962] I consider that it is important that the number of scientific professorial posts be increased during the next decade to about 1 in 5 of total staff, thus achieving the general university figure of 1938.

Let us consider the position in the thirty-five university departments of physics in Great Britain. In 1961 there were about 700 total academic staff and some 65 professors, giving a ratio of about 1 : 10. So to achieve the above objective, about 60 extra chairs are needed (that is, nearly a doubling) apart from the number required by the planned general expansion of the science departments by 70 per cent in the next decade. So the total number of physics chairs by the early 1970's should be more than 200. To-day, out of 35 university departments of physics in Great Britain, 17 have a single professor, 8 have two, 5 have three, 3 have four, 1 has five and 1 has nine. Taking all sciences, technology and medicine together, the number of chairs has increased from about 870 in 1958 to 975 in 1961, that is, by about 105 in 3 years or 4 per cent a year. More than a doubling of this rate of increase will be required in the next decade to compete with the planned increase of the universities as a whole and with the restoration of the former ratio of professorial to total staff.

What are the reasons for the failure of the universities to increase the number of chairs to keep pace with the expansion of the departments ? In a few cases there may have been a lack of suitable candidates: however, I very much doubt if this is an important factor except perhaps in the smaller, more remote and least well-endowed departments. Nor is it often a matter of finance: for example, the cost of upgrading a readership to a chair need only be a few hundred pounds a year. Nor does there appear to be any rigid University Grants Committee regulation against an increase of professorships.

The real reason, I think, for this serious failure is to be found mainly in the innate conservatism of universities as a whole. Of course, the universities other than Oxford and Cambridge have been influenced historically by the Continental tradition of a department with one well-paid professor and a number of badly paid juniors. In Scotland, where this tradition was strongest, I am told the creation of new chairs in some Scottish universities still requires an alteration of the statutes and the agreement of the other Scottish universities.

Fears have often been expressed that too many professors will make university senates too big and so unwieldy, and too many science professors will alter the balance of the government of a university. The first difficulty can be mitigated by suitable administrative changes and, as regards the second, it is to be remembered that to-day there are 520 arts professors to only 340 science professors. The higher professor/total staff ratio of 1 : 6 for arts compared with 1 : 9 for science, arises, I think, mainly because of the larger number of small arts departments with a professorial head but few other staff.

In the cases of a few individual professors of physics opposition to increasing their number may have been based on a reluctance to share power and prestige. More reputably, there may have been some fear of the administrative problems of the multi-professorial department. These problems exist, and I will now consider them, specifically in relation to physics. Other big experimental departments such as chemistry and engineering clearly have somewhat similar but not identical problems. But mathematics departments again have fewer problems because of the lack of experimental research.

I would like to emphasize here that my advocacy of many more professorships in no way implies that I think a professorship should be a normal promotional grade for all members of the staff. I strongly oppose this: but I do think that there will be found available men and women of outstanding gifts to fill the new posts which I think are needed over the next decade.

Consider a moderately large physics department with an average of 100 undergraduates taking honours physics in each of three years, giving an undergraduate population (not counting students from other departments) of 300. Taking the present average staff/student ratio as 1 : 7 or 8, as seems accepted as reasonable, there will be about 40 staff in the department. Accepting further that 1 in 5 of the staff is a professor, there will be 8 professors. What should be the relation of these professors to each other and what part should they play in the three main functions of the staff—undergraduate teaching, postgraduate teaching and direction of research? There are clearly several possible arrangements which can give satisfactory results.

My own preference at present is on one hand for a completely unitary undergraduate teaching organization for a single unspecialized B.Sc. degree in which every member of the staff, including professors, takes part in the teaching: and, on the other hand, for a number of research units with the maximum possible autonomy at the postgraduate-level. In general, I think that every professor, if he wants to, should direct such a unit: in our imaginary department there will then be about eight such independent research units with an average total of five academic staff each.

Experience shows that on the average such a research unit with five academic staff may require half a dozen technicians of different grades, and if successful should attract a dozen or more research students and other research workers, so giving an average total population of, say, 25. In the Department of Physics in the Imperial College of Science and Technology, where there are 11 units, the total numbers in each range from about 12 to 45.

I would like to mention here an experiment in architectural planning a large physics department which we have made at Imperial College. In order to promote the maximum internal scientific and social cohesion of these research units, the new Physics Building was designed to allow so far as possible the allocation of a compact area of the building to as many units as was architecturally possible. The academic staff, including the professor together with the technicians and research workers, of a unit are all accommodated in this area. This architectural division of the research space of the whole department into more or less defined areas, housing separated research units, has a possible disadvantage in perhaps making more difficult re-allocation of space at a later date. However, its advantages are considerable over the extreme alternative, which is to house all the heads of groups in one area of the building and to distribute their respective research laboratories more or less at random over the whole building.

In my experience to-day, the average minimum size for a research unit is just about what I have mentioned, that is, five academic staff with supporting technicians and research students. Remembering that the staff are only part-time research workers, and in view of the problems of keeping up with the literature, attending conferences, etc., it is seldom possible for a much smaller group to make an outstanding contribution to a research subject in the front line of advance. Smaller and weaker groups can easily become sub-viable: they can fall below the critical size for self-sustaining research progress. These considerations apply, of course, only to groups of normally gifted workers: special arrangements can always be made for the outstanding lone worker, a C. T. R. Wilson, a Glaser or a Mossbauer.

Such a multi-professorial department can be directed in one of three main ways: one of the professors can be permanent head of the department—this is the traditional Continental solution: one of the eight can be temporary chairman—this is often done in the United States—or some form of committee can run the department. There are advantages and disadvantages in all these methods. It must be remembered that excessive departmental democracy can be very time wasting and so impede research. The main function of the central direction in relation to the research activities of the eight units is to distribute to the units the available money for research expenses received from the university, to allocate the academic staff vacancies, and, in conjunction with the university, to fill them: and finally, to allocate each summer to the units the new research students from the main teaching department. On the other hand, I think that the heads of the research units should take almost complete responsibility for acquiring outside finance, for example, from research councils or industry, etc., and for attracting research students and visitors from other institutions at home and abroad.

I would like now to refer to university research in border-line subjects. There is no doubt that the present method of finance by the University Grants Committee, which involves basing its grant to a university mainly on the number of undergraduates, does make difficult the development of specialized and border-line fields of research. Since there are few, if any, undergraduates, there are few staff and so little research, unless large outside funds are available. On the other hand, it seems likely that the importance of, and the interest in, these ever-arising new border-line subjects is likely to increase in the future.

In my view, it is generally a mistake to attempt to found an undergraduate school, giving a specialized B.Sc., at too early a stage of a new subject, simply in order to acquire the staff to make a research school. For one thing, the best students from school will seldom enter for such unfamiliar subjects: of more importance is the new general recognition that the undergraduate teaching course should be mainly concerned with the basic sciences, with only a minor degree of specialization in the last year. Training in the vitally important, but highly specialized new subjects, comes much more efficiently in the early post-doctorate years. A convenient way of dealing with some of these new subjects is to make a group working in such a field into a unit in one of the major departments of physics, chemistry, etc. As already outlined, the academic staff of these units would all take part in the general undergraduate physics teaching, but would be free to develop, as thought fit, the postgraduate specialized training required by their subject. A great advantage of this organic relation between a highly specialized research unit and a major teaching department is that it provides a potential supply of home-grown postgraduates. For example, in our imaginary department of physics with an output of about 80 graduates a year there will usually be up to 20 who can be considered as fit for a research studentship, and so an average of 2 or 3 for each of the 8 assumed research units. In addition, a good research group will normally attract at least as many or more from other universities, particularly those overseas. In the past it has been the lack of direct and formal right of access to a major teaching department which has often made such subjects as astronomy, meteorology and geophysics into the cinderellas of our university system. Sometimes, such departments have very few British research students, so that they have to rely mainly on overseas students. It is interesting to note that, because radioastronomy in both Cambridge and Manchester did grow up as sub-departments of major departments of physics, there has been a continuous flow of first-rate British physicists into this subject.

ØKSFJORD EXPEDITION, NORTH NORWAY

TWENTY graduates and undergraduates from the University College of Swansea spent more than ten weeks during the summer of 1961 on the isolated peninsulas and islands between Skjervøy and Øksfjord, West Finnmark. A comprehensive research programme included geology, biology, ornithology and mountaineering.

Two main camps were established at Sandland and Olderfjord with smaller or temporary camps at Andsnes, Segilvik, Sor Tverfjord and Langfjord Botn (geology); at Nor Tverfjord (mountaineering and geology) and Loppa (ornithology and geology). Movement between camp sites and the geological work along shore sections was greatly facilitated by the Expedition's dinghy and outboard.

The team of six geologists completed a preliminary geological map of the triangular area from Silda Island in the north to Jøkelfjorden in the south and Andsnes in the west. Because no accurate large-scale topographical maps were available, the geology was recorded on traces overlying air photographs. The structural sequence of Eocambrian and Cambro-Silurian metasedimentary rocks west of Sandland Village is massive quartzites, often including broad amphibolitic bands, followed upwards by purple staurolite-garnet schists and a sheeted sequence of limestone and gabbro at the top. Basic dykes occur in all three units, and this part of the area has been metamorphosed to the amphibolite facies. Eastwards, the metasediments are separated from the main gabbro-garnet gneiss complex by a zone of intensive faulting passing through Sandland Valley.

Broad alternating bands, from 100 to 500 ft. thick of hypersthene gabbro and coarsely banded garnet gneiss constitute the gabbro-gneiss complex, the western margin of the huge layered Seiland Gabbro. In places the gabbro is choked with metasedimentary material and a granulitic variety of hypersthene gabbro outcropping along the sides of Langfjord includes narrow bands of quartzo-feldspathic and limestone metasediments, less than 20 ft. thick but traceable for more than a mile parallel to the foliation. This part of the area has been metamorphosed to the granulite facies. Into the gabbro-gneiss complex are intruded a coarse olivine gabbro, a periodotite-anorthosite complex, ultrabasic dykes, basic dykes and syenites.

At least three-fold systems can be distinguished in the metasediments, the two most important striking N. 20° and N. 340°. The predominant structural trend in the gabbro-gneiss complex curves gently from N. 20° in the north to N. 340° in the south. Mylonite zones, concentrated along the gabbro-garnet gneiss contacts indicate a number of thrust planes, parallel to the foliation, dipping between 60° and 30° west. East of Langfjord the dip of the foliation steepens and passes through the vertical to dip steeply east.

Much work remains to be done. This year's preliminary map must be extended eastwards to Øksfjord to join up with areas mapped by other workers. More detailed investigations of the metasediments and their structure and of the origin of the various basic and ultrabasic rocks must be completed before a comprehensive reconstruction of the geological development of the area is attempted.

Biological studies were carried out in the area of Olderfjord, Reinfjorddalen and Kanesdalen. Extensive collections of angiosperms and bryophytes were made and particular attention was paid to the variation of *Salix* species. A general botanical survey was made as a basis for further ecological studies and future work on the variability of breeding systems.

General collections were made of small mammals in order to study their parasites and of insects, the distribution of which was related to vegetation, altitude and other factors. Sea-shore collections were made with particular reference to the taxonomy of the isopod crustacean *Jaera*.

A specially designed apparatus was used for quantitative sampling of the soil fauna at some 72 different sites, involving more than 400 sample units. Apart from obtaining zoogeographical data on the distribution of arctic invertebrates the intention had been to relate detailed distributions of mites and collembola to vegetation and altitude. However, the effects of exposure and aspect were so extreme that simple relations could not be detected and a phenological reference system was used instead. The effects of solifluation patterns and related phenomena were studied and work on the identification of the soil fauna is now in progress. This has proved interesting; 50 species of mites and 25 of collembola have been identified and some of these have not been described previously.

The three ornithologists collected data on the occurrence and distribution of birds from the Sandland and Olderfjord areas and from the Island of Loppa. A short visit, in bad conditions, was made to the large auk colony on Nord Fugløy.

Some 220 birds were trapped and ringed. The largest group was of redpolls, *Carduelis*, and the detailed descriptions made at the time are now being compared with those from skins in an attempt to determine the taxonomic position of the Øksfjord Peninsula birds. One ringed redpoll has already been recovered in Russia and a fieldfare from the south of France.

A small colony of arctic tern, *Sterna macrara*, on an islet off Sandland Peninsula had an extended breeding period and 25 chicks were ringed. On the Island of Loppa a detailed survey of the bird population was made.

Geomorphological work included the mapping of morainic features around the northern and western sides of the small ice-cap. Three major retreat stages are recognized, but the final analysis is not yet complete. Detailed work on the numerous raised beaches around Langfjord and Nord Bergsfjord was initiated and will be continued during 1962.

Finally, a party of three mountaineers spent several weeks in the Nor Tverfjord area, making a number of new ridge traverses and first ascents. Svartfjell (1,218 m.), the highest mountain in Finnmark, was climbed and a number of days were spent on the dramatic gabbro ridge separating the Sandland and Sor Tverfjord valleys. Gabbro rock, abundant in the area, provides a great deal of interesting, sometimes spectacular, climbing, but semi-frozen scree and loose blocks constitute an unwelcome hazard on all but the steepest faces.

The expedition was sponsored by the University College of Swansea and generous financial assistance was received from the Royal Society, the Royal Geographical Society, the Everest Foundation, the Gilchrist Educational Trust and the C. H. Foyle Trust among many others.

H. Dickinson
P. R. Hooper

DISTRIBUTION OF ARSENIC IN NAPOLEON'S HAIR

By Dr. HAMILTON SMITH, Dr. STEN FORSHUFVUD
and Dr. ANDERS WASSÉN

Department of Forensic Medicine, University of Glasgow, and Vasagaten 33, Goteborg C, Sweden

THE illness Napoleon suffered during most of his captivity on St. Helena was actually the syndrome of chronic arsenic intoxication with intercurrent attacks of acute arsenic poisoning, as reported briefly in *Nature*[1] and discussed at length in a reassessment of the disease history by one of us (S. F.)[2]. Among the facts adduced in support of this theory was the finding that a few rather short hairs known to have been taken from Napoleon's head, presumably the day after death, showed a total arsenic content of 10·38 p.p.m., a value approximately thirteen times higher than the normal mean arsenic content of about 0·8 p.p.m. It would have been interesting to examine the distribution of the arsenic, but the sample was too small (1·72 mgm.) and we were then unable to obtain further hairs from the same source.

However, on November 6, 1961, not long after the news of Napoleon's deliberate poisoning and tragic mode of death had become public property, M. Clifford Frey, a textile manufacturer of Münchwilen, Switzerland, called at the Department of Forensic Medicine, Glasgow. He brought with him a family heirloom in the form of a small bundle of Napoleonic hairs. It was attached to a piece of paper with intricately knotted twine. We were permitted to take as many hairs as required within reason, provided the knot was not undone. Thus, although some of the brittle hairs broke when being pulled free of the knot, we were able to acquire a supply of hairs which were appreciably longer than those we had previously analysed, the longest measuring 13 cm. It could be distinctly observed that most of these hairs had been shorn off with a razor and not cut off with scissors.

The paper to which the hairs were attached was folded into a small envelope inscribed "Cheveux de l'immortel Empereur Napoléon". It was inserted in a larger envelope which, in the same characteristic handwriting, bears the names of the addressee and sender, respectively, "Monsieur Mons-Riss, St. Gall, Suisse", and "Abram Noverraz, La Violette près Lausanne, le 8^{e}, 7^{bre} 1838"; it is post-marked Lausanne, September 9, 1838. The larger envelope includes a covering letter written in the same characteristic hand and signed by J. Abram Noverraz. It states, among other things: ". . . Je me fait un plaisir aujourd'hui Monsieur Mons de vous envoyez quelques cheveux de l'Empereur Napoléon que j'ai pris sur sa tête après sa mort, c'etait le six Mai 1821".

The hairs remained in the possession of the Mons family until, according to documentary proof, it was procured by M. Clifford Frey, sen., an officer of the Swiss Army, from Mme Mons-Im-Hoff, the widow of the aforementioned Monsieur Mons's grandson.

A Swiss, M. J. Abram Noverraz, born in the neighbourhood of Vaud, was appointed to Napoleon's domestic staff in 1809 and became his valet throughout the captivity on St. Helena. After Napoleon's death he returned to Switzerland. Noverraz has the reputation of being completely reliable and absolutely faithful to his master and his memory. Napoleon died on May 5, 1821, and during the following night his entire head was shaved, with the alleged two-fold aim of providing as much hair as possible for distribution as souvenirs and of facilitating the making of a death mask. The barber on this occasion was none other than Noverraz[3].

Accordingly, it seems beyond doubt that the hairs put at the disposal of the laboratory by M. Clifford Frey came from an authentic souvenir of Napoleon.

The average daily growth of hair on the scalp is about 0·35 mm. (ref. 4). On this basis a 13-cm. hair should register a record of the exposures to appreciable amounts of arsenic for a period of a little less than a year. Hence, the longest hairs in Clifford Frey's sample should tell us whether Napoleon was exposed to significant doses of arsenic over the last year.

The hair was irradiated for 24 hr. by a flux of 10^{12} thermal neutrons/cm.^{2}/sec. in a nuclear reactor at the Atomic Energy Research Establishment, Harwell. On return from the pile the hair was fixed by means of self-adhesive tape to a piece of graph paper and then cut into 5-mm. lengths, suitable for counting by an end-window Geiger counter. The results were plotted in a graph against distance from an end of the hair.

The graph in Fig. 1 shows the distribution of activity along a hair, the distal end of which is at *A*. This was a hair that had never been cut before and the very fine end (responsible for the sudden fall in activity) was easily seen. We have estimated that the line *C* is the usual average for the hair. It is certain that the peak marked *P* is due to some trace element being present in larger amounts than normal, because its height lies more than 50 per cent above the average. Accordingly, it is evident that for a period of about 4 months Napoleon was exposed to abnormally large amounts of a substance which was transformed into a radioactive isotope by the irradiation. Other hairs gave similar graphs.

In order to ascertain whether the isotope really was arsenic-16, the arsenic content was estimated[5] in consecutive 1-cm. lengths of a 9-cm. hair, the respective values being 11·0, 7·70, 4·20, 3·79, 1·06; 4·46;

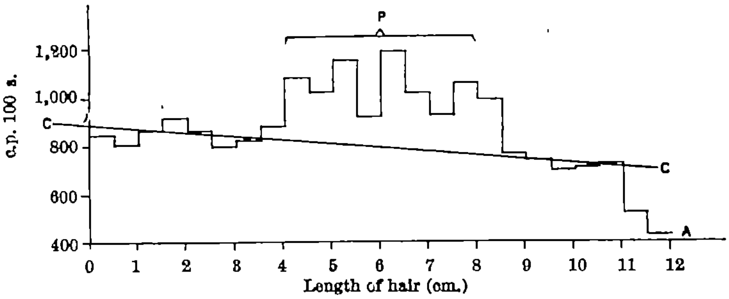

Fig. 1

5·80, 2·79 and 3·44 p.p.m. (average 4·91). Since this hair was 3 cm. shorter than that yielding the graph in Fig. 1, it lacked the older portion which was relatively free from arsenic.

The arsenic content was estimated in two pooled samples of broken hairs, the respective values being 3·75 and 3·27 p.p.m.

Estimation of arsenic by activation analysis is an extremely sensitive method. Nevertheless, the method has its limitations. When it is used on less than about 1 mgm. of ordinary hair, it yields values which tend to be obscured by the background. Accordingly, it is rather risky to apply this method to 1-cm. lengths of Napoleonic hair which weigh about one-thirtieth of a mgm. We have done so merely to provide a general picture of the distribution of arsenic in Napoleon's hair, knowing that any quantitative error will be well on the conservative side.

These distribution studies show that Napoleon was exposed to arsenic intermittently. Indeed, judging by the only hair with the distal end uncut, the periodicity of the exposures agrees remarkably well with what can be deduced about the course of Napoleon's disease from the accounts of the eye-witnesses.

The arsenic cannot have been added afterwards, by spraying, dusting or dipping, as suggested by some critics[6]. No estimate of the size of the arsenic dosage given to Napoleon can be made on the basis of our results; such very desirable information could probably be obtained after exhumation of the corpse.

In a forthcoming paper it is hoped to publish the results of similar distribution studies on hairs from other sources and other periods of Napoleon's life.

The work of one of us (H. S.) was supported by the Medical Research Council (Great Britain).

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GOLDEN JUBILEE OF THE ROYAL ONTARIO MUSEUM, TORONTO

By Dr. W. E. SWINTON

Head of the Division of Life Sciences in the Museum

THE Royal Ontario Museum celebrated its golden jubilee on April 16 with a special exhibition, entitled "Search and Research", which illustrated advances in knowledge in which the Museum had participated during the past fifty years. The exhibition was opened by the Lieutenant-Governor of Ontario, the Hon. Lieut-Colonel J. Keiller MacKay. There was also a special series of lectures by distinguished scientists given in February, March and April. April 16 was the anniversary of the Museum's foundation only on paper, for it was on that date in 1912 that the Legislative Assembly of the Province of Ontario established the Museum for "(*a*) the collection and exhibition of objects of every kind calculated to illustrate the natural history of Ontario, and thereby to aid in a knowledge of what it is able to contribute to science and industry; (*b*) the collection and exhibition of objects of any kind calculated to illustrate the natural history of the world and the history of man in all ages; (*c*) such other objects as may be authorized by the Lieutenant-Governor in Council".

It may be important to repeat this enactment now, for it received no comment at the time, the event being overshadowed throughout the world, and especially in North America, by the sinking of the *Titanic*.

Within two years material was assembled, an unprepossessing building was made ready and officially opened in March 1914 by H.R.H. the Duke of Connaught, Governor-General of Canada.

Most of the materials came from the departments of the University, and right from the start emphasis was placed on art and archæology. Yet the Board had designated five museums within the whole: the Royal Ontario Museums of Archæology, Geology, Mineralogy, Palæontology and Zoology. Each had its director; its somewhat limited space; its even more limited funds; and a pride peculiarly its own which did much to ensure the rapid increase of the individual collections in size and in importance for teaching. There was an intimate connexion with the University of Toronto from the beginning, and the directors were usually the heads of the appropriate University departments.

The Geological Museum began with a fine series of Canadian silver ores, and with a remarkable collection of ornamental and building stones collected by Prof. W. A. Parks, later to be director of the Museum of Palæontology. The exhibits were, however, housed in a basement so dark and unsuited for the purpose that the public scarcely ever penetrated its murkiness. Yet the forbidding conditions do not seem to have depressed its directors. The first was Prof. A. P.

Coleman, who ministered over its unattractive cases for eight years and managed to attract many more specimens. He was succeeded by Prof. E. S. Moore, who greatly amplified the Museum's outlook. Both this Museum and its fellow of Mineralogy were allotted more equitable accommodation in 1932 when the new Museum extension, with its imposing Queen's Park frontage, was built. In 1959, in another of many reconstitutions, the Museums of Mineralogy and Geology were united as the Division of Earth Sciences, and this, under its head, Dr. V. B. Meen, has made remarkable advances both in the size of its collections and the methods of their display. Its attractive galleries are at the moment being made even more attractive with the aid of a substantial grant from the Bickell Foundation.

The Museum of Palæontology owes its history to the brief but productive professorship of Henry Alleyn Nicholson, the well-known palæontologist, who went from Edinburgh to Toronto in 1871 and who built up the nucleus of a great collection which, unfortunately, was almost wholly destroyed by a fire at the University. His example was followed fortunately by others, so that when the Royal Ontario Museum was founded there was a plenitude of materials. The first director, who had an enormous influence on the Museum, was Dr. W. A. Parks, who started off, as has been seen, with a collection of building stones, who became head of the Department of Geology in the University, and was an authority on invertebrate palæontology. He was also the progenitor of the fine collection of dinosaurs which is one of the claims to fame of the Museum to-day.

Royal Ontario Museum expeditions, especially those under the charge of Mr. Levi Sternberg, have made remarkable discoveries in Alberta and Saskatchewan, and the dinosaur galleries have an almost unparalleled collection of hadrosaurs, with excellent samples also of the theropods *Gorgosaurus*, *Albertosaurus*, and the slender *Ornithomimus* (which now includes *Struthiomimus*). This fauna is, of course, essentially Upper Cretaceous in age and the Canadian museums therefore lack sauropods and stegosaurs, though the fine series of ceratopsians must be regarded as some compensation.

The Museum of Palæontology was one of those which especially benefited from the 1932 addition. Four large galleries became available: two for vertebrate fossils and two for the invertebrates. Unfortunately, in the past year or two, one of these galleries, so necessary for instruction in a university museum, has been taken over for a museum library. This is all the more regrettable since this hall, and the present invertebrate gallery, have a splendid series of murals of life through the ages, painted by Mr. G. A. Reid.

Dr. Parks was succeeded as director by Dr. Loris Russell, now director of the National Museum of Canada in Ottawa.

The fifth museum was that of Zoology. Housed after 1932 on the spacious top floors of the new addition, it benefited from the imaginative planning of two directors who were also successive professors of zoology. Dr. E. M. Walker, the senior of these, is still active in the Department of Entomology and Invertebrate Zoology and is at present engaged in the preparation of the third volume of his extensive studies of the Odonata. Dr. J. R. Dymond, his successor in the Museum and the professorship, is also very active and occupies an office in the Department of Ichthyology.

A few years ago the Museums of Zoology and Palæontology were united. In 1959 they became the Division of Life Sciences.

Traditionally, botany has received little attention in the Museum. Happily this state of affairs now appears to be ending, and the University Department of Botany, under its chairman, Prof. N. P. Badenhuizen, has generously offered the Museum its cryptogamic and phanerogamic herbaria. At the moment, lack of appropriate space in the Museum has prevented their transfer.

A more positive recent achievement is to be recorded. The Carling Gallery of Reptiles was officially opened by Mrs. C. O. Dalton, wife of the president of Carling Breweries. Without the financial generosity of this Company the gallery of twenty-four dioramas and internally lit cases could not have been prepared and the resulting exhibits show new techniques of casting, recently developed in Toronto by the Display Department under Mr. T. M. Shortt, whose own artistic gifts are seen in the splendid backgrounds to the dioramas.

Fifty years of museum work would not necessarily be of special merit if it were not to be the foundation of things to be continued or to be inaugurated. The Earth Sciences and the Life Sciences can view the future with some confidence. A splendid new gallery of geology will soon be opened. Life Sciences is engaged in planning a series of new dioramas illustrating environments in various zoogeographical zones. These will incorporate materials now in the Museum and the results of expeditions still to be undertaken to several parts of the Old and New Worlds.

Concurrently, the systematic series are being rearranged with emphasis for teaching morphology and taxonomy. In preparation for all this, members of the scientific staff will be visiting type-collections and new exhibits in Europe and the United States and there will be expeditions this year in Alberta and Saskatchewan and field parties to Arctic Canada, the Maritimes and the United States.

The series of special lectures given earlier in the year directed attention to outstanding scientific problems and efforts to solve them. Prof. A. S. Romer inaugurated the series with a masterly account of the evolution of Amphibia. Following speakers dealt with the development of the Pacific Ocean (Prof. H. W. Menard); volcanoes (Dr. Howel Williams); permafrost (Mr. R. Leggett); animal clocks and compasses (Dr. J. Carthy, University of London); and the Moon and space programmes (Dr. G. P. Kuiper).

This heterogeneous series emphasized in a special way the advances in both knowledge and techniques of the past fifty years. Another lecture, given by me in the middle of the series, emphasized the place of the Museum in the interpretation of such advances and pleaded for a new place and regard for museums in the days of the increasing tide of scientific knowledge. How otherwise can the public see the roundness of science and the integration of discoveries? The scientific journals on one hand and the newspapers on the other exhibit extremes for quite different purposes than the elucidation of the whole for lay readers.

The adult population of to-day, who are the responsible body of the electorate and the financial backbone of the State, are largely excluded from knowledge of the circumstances that more and more control their world. Even the graduate of several years standing finds himself in a world in which his own education has often become obsolete. The structure of physics, the range of the Periodic Table in chemistry, the concepts of biochemistry and the more complete series of fossils illustrating evolution, are of fairly recent development. The radio telescope and the electron microscope give new horizons to the universe and its life. Is the public to be excluded from the knowledge and appreciation of the trend and consequences of such advancements ? Although adult education makes an attempt in the largest centres to fill the gaps, television has so far failed to consider meeting the challenge. The museum, with scientifically trained staffs and its three-dimensional exhibits, particularly as in Toronto where there are the resources of a great University behind it, has an almost unrivalled medium of presentation. If such a museum's services be amplified by a planetarium and access to botanical and zoological gardens, a vastly increased service to the public could be given.

In making this 'free university' suitable for public instruction, aided by the Museum's Division of Education, one may tend to overlook its very considerable value to the undergraduate and the graduate student also.

The museum profession, taken eclectically, is competent to make and to maintain such services, but the attainment of the necessary buildings and finance, neither of which is excessive or unreasonable compared, for example, with those of zoological gardens, will never be obtained until a new conception of the museum's place in education and its potential for public instruction is born in the minds of committees and municipal councils.

Perhaps, in Toronto, the new permanent galleries and the temporary special exhibitions, complete with the manifest achievements of fifty years of search and research in many parts of the world, will help toward this desirable end, without which there may be less security for all other institutions in the next few determinant years.

NEWS and VIEWS

Ministry of Works Chief Engineer :

Mr. A. B. Mann, C.B.E.

Mr. A. B. Mann, chief mechanical and electrical engineer of the Ministry of Works, retires on October 26. He joined the Ministry in 1923. Prior to and during the Second World War he was extensively engaged in a senior capacity with the engineering installations and equipment for propellant and high explosive factories at Bishopton, Wrexham, Ranskill, Pembrey and Sellafield, and the emergency military hospitals for the Normandy invasion. In the immediate post-war years he was concerned with the Ministry's programme on behalf of the Ministry of Supply for the construction and equipment of the atomic research establishments at Harwell and Aldermaston, and the factories at Springfields, Capenhurst and Windscale. He was appointed chief engineer in 1954 and assumed responsibility for both the Maintenance and New Works Branches of the Engineering Division. During his term of office a system of planned maintenance was introduced within the Maintenance Branch, and the New Works Branch carried out large-scale and unusual engineering work embracing the new wind tunnels at the Royal Aeronautical Establishment at Bedford, engine test facilities at the National Gas Turbine Establishment at Pyestock, the Rocket Research Establishment at Spadeadam, Admiralty Research Establishments at Bushy Park and Haslar, and Department of Scientific and Industrial Research projects at Feltham, Howbery Park, and the National Physical Laboratory.

Mr. W. L. Wilson, O.B.E.

Mr. Mann is to be succeeded by Mr. W. L. Wilson. He was trained by Imperial Chemical Industries, Ltd. (Billingham Division), and joined the Ministry in 1936. From that time until the end of the Second World War he was engaged at increasing levels of responsibility in the design of Government factories and establishments. He was promoted to the rank of assistant chief engineer in 1954 and since that date has been in control of engineering works undertaken by the Ministry on behalf of the United Kingdom Atomic Energy Authority, the Ministry of Aviation, the War Office and the Department of Scientific and Industrial Research.

Botany at Glasgow : Dr. Samuel Williams

The retirement of Dr. S. Williams at the end of the present academic session after thirty-nine years service on the staff of the Department of Botany, University of Glasgow, will mean a real break with the past. Williams, a native of Nantwich, graduated from Manchester at a time when the staff of the Department of Botany there included Weiss, Lang, Adamson, Thoday, Robinson and Howarth. His appointment to the Glasgow Department came at a period when the staff had been seriously weakened by appointments to chairs elsewhere, with the result that Williams had right away to shoulder a large share of the teaching, which he continued to do with great success until the post-war increase in establishment brought some relief. He has given loyal support to the three heads of Department (Bower, Drummond and Walton) under whom he has served, and has on several occasions taken temporary charge of the Department. Though a botanist of very broad outlook, Williams is, of course, primarily a morphologist and is recognized as an authority on the morphology and anatomy of the Pteridophytes, aspects on which he has published some twenty papers, all of the highest quality, the Neill Medal of the Royal Society of Edinburgh having been awarded to him for this work. He has always been held in the highest esteem by his colleagues because of his botanical competence, his personal integrity and sincerity, and his friendly, generous nature. Mrs. Williams by her most effective demonstrating over many years has also contributed notably to the maintenance of teaching standards in the Department, and it is certain that the gap left by the departure of Dr. and Mrs. Williams will be very hard to fill.

School of Applied Science, University College of North Wales, Bangor

The demand for the course in electrical science at the University College of North Wales has increased considerably in recent years and there is now an annual intake of fifty students. It is proposed in plans for further development to set up a School of Applied Science with three main divisions. In addition to the existing subjects of electronic engineering there will be two new subjects of control engineering and materials technology. Something of these subjects is already included in the present course, but a major step forward has been made by the creation of a new chair of materials technology. Dr. R. W. Cahn, reader in physical metallurgy at the University of Birmingham, has been appointed to the chair of materials technology. Dr. Cahn is a graduate of the University of Cambridge. After three years in the Metallurgy Division of the Atomic Energy Research Establishment at Harwell, he joined the Department of Physical Metallurgy at the University of Birmingham in 1951, where he has held in turn the posts of lecturer, senior lecturer and reader. Dr. Cahn's research work has covered numerous aspects of the crystallography and physical properties of metals and alloys. A chair of control engineering will also be set up in due course. In the development of the new School of Applied Science with the divisions of electronic engineering, control engineering and materials technology it is intended that all technological undergraduates will spend some time in each of the three divisions. It is intended that the emphasis on electronics will remain in the early stages of the new developments. An essential feature of the new plans is to provide opportunities for further study, after the first degree, with M.Sc. courses in various aspects of electronics, control and materials.

Courtaulds, Ltd.: New Research Laboratories

Courtaulds, Ltd., are to transfer the work on basic research now being carried out at Maidenhead to their new laboratories at Coventry. This will ensure closer liaison between basic and applied research staffs and will enable full advantage to be taken of the wide range of facilities available in Coventry. The transfer of staff and equipment, scheduled to begin during the summer, will be completed by the autumn of this year. It has also been decided to concentrate research on petrochemicals and plastics in a new British Celanese laboratory now being built at Spondon, and due to be completed by September. Work in these fields now undertaken in smaller existing research units at Spondon and at Putteridge Bury, near Luton, will be transferred to the new laboratory. After September 30 correspondence should be addressed to the Basic Polymer Research Group, A. and S.F. Laboratory, Courtaulds, Ltd., Coventry, in place of Maidenhead, and to the Research Laboratory, British Celanese, Ltd., Spondon, in place of Putteridge Bury.

London and Home Counties Regional Advisory Council for Technological Education

The thirteenth annual report of the Regional Advisory Council for Technological Education, London and Home Counties, covers the year ended August 31, 1961, and includes a statement of evidence submitted to the Robbins Committee (Pp. 39. London: London and Home Counties Regional Advisory Council for Technological Education, 1962). A plan for the regional development of courses leading to the Diploma in Technology was prepared and published during the year, and comments have been invited from the Regional Academic Board on a draft statement of policy regarding sandwich degree courses. A regional survey of B.Sc. courses has been made. Recommendations have been made and adopted regarding the geographical distribution and demand for degree courses in chemical engineering, as well as courses for the Diploma in Technology and in Technology (Engineering). A report on education for civil and structural engineering in technical colleges in the region is being printed.

Organization and Method in Local Government

The valuable contribution which an organization and methods unit can provide in improving the efficiency of local government services has been described by J. L. Hampshire, County Treasurer of Kent (*Productivity*, 1; January/February 1962. An International Journal and Abstracting Service for Industrial and Business Managers to help Increase Productivity in Factory and Office. Pp. 56. London and New York: Pergamon Press. Annual subscription, six issues, 70*s*.; United States and Canada, 10 dollars). In Kent the Organization and Methods Unit was installed in 1947 and, since then, has carried out investigations in many departments. These include a general reorganization of the Finance Department including the Licences Division, and a general survey of the relationships between the Finance Department and other Departments in such matters as the control of requisitions for supplies and the certification of accounts for payment. Staff members in the Finance and Licences Divisions of the Department were reduced at a time when considerable additional work was being absorbed and the financial savings were considerable. Since 1950, a large number of assignments of a varied character have been carried out. The work has at some time or other affected every County Department, and, although many of the assignments have resulted in ascertainable financial savings, there have been instances where increased effectiveness of the work has been the only aim, and savings, if any, have been of an indirect character. During 1955–56 it was decided to place emphasis on overall departmental investigations while continuing to work on purely subject assignments to meet specific needs and requests. From the beginning it was realized that savings would result from the technique involved. The aggregation of the figures shows that, up to March 31, 1961, realized savings attributable to investigations made by the Organization and Methods Unit amount to approximately £75,900 a year and that suggestions are now under consideration which may involve further savings.

Royal Observatory Annals

Most scientists are aware that fashions in publication have changed in recent decades, and that the general trend is one of publishing less and less detail. Indeed, the growing costs and volume of material that is suitable for publication have compelled many scientific journals to restrict their acceptance of tabular matter to very modest proportions. This is, in many cases, reasonable, but it has thrown a burden on workers in certain branches of astronomy. It is still necessary to make extensive catalogues and observations available to other workers. It is not, therefore, surprising that it has become necessary to start a new publication, *Royal Observatory Annals*, joint publications of the Royal Greenwich and Cape Observatories, published by H.M. Stationery Office (No. 1: *Nutation*, 1900–1959. Prepared by H.M. Nautical Almanac Office. Pp. vii+41. London: H.M.S.O., 1961. 7*s*. net). No. 1, 1961, contains numerical tables of nutation in longitude and obliquity for each day of the period 1900–59, based on new series expansions by E. W. Woolard. The numerical calculations were performed by H.M. Nautical Almanac Office. The new series expansions are being used in the national ephemerides from 1960. The calculations have been extended backwards to enable observers to make improved reductions of recent observations of the Sun, Moon and planets.

New High-power Xenon-Arc Lamp

A new lamp, a compact 5,000-W. xenon-arc lamp, which is nearly three times as bright as the Sun and which will have applications in searchlight and projection equipment, has been developed by the U.S. General Electric Company. The light produced by the lamp totals 275,000 lumens, or 55 lumens/W. The lamp is the second of its kind; the first was a 2,000-W. xenon arc lamp and has been available for several months. Its first use is in a solar simulator for space vehicle investigations. In the visible, the light simulates sunlight, and its total spectrum, including the ultra-violet and infra-red, approximates closely to solar radiation. The lamp has a high degree of stability and of freedom from noise, smoke and fumes. Housed in a parabolic reflector, the lamp throws a powerful beam of light, and, used as a large searchlight, could throw a beam of 10^9 candle-power, sufficient to enable a person to read a newspaper at a distance of about 15 miles from the source. Another expected application is to optical masers. As a source of intense infra-red radiation it will be useful in connexion with solar-type furnaces. The lamp is a direct-current light source in an ellipsoidal-shaped quartz bulb. The bulb is only about 3½ in. in diameter and 4¼ in. in length although the overall length of the lamp is slightly above 19 in. The bulb houses two tungsten electrodes with an arc gap of 8 mm. and the arc operates in a high pressure of xenon. A high voltage is applied to start the lamp and a current of 145 amp. flows across the arc from the cathode to the large anode, which weighs ¾ lb. The anode reaches a temperature of 6,000° F. The lamp is expected to have an operating life of 1,000 hr. Further details of the lamp are available from the International General Electric Co. of New York, Ltd., 296 High Holborn, London, W.C.1.

The First Bragg Lecture

The first public Bragg Lecture (admission free) will be given by Prof. P. P. Ewald, president of the International Union of Crystallography, on the subject "William Henry Bragg and the New Crystallography" at 5.15 p.m. on June 7, in the Arts Lecture Theatre of the University of Leeds, and at 5.15 p.m. on June 13, at the Royal Institution, 21 Albemarle Street, London. The Lecture this year is part of the centenary celebration of the birth of Sir William Bragg. It is also a prelude to the jubilee celebration of the discovery of X-ray diffraction by crystals, to be held in July in Munich, where Prof. Max von Laue suggested and Drs. Friedrich and Knipping carried out their first famous experiment, an experiment which was the result of a question asked by the young research student, Paul Ewald, fifty years ago. The Bragg Lecture will be given once in three years at one or more of the Universities of Cambridge, Leeds, London and Manchester or at the Royal Institution, London, in which places Sir William Bragg and his son, Sir Lawrence Bragg, have carried on their research work. Sir Lawrence Bragg will take the chair on June 13.

British Electrical Power Convention

The fourteenth British Electrical Power Convention will be held in Brighton during June 4–7. The theme of the Convention will be "The British Electrical Industry in the World Scene", and the programme will include: British engineering services overseas (Mr. A. E. Powell, Merz and McLellan); planning for expansion in electricity supply (Mr. R. S. Edwards, Electricity Council, and Mr. D. Clark, Central Electricity Generating Board); automation—the central industrial theme of the '60's ? (Sir Leon Bagrit, Elliott Brothers (London), Ltd.). The Citrine Lecture, "The Snowy Mountains Scheme", will be delivered by Sir William Hudson, commissioner of the Snowy Mountains Hydro-Electric Authority. Further information can be obtained from the press officer, Mr. Stanley Oxley, British Electrical Development Association, 2 Savoy Hill, London, W.C.2.

Announcements

Dr. B. K. Davison has been appointed development chemist in the Development and Research Department of the International Nickel Co. (Mond), Ltd. He is concerned with the chemical and chemico-physical aspects of the Department's work, with particular reference to the established and potential industrial applications of the Company's chemical products. Dr. Davison was formerly organic research manager at Hardman and Holden, Ltd., of Manchester. Previously he was with Imperial Chemical Industries, Ltd., Billingham, and the Imperial College of Tropical Agriculture, Trinidad.

The seventh International Congress on Alpine Meteorology will be held in Sauze d'Oulx-Sestriere, Italy, during September 3–6. Further information can be obtained from the Secretary, Società Italiana di Geofisica e Meteorologia, P.O. Box 3145, Genova.

The National Vegetable Research Station is holding an open day on July 19, when visitors will be able to inspect the laboratories, and see some of the techniques used in a modern research station. Further information can be obtained from Mr. G. Lesson, National Vegetable Research Station, Wellesbourne, Warwick.

A symposium on "Reproductive and Parental Behaviour", arranged by the Association for the Study of Animal Behaviour, will be held in Oxford during July 11–13. The programme will include a visit to the Animal Behaviour Research Group of the Department of Zoology, University of Oxford. Further information can be obtained from Dr. J. B. Free, Rothamsted Experimental Station, Harpenden, Herts.

Errata. In the article appearing in the March 31 issue of *Nature*, entitled "Classification and Nomenclature of Enzymes and Coenzymes", by Prof. R. H. S. Thompson, on p. 1229, col. 1, line 3 from bottom (footnote), "K_{+n}, K_{-n}, velocity constants of . . ." should read "k_{+n}, k_{-n}, velocity constants of . . .", and on p. 1229, col. 2, line 11 from top, "Thus K_{+n} will denote . . ." should read: "Thus k_{+n} will denote . . .".

In the article appearing on p. 372 of the April 28 issue of *Nature* entitled "Determination of Molecular Weights of Gelatin by Scattered Light Measurements", by B. E. Tabor, lines 20–23 should read ". . . $\overline{M}_w$ with respect to the intrinsic viscosity. The replacement of the 0·5 M saline, used by Stainsby, with 1 M potassium thiocyanate . . .".

EXTENSION OF AGRICULTURAL RESEARCH IN QUEENSLAND, AUSTRALIA

A NEW research centre for the Commonwealth Scientific and Industrial Research Organization is to be established at Townsville, in northern Queensland. The centre will fill an important gap in northern Australian research. It will consist of research laboratories and an associated field station of 5,000 acres. The Government will provide a capital sum of £300,000 to finance the venture, and the annual expenditure on research will rise within a short time to £110,000.

The programme of research at Townsville will combine pasture, soil and animal studies. Scientists based at the new centre will work in an area of about one-third of Queensland, extending from Rockhampton to Cape York.

The Organization already has pasture research stations in the Kimberleys, at Katherine, Darwin, Alice Springs, Brisbane, and in the southern States. When the Townsville project is completed, the Organization will be working in every large area of Australia which has a high pastoral potential.

The new laboratories will be built on land adjacent to the University of Queensland's Townsville site. The Government has provided £50,000 this financial year for the purchase of land. The laboratories will have a staff of about fifty, including ten research officers.

The new laboratories and station will become part of the Organization's Division of Tropical Pastures which, under the direction of Dr. J. Griffiths Davies, has carried out pasture research in south-eastern Queensland for several years. The results achieved by this Brisbane-based group have been so promising that the Government now feels it is time to extend the work to northern Queensland.

The research programme will be carried out in the Townsville hinterland, the Cairns hinterland, the northern brigalow lands, the northern speargrass region, and the coastal regions. South of Townsville, the area will extend into central eastern Queensland as far as the northern limits of the area being studied from Brisbane.

The research work will be carried out in collaboration with the Queensland Department of Agriculture and Stock and local graziers. It will include a vigorous programme of testing new introduced pasture species and mixtures. Soil scientists will undertake research into soil deficiencies, and animal and pasture management practices will be studied.

By overcoming stock nutritional deficiencies through the development of improved pasture and fodder crops, this new research programme should help to raise stocking-rates, improve calving percentages, reduce losses to breeders, produce marketable animals at younger ages, increase the percentage turn-over, etc.

At present, more than two million beef cattle, or about one-third of Queensland's beef cattle population, are reared in these regions. It is confidently expected that, in many places, present production can be increased several-fold.

The Organization's Division of Land Research and Regional Survey has already conducted extensive regional surveys in the Townsville and Gulf of Carpentaria areas and is at present making a survey of the Fitzroy Basin in central eastern Queensland. These surveys will provide a basis for future pasture development work.

The research programme will be complemented by the work of the Organization's National Cattle Breeding Station at Belmont, near Rockhampton, and the Division of Entomology's cattle tick research station at Ingham. At Belmont, scientists are studying the selection of cattle for better adaptation to tropical conditions, faster growth-rate, and greater efficiency of food utilization.

THE PRIMATES

A SYMPOSIUM on the primates was held during April 12–14 at the Zoological Society of London, sponsored by the Society in collaboration with the Anatomical Society and the Society for Human Biology. Dr. J. R. Napier, the organizing secretary, had arranged a diverse programme and had invited numerous overseas specialists, some of whom, unfortunately, were unable to be present. This necessitated some changes in the published programme.

The first day, under the chairmanship of Sir Solly Zuckerman, was devoted to aspects of primate behaviour in the wild and in captivity, and included papers by Prof. K. R. Hall (ecology of the chacma baboon, *Papio ursinus*), Jane Morris-Goodall (ecology and behaviour of free-ranging chimpanzees), Rosalie Osborn (behaviour of mountain gorilla), A. Kortland (protohominid behaviour), R. Andrew (evolution of vocalization), J. A. H. van Hooff (facial expression), and the Rev. J. Cole (research on *Macaca nemestrina*). A short film on the so-called ufiti of Nyasaland was shown by Dr. W. C. Osman Hill.

Prof. Hall's contribution added considerably to our knowledge of the social structure, group-composition, feeding behaviour and daily activities of the chacma in several areas of South and South-west Africa, and Southern Rhodesia. Of interest was his observation that in the Drakensbergs baboons range up to the snow-line. Rosalie Osborn exhibited the gorilla film prepared by Dr. Schaller and kindly loaned by the New York Zoological Society. In addition to her commentary, she added remarks on her own first-hand acquaintance with the gorillas in Western Uganda. Emphasis was laid on their essential quadrupedality and lack of brachiation; data were given on feeding activities and nesting procedure.

The well-documented contribution from Miss J. Morris-Goodall was received with much enthusiasm. Her sixteen months close contact with wild chimpanzees in Tanganyika was fully recorded in film and photograph, and the results included convincing evidence of tool-using by the ape. especially application of sticks and straws to probe ants nests for the purpose of collecting the ants for food. There was a noted preferential use of the left hand in this procedure. Also remarkable is the chimpanzee's avidity for salt, which, if available, it will steal. On occasion, flesh is eaten, presumably the local hare or some new-born artiodactyl, and in this there was evidence of sharing of food by a dominant male with his partners.

The morning session concluded with some general discussion opened by Dr. W. C. Osman Hill (who here showed a film and demonstrated a tape recording of the ufiti—a lone chimpanzee recently reported from the Nkata Bay area of Nyasaland). This was followed by an appraisal of the papers by Dr. N. Tappen (Tulane).

The afternoon was devoted to papers by Dr. A. Kortlandt (Amsterdam), Dr. R. Andrew (Yale), J. A. H. van Hooff (Utrecht) and the Rev. J. Cole (Oxford). Kortlandt explained his views on protohominid behaviour based on his field studies of chimpanzees. He posed the question why the great apes have not developed more human behaviour since the basic qualities are available, and compared their cultural progress with that of the australopithecines. His conclusion was that the protohominids were savannah dwellers, an environment evoking new ideas not demanded of forest dwelling pongids. He opined that the great apes could well have been protohominids that had been driven back to the forests.

Dr. Andrew contributed an account of an analysis of the vocal emissions of various primates illustrated by audiograms. He distinguished fundamentals from resonances, giving examples of various utterances by *Cercopithecus, Macaca, Theropithecus* and *Pan*. He concluded that the voice of *Cercocebus* was closer to that of baboons than to *Cercopithecus*, etc.

Mr. Van Hooff outlined the historical development of studies in facial expression since Darwin's classical observations, and gave an account of his personal comparative ethological studies on the higher primates with reference to ætiology, motivation and function. Facial expression could be analysed into movements of brows, eye-region, ears, the erection of coronal tufts and modifications of the mouth, angulus oris and lips Some of the expressions served as social signals.

The Rev. J. Cole held a high opinion of the pig-tailed macaque (*Macaca nemestrina*) as a subject for experimental research, especially in the field of neurophysiology. Outlining some of his experiments, he concluded that: (1) pig-tails show a dominant hand and foot individually varying from right to left; (2) colour has more significance than shape in choice experiments. A decreasing importance of the three sense organs, vision, touch and hearing, in that order, seemed to stem from the effects of the natural environment.

Dr. J. S. Weiner showed a short film loaned by Dr. G. M. Vevers depicting the use of a tool by a captive capuchin monkey. The session terminated in a discussion opened by Dr. M. R. A. Chance.

The morning of the second day, under the chairmanship of Prof. J. Z. Young, was devoted to more specialized topics. In the morning, papers on functional anatomy were delivered. Dr. J. Scott (Belfast) discussed factors determining skull form, paying special attention to the symphyseal region of the mandible, the development of brow-ridges and the recession of the snout, with subsequent changes in the direction of facial growth. In connexion with the latter, he emphasized the role of the septal cartilage which serves as a built-in epiphysis allowing for downward and forward growth and the production of a muzzle. Only in man is the growth vertical in direction.

Dr. C. E. Oxnard (Birmingham) followed with an account of his comparative osteometrical and myological studies of the pectoral limb in reference to locomotor adaptations. This led to a brisk discussion on the definition of brachiation, semi-brachiators and kindred matters. The session concluded with a paper by Dr. G. E. Erikson (Harvard) on brachiation in New World monkeys contrasted with the phenomenon in anthropoid apes. It was pointed out that the higher platyrrhines (*Lagothrix, Brachyteles, Ateles*) are true brachiators. Evidence was specially deduced from studies of the little-known *Brachyteles*. The concluding discussion was led by Prof. A. J. E. Cave.

The afternoon session was devoted to various problems of genetic interest, under the chairmanship of Prof. L. S. Penrose, and opened with a discussion by John Hamerton (Guy's Hospital) on primate chromosomes. Some excellent photographs of gorilla chromosomes were exhibited. Dr. D. Franks (Cambridge) followed with remarks on blood-groups, and admitted that their study gave little information with regard to relationships. He reported that the B group does not appear in *Pan*, while the O group is lacking in *Pongo* and *Hylobates lar*. In *H. moloch* and *Symphalangus* only the B group is present. The A substance in *Pan* is not identical with human A^1 or A^2. The M antigen occurs in the rhesus monkey. As regards the Rh factor, *Gorilla* aligns with man, the other anthropoids do not.

Dr. L. Backman (Uppsala) described new techniques for separation of protein mixtures and discussed results as derived from their application to serum proteins in primates. In electrophoresis experiments he found the speed of transfer of haptoglobin bands was similar in man and baboon, but faster in *Cercopithecus* and some species of *Macaca*; in *Pan* it is slower than in man.

Dr. N. Tappen (Tulane) discoursed on the relations between genetics and systematics in the study of primate evolution, drawing his data from material collected in East Africa. He discussed the chromosome numbers in *Cercopithecus, Erythrocebus* and *Cercocebus* and indicated three or four patterns within the first mentioned. He also drew evidence from paper electrophoresis, which indicated an apartness for *Cercocebus* which had a phenomenally slow-moving hæmoglobin.

The last day, under the chairmanship of Prof. W. Montagna, was devoted to films showing various aspects of primate behaviour. Dr. D. Morris exhibited and made comments on a film depicting chimpanzee behaviour. Dr. W. C. Osman Hill commented on a film taken by Mr. David Attenborough (British Broadcasting Corporation) on his recent trip to Madagascar, and depicting *Microcebus, Lemur fulvus. L. variegatus, Propithecus verreauxi* and *Indri*, the last-mentioned accompanied by a sound track of the peculiar vocalizations. Prof. Hall commented on two films depicting baboon behaviour, one taken on his own expedition, the other originating from Dr. S. L. Washburn (California). The session concluded with Dr. Kortlandt's film of wild chimpanzees.

W. C. OSMAN HILL

VITAL STATISTICS OF ENGLAND AND WALES

THE two volumes of tables for 1960 [1,2], and the commentary for 1959 [3], which the Registrar General issues annually, provide the opportunity for an assessment of the position relating to the population of England and Wales. There are no important changes in the presentation of the statistics, but in the commentary there is one major innovation in the section on mortality. In place of the customary detailed review of mortality by cause of death, there is now a general section dealing with the salient features of mortality, followed by notes on selected special topics; those selected in 1959 being mortality by marital status and deaths from congenital malformation.

Mortality in general continued to decline. One of the principal features of mortality in the 1950's has been the relative stickiness of the death-rates of men over 45 compared with those of women. The following figures illustrate the position, and show that the death-rate of men aged 55–64 is now more than double that of women of the same age:

		1948–50		1958–60	
		Men	Women	Men	Women
Deaths per 1,000	45–54	8·8	5·3	7·3	4·4
population	55–65	22·3	19·5	21·7	10·8
aged	65–74	51·8	34·1	58·5	30·3

Considerable reduction in the mortality from particular causes has been achieved; thus, mortality from tuberculosis has fallen by two-thirds for men, and four-fifths for women in the decade. The growth in mortality from lung cancer in the same period which has increased by 50 per cent for men and 25 per cent for women has already been widely commented on.

The interesting feature of the nineteen-fifties, however, is the behaviour of the birth-rate and of fertility. After the peak of births in 1947, when there were 881,000 live births, the number fell to 668,000 in 1955, but this was followed by an upturn, and from 1955 the number has risen steadily to 785,000 in 1960, a figure which (except for 1947) has not been surpassed since 1921. In his commentary on the 1959 data, the Registrar General still does not commit himself, however, on the question whether this increase in the number of births is a reflexion of an upward trend in family size or of a merely temporary change in the timing of births within marriage. Whichever is true, the rise in the absolute number of births will, of course, result in stresses on the educational system in the late nineteen-sixties and the nineteen-seventies.

It is clear that there is at present no prospect of population growth ceasing, and the projections in the 1960 Population Tables are based on the assumption that after an annual figure of 750,000 births in 1960–65, this figure will gradually rise to 925,000 by the end of the century, and that the population will by that time have reached a figure of 55·6 millions—an increase of nearly 10 million in 40 years. These figures make it all the more important to carry on research into the social and economic factors associated with reproduction. Now that practically all married couples use some form of family planning at some stage of their marriage, predictions about the numbers of future births must take much more account of these factors, if they are to be at all realistic.

E. GREBENIK

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[1] General Register Office. *The Registrar General's Statistical Review of England and Wales for the year 1960. Part 1: Tables, Medical.* Pp. x+332. (London: H.M.S.O., 1962.) 22*s*. 6*d*. net.

[2] General Register Office. *The Registrar General's Statistical Review of England and Wales for the year 1960. Part 2: Tables, Population.* Pp. xi+180. (London: H.M.S.O., 1962.) 12*s*. 6*d*. net.

[3] General Register Office. *The Registrar General's Statistical Review of England and Wales for the year 1959. Part 3: Commentary.* Pp. xvi+259. (London: H.M.S.O., 1961.) 18*s*. 6*d*. net.

THE AMERICAN SCIENTIST

THE March 1962 number of the *American Scientist*, besides celebrating its fiftieth year of publication, celebrates the seventy-fifth jubilee of the Society of the Sigma XI. The president of the Society, Dr. W. R. Brode, contributes a long article discussing the growth of science, as measured in terms of manpower, financial support and facilities, and the implications of a national science programme in terms of changing sources of support and in responsibility for administration and planning. Dr. Brode's comments on the production of publications, the expansion of professional organizations and on the effects of the growth of science on supporting and performing institutions are of wide interest. An article by Dr. E. R. Weidlein, entitled "Co-operation—a Responsibility of the Scientist", starting with the development of the Mellon Institute, reviews the growth of co-operative research in the past half-century.

These two general articles are followed by a series of articles looking to the future of particular fields of science. Prof. G. G. Simpson in "The Status of the Study of Organisms" and Prof. C. Grobstein in "Levels and Ontogeny" look to the future of biology. Prof. S. C. Brown in "Plasma Physics" considers the potentialities of controlled thermonuclear reactions as sources of energy for peaceful purposes. Dr. N. B. Hannay writes on "Chemical Equilibria and Reactions in Semiconductors", Prof. M. Ryle on "Radio-astronomy and Cosmology", including the steady-state theory, and Dr. E. M. Shoemaker describes the geologist's ideas on the exploration of the Moon's surface. Prof. R. G. W. Norrish contributes, under the title "Kinetic Spectroscopy and Flash Photolysis", a summary of his presidential address to Section B at the meeting of the British Association for the Advancement of Science at Norwich last year; J. R. Fresco and D. B. Straus describe the production of synthetic nucleotides as models of the naturally occurring nucleic acids, and Dr. C. D. Cone, jun., the thermal soaring of birds. S. Brunauer contributes an article, "Tobormorite Gel—the Heart of Concrete", and H. M. Wormington a "Survey of Early American Prehistory", while, under the title "European Scientists at Work, 1961", Dr. S. Marcson describes a visit to the Department of Scientific and Industrial Research, to the Philips Research Laboratories at Eindhoven, and to the French Petroleum Institute, Malmaison.

GEL FORMATION IN POLYVINYL CHLORIDE ON IRRADIATION

By M. A. CROOK and B. J. LYONS

British Insulated Callender's Cables, Ltd., Research Organization, London, W.12

CHAPIRO[1] has shown that a large discrepancy exists between the cross-linking and hydrogen chloride yields obtained on irradiation of polyvinyl chloride. He suggested that cross-linking in this polymer occurs through reaction of radicals with unsaturated groups and not by recombination of these radicals.

In recent years a number of papers has been published pointing out that large increases in cross-linking in polyvinyl chloride[2,3] and other polymers[4,5] occur when unsaturated compounds are added, and it seems that some form of polymerization or other chain reaction may be involved.

Recent work carried out in this laboratory has shown that the decay of unsaturation in polythene may be represented as a type of chain reaction[6]. A comparison of gel formation in polythene, polymethyl acrylate, polyvinyl acetate and polyvinyl chloride in the presence of triallyl cyanurate suggests that similar mechanisms of decay of added unsaturation are followed in all four polymers[7].

Charlesby[8] has derived equations relating gel formation to dose for polymers in which insoluble networks are formed by a chain reaction. For a polymer, initially of uniform molecular weight, in which the cross-linking index is proportional to the radiation dose:

$$S \propto \exp(-\gamma) \propto \exp(-KR) \qquad (1)$$

where S is the sol fraction, γ the number of units cross-linked per number average molecule, K a constant and R the dose in megarads. He notes that only slight deviations from this relationship will occur for other molecular weight distributions. Fig. 1 shows that equation (1) represents the variation of sol fraction with dose in the case of a polyvinyl chloride resin examined in this laboratory and for results taken from ref. 9.

Fig. 1 shows that the powder sample gels at a considerably lower dose than the film. This difference may result from: (*a*) greater increases in temperature of the powder during irradiation; (*b*) differences in the surface/volume ratio of the polymer; (*c*) decreases in the molecular weight of the polymer during preparation of the films.

The polymer films were obtained by casting from a solution of the polymer in tetrahydrofurane (distilled twice) at 50° C., all operations being carried out under oxygen-free nitrogen. The films were dried to constant weight in vacuum at room temperature. It is unlikely that large changes in molecular weight occurred during this preparation.

Samples of film surrounded by the polymer powder were sealed under vacuum in ampoules and irradiated. Representative results are given in Table 1.

It appears that the differences result from changes in the surface/volume ratio of the polymer and hence that hydrogen chloride inhibits cross-linking in polyvinyl chloride. Wippler[10] has noted that rigid samples of polyvinyl chloride are more soluble in tetrahydrofurane inside the polymer samples than at the surface.

Table 1. IRRADIATION OF POLYVINYL CHLORIDE POWDER AND FILM TOGETHER

Dose (megarads)	Gel fraction of Powder	Film
10	0·160, 0·149	0·000, 0·000
20	0·780, 0·850	0·534, 0·475

Charlesby also points out that the gel point (δ_{gel}) is related to the gelling dose (R_{gel}) by:

$$R_{\text{gel}} = \frac{\delta_{\text{gel}} i}{1{\cdot}04 \times 10^{-6} G.M_w} = \frac{0{\cdot}5 \times 10^{-6} i^2}{G.M_w} \qquad (2)$$

where the gelling dose is expressed in megarads, G, is the value for chain initiation in events per 100 eV., M_w is the weight average molecular weight and i is an 'amplification' factor which is inversely proportional to the number of links arising from each initiation. When i is below 0·1 gelling doses under 1 megarad are obtained even with quite low values for M_w.

If it is assumed, for the sake of argument, that no chain reaction occurs in polyvinyl chloride alone and a chain reaction with additives is initiated by cross-linking radicals, a comparison of gelling doses with and without unsaturated additives will give an estimate of i. Such a comparison gives i, 0·1: this value is not markedly dependent on the nature of the added unsaturation. It has been suggested above, however, that polyvinyl chloride alone does cross-link by a chain reaction, hence the value for i quoted should be an upper limit. The high gelling dose of the polyvinyl chloride resins examined despite the low value for i suggests that the chain mechanism requires the prior radiolytic formation of unsaturation.

A considerable enhancement of gel formation in polyvinyl chloride when samples are heated to 100° C.

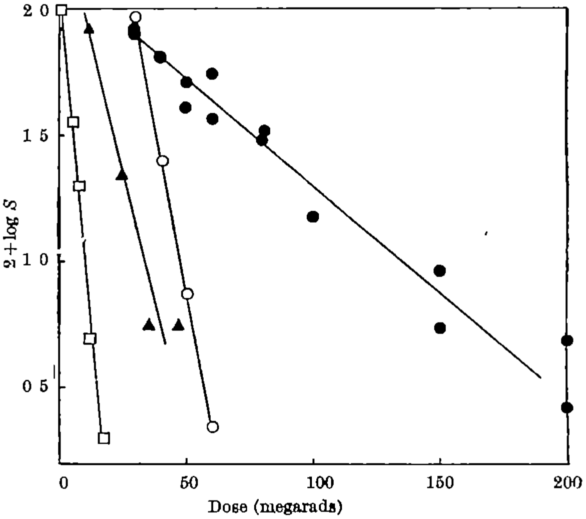

Fig. 1. Variation of log S with dose for polyvinyl chloride polymers. ●, polyvinyl chloride as film, water-cooled vacuum cell, 2 MeV. electrons; ○, polyvinyl chloride as film, in evacuated ampoules 2 MeV. electrons; heated at 50° C. for 30 min. after irradiation; ▲, polyvinyl chloride as powder in evacuated ampoules, no cooling, 2 MeV. electrons, □, polyvinyl chloride powder in vacuum, cobalt-60 gammas, heated at 100° C. for 10 min. after irradiation (ref. 9).

in vacuum after irradiation has been noted by Shinohara *et al.*[9]. These workers estimated the glass transition temperature to be 80° C. and concluded that the increases resulted from a dependence of radical recombination on Brownian motion of the polymer segments. This conclusion is supported by the somewhat smaller increases in gelation which result when polyvinyl chloride is slightly swollen in degassed solvents after irradiation. We have observed significant increases in gel formation accompanied by increased evolution of hydrochloric acid when polyvinyl chloride is heated in vacuum to 50° C. after irradiation. This suggests that the enhancement may be partly due to an increase in unsaturation resulting from the thermal treatment which leads to an increased participation of the 'chain' cross-linking process.

We thank Dr. R. M. Black for his advice and Dr. L. G. Brazier, director of research, British Insulated Callender's Cables, Ltd., for permission to publish this article.

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EFFECT OF IMPROVED CARE ON THE PREDICTED ADULT HEIGHT OF UNDERNOURISHED CHILDREN

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LONGITUDINAL investigations in man and experimental animals have indicated that illness, undernutrition and other forms of environmental adversity can stunt adult stature[1]. It has been suggested that this is because growth in stature is relatively slowed more during the period of adversity than is skeletal maturation[2-4]. This article is concerned with the potentiality for, and mechanism of, recovery in a group of German children who were observed during a period of improved care after the deprivations of war[5-7]. The physical development of this experimental group is compared with that of a random sample of children of underground coal-mine workers and of men in professional occupations (drawn from the Medical Research Council's private census[8,9]), who were living a normal life in their own homes in the Rhondda Fach, and who were also examined over a year. Information about the two groups of children is given in Table 1.

Table 2 shows the physical development, by sex, of the two groups of children. Growth represents the increase during the period of observation of standing height in inches, and skeletal maturation is the increase in skeletal age assessed from X-rays of the hand and wrist against the standards of Greulich and Pyle[10]. (Every film was read independently by two of us (R. M. A. and G. B. F.) without knowledge of chronological age, and the two skeletal age values were averaged.) It can be seen that the experimental group grew and matured more rapidly than the comparison group.

Bayley and Pinneau[11] have published tables from which adult height can be predicted on the basis of skeletal age and present height for children aged about seven years and over. These tables assume that the rate of growth relative to skeletal maturation will remain constant (as calculated from a healthy population of American children) from the time of prediction until growth ceases. Therefore, if any environmental factor interferes with the rates of growth and skeletal maturation relative to each other, the predicted height estimated before the interference of the factor will be different from that estimated afterwards. Thus, slowing of growth more than maturation will reduce the predicted height and vice versa. Table 3 shows the changes in predicted adult height in the two populations at the beginning and end of the year; and it can be seen that the predicted adult height of the German boys increased by 0·63 in. during the experimental year ($P < 0.05$), but that none of the other groups of children differed significantly from the expected value (on the basis of the null hypothesis) of zero.

These findings suggest that children of both sexes are capable of growing and maturing at an accelerated rate when their care is improved after suffering from undernutrition. It also suggests that boys, but not girls, increase their predicted adult height during such a period, presumably because they not only grow more rapidly than is usual relative to chronological age, but they also grow more rapidly relative to their skeletal maturation. This is interesting both because previous work[1-3] has shown that boys are more readily retarded than girls in growth and maturation by environmental vicissitude, and because the present evidence bears out an earlier suggestion[3]

Table 1. Age, Skeletal Age, and Predicted Adult Height of 42 Welsh and 42 German Children at the Beginning of the Year

	Chronological age (yr.)						Predicted adult height (in.)				Skeletal age (yr.)			
	Boys			Girls			Boys		Girls		Boys		Girls	
	Mean	*n*	*S.D.*	Mean	*n*	*S.D.*	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*
Welsh	12·16	21	2·39	11·35	21	3·38	68·54	2·41	63·58	2·17	11·40	2·64	10·88	3·54
German	11·53	23	1·96	10·45	19	2·23	68·29	2·41	63·76	2·43	9·85	2·07	8·56	1·86

Table 2. Growth and Maturation of the Children during the Year

	Growth (in.)				Skeletal maturation (months)				Mean interval between assessments (months)
	Boys		Girls		Boys		Girls		
	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*	
Welsh	2·12	0·89	1·53	1·08	9·71	6·14	9·88	5·43	11·02
German	2·91	0·59	2·81	0·87	15·78	7·25	15·18	6·53	11·38

Table 3. CHANGE IN PREDICTED ADULT HEIGHT DURING THE YEAR

Difference between 2 assessments of predicted adult height (in.)

	Boys			Girls		
	Mean	*S.E.*	*P*	Mean	*S.E.*	*P*
Welsh	+0 19	0·34	>0·5	+0 31	0 32	>0 3
German	+0 03	0 29	>0 02 <0 05	+0 10	0 32	>0 7

that environmental interference with physical development is more likely to have a permanent effect on the adult physique of the female than on that of the male.

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IODINE-131 AND OTHER FISSION PRODUCTS IN DRINKING-WATERS

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THE appearance of significant amounts of iodine-131 in air, rain-water and milk as a result of the 1961 series of nuclear weapon tests prompted interest in the amount of this nuclide which might be ingested by man from drinking-water. We have measured γ-emitting fission product concentrations in a number of waters, concentrating on iodine-131 owing to its biological importance.

Samples were selected from various parts of England and Wales in two ways. In the first instance, we concentrated on the large cities of Birmingham, Manchester and Liverpool, which draw most of their water from open reservoirs in high-rainfall districts. Secondly, we have considered regions where the transit time from rain-water to consumer is short, such as the Lake District, Southampton and districts supplied from the River Severn. A rough guide to the areas which might show high levels was obtained from the report on *Radiostrontium and Radiocæsium in Drinking Waters*, by Crooks *et al.*[1]. The samples were obtained through the co-operation of the Ministry of Housing, local water engineers, the United Kingdom Atomic Energy Authority, and, in some cases, were drawn directly by us from domestic supplies.

In the first investigations in November 1961, when a guide to the levels of iodine-131 was required quickly, about 1 litre of each sample was assayed using a low-background γ-spectrometer incorporating a 3-in. × 3-in. sodium iodide crystal with a photopeak efficiency for iodine-131 of 3·8 per cent. A range of samples from widely separated points in England and Wales which are listed in Table 1 were assayed during November 1961. In no case was there a counting rate significantly above background in the channels corresponding to the 0·364 MeV. γ-ray of iodine-131. We calculate that under these conditions the limit of detection is 15 $\mu\mu$c./litre.

To increase the sensitivity, an ion exchange method was used to remove the iodine from the water after it had been measured on the 3-in. crystal spectrometer. The resin containing the iodine-131 was then assayed, with a photopeak efficiency of 66 per cent, by counting in a 5-in. diam. × 6-in. high well-type sodium iodide crystal detector coupled to a 100-channel pulse-height analyser.

For the ion exchange separation, carrier iodide (0·5 ml. 0·15 per cent sodium iodide in 0·5 per cent sodium thiosulphate per litre of water) was added together with about 0·1 gm./litre of hydroxy ammonium chloride. After standing for 15–30 min. the water was allowed to flow through a column, 1 cm. diam. × 25–30 cm. high, of the anion exchange resin 'Deacidite *FF*' (Permutit Co., Ltd.), mesh size 15–52, in the chloride form. The flow-rate was adjusted to about 20 ml./min. When all the water had passed through the column the resin was expelled into a suitable sample tube for assay of the iodine-131. Experiments in which water samples 'spiked' with

Table 1. SAMPLES WITH IODINE-131 CONCENTRATION LESS THAN 15 $\mu\mu$c./litre

	Sampling site	Origin	Date of sampling
1.	Birmingham, at water works, prior to filtration	—	31.10.61
2.	,, domestic supply	—	31.10.61
3*.	Liverpool, Prescott Reservoir inlet	Rivington (Pennines)	31.10.61
4.	,, ,, ,,	Lake Vyrnwy (Central Wales)	31.10.61
5*.	,, ,, ,,	,, ,,	6.11.61
6.	Central London, domestic supply	—	1.11.61
7.	Sutton, Surrey, ,, ,,	—	1 11.61
8.	Manchester, Godley Reservoir inlet	Longdendale (Pennines)	31.10.61
9.	,, Denton Reservoir inlet	Haweswater (Lake District)	1.11.61
10	Southampton, domestic supply	—	5.11.61
11*.	Shrewsbury	—	6.11.61
12*.	Lake District, Linbeck Gill	—	6.11.61
13*.	,, ,, Whillan Beck, Eskdale	—	6 11.61
14*.	,, ,, Worm Gill, Blengdale	—	6 11.61

* Less than 5 $\mu\mu$c./litre for these samples.

1,000 μμc. iodine-131, as sodium iodide, were processed, showed that the resin removed more than 95 per cent of the iodine-131. This method of measurement on about 1·5 litres of six of the samples was insufficiently sensitive to reduce the limit of detection for iodine-131 to less than 5 μμc./litre.

The sensitivity was then further increased by processing 20 litres of each of an additional series of samples by the same method but using a double-sized column. The counting time was increased from 2 to 16 hr. As a check on the method the iodine-131 concentration in a 4·65-litre volume of one of the samples was estimated radiochemically. After addition of carrier iodide and concentration to small volume, the iodide was oxidized with sodium nitrite and nitric acid, and the free iodine extracted into carbon tetrachloride followed by back-extraction into water saturated with sulphur dioxide. The final aqueous extract was assayed for iodine-131 in the 5-in. well counter and the final yield of iodine determined volumetrically after oxidation to iodate. The concentration of iodine-131 in this water sample was found to be 1·8 ± 0·2 μμc./litre by the ion exchange method and 1·8 ± 0·4 μμc./litre by the radiochemical method.

It was observed that, in the ion exchange procedure, ruthenium-103 and the zirconium-95 plus niobium-95 pair were also adsorbed by the resin. Thus values for the concentration of these nuclides were also obtained, using the 0·498 MeV. photopeak of ruthenium-103 and the composite photopeak at about 0·75 MeV. due to the zirconium and niobium. The efficiency of the column in removing these nuclides was estimated by carrying out a similar extraction on rain-water which, on account of its higher specific activity, could be measured both before and after the ion exchange separation. Rain-water was used on the assumption that the elements would be in the same chemical form as in the drinking-waters. The extraction efficiency for ruthenium was found to be 86 per cent and for zirconium and niobium together, 81 per cent.

The results of the measurements made on the 20-litre samples are shown in Table 2 and the activities have been corrected for decay to the time of sampling. When these low-activity samples were measured for 16 hr., the counting error was equivalent to 0·5 μμc. in the case of both iodine-131 and ruthenium-103 and 0·7 μμc. for zirconium + niobium-95. For a 20-litre sample, this corresponds to 0·025 and 0·035 μμc./litre respectively, but the presence of more than one nuclide increases this error. The highest iodine-131 activity measured, 1·8 μμc./litre, was in a sample of water drawn directly from the River Thames at Sutton Courtenay, Berkshire, at a point upstream from the site of discharge of effluent from the Atomic Energy Research Establishment at Harwell. The highest ruthenium-103 and zirconium and niobium-95 values also occurred in river water from the same site. It should be noted that by January 1962 the iodine-131 levels had fallen considerably.

Table 2

Sample		Date sampled	Vol. (l.)	Concentration μμc./l.		
				^{131}I	^{103}Ru	^{95}Zr + ^{95}Nb
Sutton Courtenay, Berks	I.	13.11.61	20	1·8	4·2	3·0
	II.	4.1.62	20	< 0·2	5·9	9·5
Central London	I.	17.11.61	20	0·4	1·8	1·1
	II.	11.12.61	20	< 0·1	2·6	1·6
Sutton, Surrey	I.	19.11.61	20	< 0·3	< 0·05	1·0
	II.	8.2.62	20	< 0·05	< 0·05	~ 0·07
St. Ives, Cornwall		15.1.62	10	< 0·1	~ 0·8	0·5
Southampton		11.2.62	20	< 0·05	~ 0·07	~ 0·3
Bookham, Surrey		11.2.62	20	< 0·05	< 0·05	~ 0·1

The concentration of iodine-131 in all the waters examined is very much less than the maximum concentration of 130 μμc./litre allowed by the Medical Research Council[2] for continuous ingestion in milk by large populations. The concentration in all waters measured is also much less than 82 μμc./litre, which was the mean concentration of iodine-131, measured by us, in milk purchased in the London area throughout the month of November 1961. The concentrations of ruthenium-103 and zirconium plus niobium-95 are an even smaller proportion of the maximum permissible levels (not less than 10^4 μμc./litre (ref. 3)) for these nuclides in drinking-water.

It can be concluded, therefore, that the amounts of iodine-131, and the other nuclides quoted, likely to be ingested in drinking-water from the sources we have examined constitute a negligible hazard to the population.

We wish to acknowledge the interest shown by Prof. W. V. Mayneord and Mr. W. Binks in this investigation.

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NATURE OF THE PRECURSORS OF MOLECULAR PRODUCTS IN THE RADIATION CHEMISTRY OF WATER

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THE nature of the precursors leading to the formation of molecular hydrogen has been much debated since it was found about ten years ago that molecular products are formed in the radiation chemistry of water[1]. A few years ago it was concluded that molecular hydrogen could not be produced from the interaction of excited water molecules ($H_2O^* + H_2O^* \rightarrow H_2 + H_2O_2$), and must result from like species recombination[1], $H + H \rightarrow H_2$, since some solutes (NO^-_3, H_2O_2, Cu^{2+}, Ce^{4+}, acrylamide) could scavenge up to more than 95 per cent of molecular hydrogen. More recently it was suggested[2] that molecular hydrogen is formed from the recombination of two hydrated electrons (the alkaline form of hydrogen atoms) in neutral water, $H_2O^- + H_2O^- \rightarrow H_2 + 2OH^-$. It was thus possible to account[3] for the decrease in the yield of molecular hydrogen with increase in the hydrogen ion concentration, on the basis[3,4] of competition of H^+ for the alkaline form of hydrogen atoms, $H^+ + H_2O^- \rightarrow H + H_2O$. This reaction results in an increase in the dimensions of the 'spurs' in acid solution, compared with neutral solution, and thus reduces the probability of atom recombination. The purpose of this article is primarily to provide further evidence that the primary reducing species in the radiation chemistry of water is the hydrated electron, H_2O^-, and demonstrate that in the radiolysis of water molecular hydrogen is formed by two mechanisms. The main portion results from recombination of the alkaline form of hydrogen atoms (H_2O^-), as suggested above, and about 25 per cent of the total molecular hydrogen via recombination of reducing radicals which are generated initially in the acid form (hydrogen atoms) in the 'spurs' of the radiation.

Hydrogen peroxide and nitrate ions are known to be good scavengers for the precursors of molecular hydrogen[1]. These two solutes have also been shown to react more effectively with the alkaline form of hydrogen atoms (H_2O^-) than with the acid form of hydrogen atoms, when these reducing species are generated both in the radiation chemistry[5] and photochemistry[6] of aqueous solutions. One would therefore expect a change in the rate of scavenging of H_2 by these solutes should the yield of molecular hydrogen be formed from the two different precursors.

Sowden[7] and Mahlman[8] found, using nitrate ions as scavenger for H_2, that a plot of $G(H_2)$ versus cube root of $[NO_3^-]$ showed two linear lines with two different slopes when irradiation was carried out with mixture of fast neutron and γ-rays[7] or cobalt-60 γ-rays[8]. Using hydrogen peroxide as a scavenger for H_2, and plotting the recent data of Anderson and Hart[9] for cobalt-60 rays, $G(H_2)$ versus $[H_2O_2]^{1/3}$, one obtains again two linear lines with two different slopes. It can be seen in Fig. 1 that a major portion (E_1) of molecular hydrogen can be reduced readily by the addition of scavengers, while the remaining portion (E_2) is less susceptible to scavenger concentration. Extrapolation of E_2 to infinite dilution gives $G(H_2)$ 0·1 and 0·12 for nitrate and hydrogen peroxide solutions respectively. The E_2 portion of the molecular hydrogen is higher for H_2O_2 compared with NO_3^- solutions as it includes a $G(H_2) = 0{\cdot}022$ from irradiation of pure (98 per cent) hydrogen peroxide[9]. It seems therefore that the E_2 portion of the molecular hydrogen is a finite quantity for given radiation and that its precursors must be different from those leading to the E_1 portion.

From Mahlman's and Anderson and Hart's results one can readily calculate that the rate of scavenging (the slopes) of the two portions $k(E_1)/k(E_2)$ is $\simeq 10{\cdot}0$ and 7·0 for nitrate and hydrogen peroxide solutes respectively. For nitrate solutions irradiated with mixed neutron and γ-rays[7] $G(H_2)$ at infinite dilution and the E_2 portion are 0·84 and 0·25 respectively, giving $k(E_1)/k(E_2) = 9 \pm 2$. For hydrogen peroxide solutions irradiated with 18·9 MeV. deuterons, E_2 at infinite dilution is $0{\cdot}15 \pm 0{\cdot}02$ (including $G(H_2) = 0{\cdot}025$ from pure hydrogen peroxide), giving $k(E_1)/k(E_2) = 7 \pm 2$. Considering the precursor of the E_1 portion of the molecular hydrogen to be the hydrated electron H_2O^-, and that of the E_2 portion the hydrogen atom, then the ratio of $k(E_1)/k(E_2)$ for nitrate to hydrogen peroxide, that is:

$$\frac{k(H_2O^- + NO_3^-)}{k(H + NO_3^-)} \times \frac{k(H + H_2O_2)}{k(H_2O^- + H_2O_2)} = \frac{10}{7} = 1{\cdot}4$$

Since:

$$\frac{k(H_2O^- + NO_3^-)}{k(H_2O^- + H_2O_2)} = 1{\cdot}0 \text{ (ref. 10)},$$

it follows that: $$\frac{k(H + H_2O_2)}{k(H + NO_3^-)} = 1{\cdot}4.$$

Recently it was shown on introducing hydrogen atoms generated by an electrodeless frequency discharge of hydrogen gas into neutral aqueous solutions of hydrogen peroxide[11] and of nitrate[12] that:

$$\frac{k(H + H_2O_2)}{k(H + NO_3^-)} \simeq 5{\cdot}0$$

which is in fair agreement with the value deduced above from radiation chemistry, considering the different systems and techniques involved and the reproducibility that can be obtained in discharge work. This agreement indicates that in fact the E_1 portion must be a result of recombination of the alkaline form of hydrogen atoms (H_2O^-), and the E_2 portion recombination of the acid form of hydrogen atoms. It provides also independent evidence that the reacting primary reducing species in the radiation chemistry of water is the hydrated electron H_2O^-. This evidence is particularly significant in that it is derived solely from data obtained in neutral solution.

The case that the E_2 portion may be a result of interaction of two excited water molecules ($2H_2O^* \rightarrow H_2 + H_2O_2$ (or 2OH)) and that its reduction with solute concentration is due to energy transfer ($H_2O^* + NO_3^- \rightarrow H_2O + NO_3^{-*}$) is rather weak. Such an event would mean that energy is transferred from water to the solute in a fashion which is directly proportional to the concentration of acceptor up to 20*M*. Further, the yield of the E_2 portion of molecular hydrogen is exactly the same in liquid and frozen solution[8], and its decrease with solute concentration would necessitate that energy be transferred at a rate identical in frozen and liquid solutions. These two premises are not in keeping with present understanding of energy transfer in liquid and rigid media.

On γ-irradiation of nitrate[8] in neutral water and deuterium oxide solutions, $G(H_2)$ and $G(D_2)$ for the E_1 portion are 0·36 and 0·33 respectively; the corresponding values for the E_2 portion are 0·1 and 0·04. As was pointed out[13a] the relaxation time and viscosity are both about 30 per cent larger in deuterium oxide than in water, and this could lead to a more extended initial distribution of the reducing species in the spurs in deuterium oxide compared with water. It follows from this suggestion that in neutral solutions the distribution of the hydrated electron would be much wider in deuterium oxide than in water, thus reducing like and unlike species recombination. In acid solu-

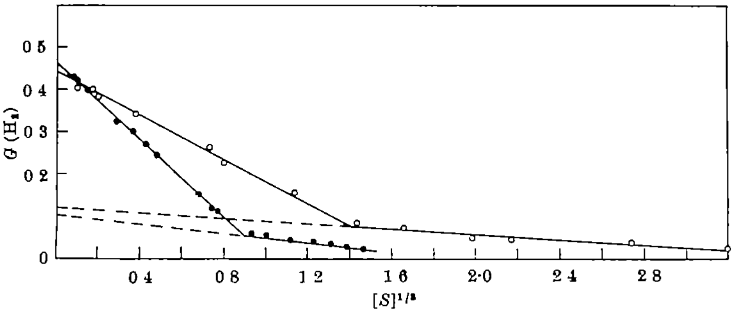

Fig. 1 Cobalt-60 γ-irradiation of neutral air-free solutions of nitrate. (●, data of Mahlman (ref. 8), plot $G(H_2)$ versus ($NaNO_3$ activity)$^{1/3}$) and of hydrogen peroxide (○, data of Anderson and Hart (ref. 9), plot $G(H_2)$ versus $[H_2O_2]^{1/3}$)

tion, the electron would also diffuse away from the core of the spur, but on reaction with acid to form hydrogen (or deuterium) atoms the rate of diffusion of the latter will be significantly less in deuterium oxide than in water. This is supported experimentally: (for γ-rays) GH is 2·9 and 3·7, and G-H_2O 3·82 and 4·44 in neutral[13b,8] water and deuterium oxide respectively; in 0·8 N H_2SO_4[13c] GH is 3·7 and 4·0 and G-H_2O 4·5 and 4·75 in water and deuterium oxide. Similarly, if the hydrogen atom is formed initially in the spurs its diffusion and probability of recombination will also be reduced in deuterium oxide compared with water, as the results[8] for the molecular hydrogen yields of the E_2 portion indicate.

The E_1 portion of the molecular hydrogen can be eliminated on irradiation of neutral aqueous solutions of nitrate[8] and hydrogen peroxide[14a] at 77° K. The yield of nitrite has also been shown[14b] to increase with increase in [NO_3^-] at 128° K. These observations suggest that some activation energy may be required for the recombination of two H_2O^-, and as a result of the greater mobility of electrons compared with hydrogen atoms they can diffuse to nitrate ions and yield nitrite[14b]. Therefore molecular hydrogen is formed via E_2 only in neutral solutions at 77° K.

In acidified aqueous solutions γ-irradiated at 77° K.[15] (as a glass) the yield of molecular hydrogen is considerably greater than in neutral solution at 77° K., indicating that the hydrated electrons are stabilized by hydrogen ions to form hydrogen atoms, most of which recombine to give hydrogen on warming. EPR measurements support the above in that unpaired-electron species related to hydrogen atoms can be detected in aqueous solutions irradiated at 77° K. in acid medium[15] but not in neutral[15,16]. On γ-irradiation of ice at 4·2° K.[16], however, the paramagnetic resonance absorption spectrum indicates the trapping of hydrogen atoms (which have not been distinguished from H_2O^- by EPR). At this very low temperature the diffusion of electrons is very much reduced[17], and hence they are trapped and measured by EPR. Livingston and Weinberger[15] also found that addition of hydrogen peroxide or nitrate to acidified aqueous solutions irradiated at 77° K. greatly suppresses the yield of atomic hydrogen (and of molecular hydrogen) as measured by EPR, though on irradiation at room temperature under otherwise similar [H^+]/[S] ratios these solutes are relatively unreactive to reducing species in acid solutions[5]. On the model presented here this means that at 77° K. the electron diffuses to reactive sites. On encountering a hydrogen ion it reacts to form a hydrogen atom (which is detected by EPR), or the electron on encountering hydrogen peroxide or NO_3^- will react with them preferentially before it has been converted to a hydrogen atom in acid solution since results (ref. 15 compare Figs. 2, 11 and 12) show that H^+ is a less effective trap for the electrons at 77° K., compared with room temperature. Furthermore, Figs. 11 and 12 (ref. 15) show that about $\leqslant$ 20 per cent of the hydrogen, that is $G(H_2) \leqslant 0·12$, is scavenged by hydrogen peroxide and nitrate at a much lower rate than the remaining hydrogen.

In the E_2 portion of the molecular hydrogen, the hydrogen atoms are generated initially in the acid form, probably from the dissociation of excited water molecules, $H_2O^* \rightarrow H + OH$. The formation of initially generated hydrogen atoms and its reaction with solutes in the bulk of the solution have recently been discussed[18,4] (Hayon, *J. Amer. Chem. Soc.*).

Table 1

	Neutral pH		0·8 N H_2SO_4		
	G-value*	Per cent of G-H_2O	G-value†	Per cent of G-H_2O	$\frac{G \text{ acid}}{G \text{ neutral}}$
G H_2	0·45	11·7	0·40‡	8·9	0·89
G H_2O_2	0·71	18·7	0·80	17·8	1·13
G H	2·90	38·1	3·65	40·5	1·26
G OH	2·40	31·5	2·95	32·8	1·23
G–H_2O	3·80	100	4·50	100	1·18

* Allen, A. O., and Schwarz, H. A., *Second Intern. Conf. Geneva*, 29, 30 (1958).

† Barr, N. F., and Schuler, R. H., *J. Phys. Chem.*, 63, 808 (1959). Sworski, T. J., *J. Amer. Chem. Soc.*, 76, 4687 (1954).

‡ Hayon, E., *J. Phys. Chem.*, 65, 1502 (1961).

The situation with regard to the nature of the precursors of molecular hydrogen peroxide is much less conclusive, owing to the scarcity of relevant data. The position corresponding to the reducing species would be that the basic form of the oxidizing species (H_2O^+) be more prevalent in acid solutions, while in neutral-alkaline solution it would be present to a larger extent as OH radicals[2]. Available experimental results are not in contradiction with the above, when these oxidizing species are formed in acid or neutral solutions in photo-chemistry as well as radiation chemistry[19]. The rate of scavenging the precursors of molecular hydrogen peroxide in dilute solutions of bromide is greater in acid solution by a factor of 1·7 compared with neutral solution[10]. This probably means that the rate of recombination of the precursors of molecular hydrogen peroxide is smaller in acid solution compared with neutral and that they are intercepted more readily by Br^- ions. On the other hand, the percentage of the oxidizing species (on the basis of net decomposition of water) that recombine to form hydrogen peroxide is very slightly larger in neutral solution compared with acid (Table 1, columns 3 and 5). This could be ascribed to the higher rate of recombination of OH radicals in neutral solutions.

Some indication can also be provided from a comparison of the hydroxylation in the o-, m- and p-position of aqueous solutions of benzoic acid, nitrobenzene, and chlorobenzene when differences in the mesomeric and inductive effects on substitution can be considered. The relative yields of the isomeric hydroxylation products follows a similar trend when these products are formed by Fenton's reagent, photolysis of hydrogen peroxide, or X- and γ-irradiation of aqueous solutions in neutral-alkaline pH (in all cases the OH radical is presumably formed), whereas the relative yields on irradiation of acidified solutions are somewhat different[20] (when the H_2O^+ oxidizing species is possibly prevalent).

Recent results[21] of the scavenging of molecular hydrogen peroxide on irradiation of oxygen-18-water in acid and alkaline solution in the presence of a number of solutes suggest that hydrogen peroxide is probably also formed from two different precursors which are scavenged by the solute at different rates. The departure of the experimental points of the yields of molecular hydrogen and hydrogen peroxide at relatively high solute concentration from the calculated curve in the Schwarz plot[22] supports also the foregoing conclusion that the molecular products are formed from the recombination of two different precursors.

From the interpretation of the results presented here it follows that the rates of the three reactions $H + H \rightarrow H_2$, $OH + OH \rightarrow H_2O_2$, $H + OH \rightarrow H_2O$ must be different from one another, and depend on pH and the kind of precursor involved. Thus the rate constant for recombination of unlike species to form water would be expected[23] to be higher in neutral than in acid solutions.

Finally, from the evidence showing that the primary reducing specie in the radiation chemistry of water is the hydrated electron H_2O^-, and since it has been shown[6] that the reducing specie formed in the photochemistry of neutral aqueous solutions of iodide ions is identical with that formed in the radiation chemistry of water, one can for the first time demonstrate in the photochemistry of aqueous solutions of ions[24] the absorption spectra of which are 'electron affinity spectra' that the reacting primary reducing species are the alkaline form of the hydrogen atom, H_2O^-. This hydrated electron seems to be identical with that formed in the radiation chemistry of water, and can be converted to a hydrogen atom on reaction with a hydrogen ion in acid solutions[25].

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PRESYNAPTIC INHIBITORY ACTIONS

Presynaptic Inhibitory Action of Cerebral Cortex on the Spinal Cord

It has long been known that activation of the cerebral cortex, particularly of the sensori-motor area, results in powerful excitatory and inhibitory actions on interneurones in the spinal cord[1,2]; but it remained for Hagbarth and Kerr[3] to demonstrate that stimulation of the somato-sensory area of the cortex and various other regions of the brain inhibited the synaptic actions that evoked discharges up several ascending tracts of the spinal cord. This whole field has recently been reviewed by Hagbarth[4]. Since then there have been many investigations[5-9] showing that, particularly in the decerebrate cat, impulses descending in the lateral columns of the cord exert powerful tonic inhibitory influences on most interneuronal pathways.

It was suggested[10] that the descending inhibitory influences reported above[3,4] might be due to a presynaptic inhibitory action on the primary afferent fibres. The present investigation has established the correctness of this suggestion in respect of the effects of cortical stimulation, and it also leads to the further suggestion that the descending tonic inhibitory influences might largely be effected by presynaptic inhibition.

The experimental procedures on cats anaesthetized with 'Nembutal' resembled previous investigations on presynaptic inhibition in the spinal cord[11,12] with in addition the exposure of the cerebral cortex, so that it could be stimulated by brief trains of repetitive pulses (usually 6 at 300/sec.) applied through bipolar electrodes with 2 mm. interpolar distance. As shown in Fig. 1*A*, stimulation of the contralateral cortex evoked a potential change in an isolated dorsal rootlet which closely resembled the dorsal root potential produced in that same rootlet by repetitive group I volleys from the nerve to a flexor muscle (*B*) and by a single volley in an ipsilateral cutaneous nerve at that segmental level (*C*). Stimulation of the arm area of the somato-sensory cortex produced a dorsal root potential of the dorsal rootlets supplying the contralateral arm, but not the leg (*D*); and similarly with the cortical leg area and the dorsal rootlets for the contralateral leg and arm (Fig. 1*E*). The diameters of the filled circles on the cortical map (Fig. 1*F*) give the effectiveness of the underlying areas in evoking dorsal root potentials in a dorsal rootlet at L_7 segmental level, and show the restriction to somato-sensory leg areas I and II. The same areas of the ipsilateral cortex were also effective in producing a dorsal root potential, but to a lesser degree. This somatotopic organization corresponds well with that reported for inhibitory influences on the ascending pathways[3,4]. No other active cortical foci have been recognized; but as yet only the convexity of the cerebrum has been explored.

Since dorsal root potentials arise due to the electrotonic transmission of a synaptic depolarizing action on the central terminals of the primary afferent fibres[11,12], it may be predicted that there would be a corresponding increase in excitability of these terminals. Using the method of testing developed by Wall[13], it was possible to identify the types of primary afferent fibre that were depolarized by various afferent inputs into the spinal cord[11]. Similarly, it has been shown (Fig. 1*G*) that dorsal root potentials elicited by cortical stimulation are produced by a depolarizing action on the cutaneous

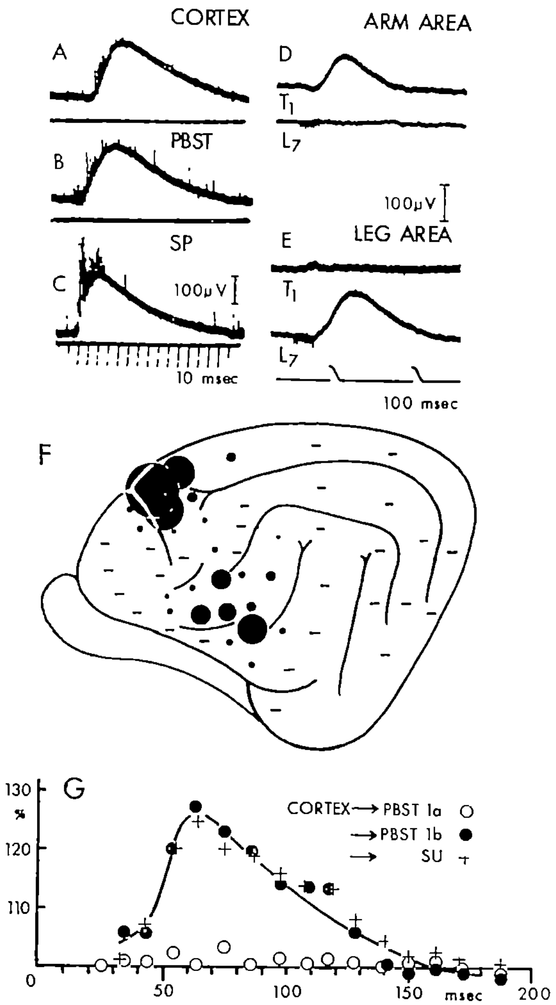

Fig 1. *A–C* show dorsal root potentials led from a L_7 dorsal rootlet which was isolated except for its cord entry and recorded by two electrodes, one about 1 mm. from the cord, the other on the distal cut end. There was brief repetitive stimulation of the contralateral cortex, *A*, and of posterior biceps semitendinosus nerve, *B* (PBST), as described in text; in *C* a single volley in the superficial peroneal nerve. *D* gives dorsal root potentials produced by repetitive stimulation of contralateral cortical arm area with similar recording from dorsal rootlets of first thoracic (T_1) and L_7 segments, while in *E* the contralateral cortical leg area was stimulated. In *F* the sizes of the dorsal root potentials produced in a L_7 dorsal root of the contralateral side are shown by sizes of filled circles for stimulating sites over the convex surface of the cortex, zero actions being shown by – signs. *G* shows that the time courses of excitability changes produced by cortical stimulation in two types of fibre (●, + points) resemble the dorsal root potential in *A*. There was no action on the third fibre type (○ points). Percentages are measured as described previously (ref. 11); time is measured from the first stimulus of the conditioning tetanus

afferent fibres (+) and on the *Ib* afferent fibres from muscle (●), but not on the *Ia* muscle afferents of either flexor or extensor muscles (○). The observed increases in excitability (range 120–135 per cent) have been almost as large as those induced by afferent volleys in large limb nerves, which corresponds to the relative magnitudes observed for the dorsal root potentials (Fig. 1*A–C*). There is convincing experimental evidence that presynaptic depolarization has a depressant action on the liberation of the synaptic transmitter, giving as a consequence the so-called 'presynaptic inhibition' of synaptic transmission[11,14–16].

It may therefore be concluded that, as a consequence of the induced dorsal root potential, cortical stimulation also achieves an effective presynaptic inhibitory action on the spinal cord, which would explain the observations already cited[3,4]. Presumably this cortical inhibitory influence acts through the same spinal interneuronal pathways[1,2,17] which are responsible for the presynaptic inhibitory action which volleys from limb nerves exert on *Ib* and cutaneous afferent fibres[11,12,15].

It has already been shown that presynaptic inhibition of *Ia* synaptic action is effected through a distinctive pathway[11,13,14], which apparently is not influenced from the cortex. It will be appreciated that this independence of the *Ia* pathway from presynaptic inhibitory action descending from the cortex parallels the independence of the *Ia* pathway from the tonic inhibitory influences that, descending from higher centres, tonically depress transmission through the central pathways of cutaneous and *Ib* afferent impulses[5–9]. The question therefore arises whether some of these tonic inhibitory influences are exerted also by the mechanism of presynaptic inhibition, and not only by postsynaptic inhibition as hitherto has been supposed.

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Presynaptic Inhibition in the Cuneate Nucleus

IN the pathway from cutaneous nerves to the sensory cortex a conditioning volley is followed by a deep and prolonged (about 150 msec.) depression of the primary cortical response evoked by a testing volley in the same nerve or in another nerve of that limb[1–4]. Much of the depression has been shown to occur in the relay of the pathway in the ventro-basal nucleus of the thalamus, and indeed Amassian[3] found little or no depression of the responses to the testing volley recorded in the medial lemniscus after synaptic relay in the cuneate nucleus. However, Marshall[1] illustrated an indubitable depression which had a time course resembling that observed for the primary cortical potentials, though much less in degree, and inhibition of about 200 msec. in duration has been observed in the discharges of cells in the gracile nucleus[5]. Cutaneous afferent volleys were found by Therman[6] to produce on the surface of the cuneate nucleus a large positive wave which had a time-course resembling the depression of synaptic transmission, but this equivalence was regarded as fortuitous because the positive wave was believed to arise

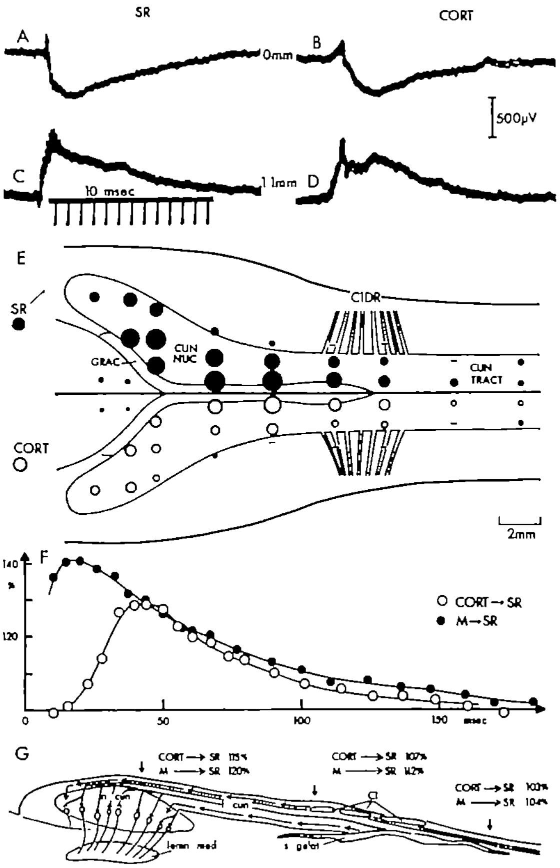

Fig. 1. *A*, *B* are potentials led by a glass microelectrode from the surface of the cuneate nucleus and evoked by a single volley in the superficial radial nerve (*SR*) and by repetitive stimulation of the contralateral sensori-motor cortex (*CORT*), while with *C*, *D* the microelectrode tip was at a depth of 1 1 mm. Potential and time-scales are for *A–D*. In a drawing (*E*) of the dorsum of the cuneate nucleus (*CUN NUC*) and its environment, the sizes of the *P* waves produced by *SR* and *CORT* are shown as described in the text, zero responses being shown by – signs. *CIDR* is first cervical dorsal root. *F* gives time courses of the excitabilities of presynaptic terminals in the cuneate nucleus which were measured as percentages of the control excitability as already described (ref. 8), and plotted against time after the first conditioning stimulus. *G* is drawn from a sagittal section of the cuneate tract and nucleus, *C*1 showing first cervical root. Excitability changes of the *SR* fibres were tested as in *F* at the three sites indicated by the vertical arrows, and the increases are shown at a testing interval of 51 msec. for *CORT* and 45 msec. for *M*

in the underlying reticular substance. The present study of the cuneate nucleus was prompted by the idea that these observations pointed to the existence of a presynaptic inhibitory mechanism in the cuneate nucleus comparable with that in the spinal cord[7–10].

Furthermore, the same interpretation seems likely for the inhibitory action exerted on the cuneate and gracile nuclei from the contralateral sensori-motor cortex[11,12].

As described by Therman[6] an afferent volley in a cutaneous nerve of the fore-limb produced a characteristic series of potential waves on the surface of the cuneate nucleus. The brief initial spike potential of the primary afferent volley was followed by a negative (*N*) wave of several milliseconds duration and a prolonged positive (*P*) wave, which reached a maximum at 20–30 msec. and was about 200 msec. in duration (Fig. 1*A*). Brief repetitive stimulation (6 stimuli at 300/sec.) of the contralateral sensorimotor cortex produced a *P* wave having a similar time course (Fig. 1*B*). When the microelectrode was advanced from the surface to the depth of the cuneate nucleus, the *P* waves evoked by cutaneous volleys and from the cortex both reversed to negative potentials having comparable time courses (Fig. 1*C*,*D*).

When the surface electrode was moved over the surface of the cuneate nucleus and adjacent parts of the medulla and spinal cord, the *P* wave was found to decline very steeply laterally and also rostrally, but it extended with a progressive decrement and for a relatively long distance caudally along the cuneate tract. The same distribution occurred for the *P* waves produced by cutaneous volleys and cortical stimulation, as indicated by the filled and open circles in Fig. 1*E*, in which the sizes of *P* waves were represented by diameters of the circles. This distribution along the presynaptic pathway to the cuneate nucleus suggests that the *P* wave is generated in the presynaptic fibres of the cuneate tract, and that in its genesis the *P* wave in the cuneate nucleus is comparable with the *P* wave of the cord dorsum. This suggestion was tested by investigating by Wall's method[13] the changes in excitability of the primary afferent fibres in the cuneate nucleus and at various more caudal levels down to the upper part of the *C*2 segment.

As with primary afferent fibres in the spinal cord[8,13], conditioning by a cutaneous afferent volley or by repetitive cortical stimulation caused a prolonged increase in the excitability of the terminals of the cuneate tract. For example, in Fig. 1*F* (● points) a volley in the median nerve produced an excitability increase in the primary afferent terminals of the superficial radial nerve in the cuneate nucleus, which had a time course resembling the *P* wave in Fig. 1*A* or its inverted (negative) counterpart in Fig. 1*C*. A comparable change in excitability was found by Wall[13] in the gracile nucleus. Similarly, repetitive cortical stimulation caused the excitability increase in the superficial radial nerve (Fig. 1*F*, ○ points) with a time course resembling that of Figs. 1*B* and *D*. It can be concluded[8,13] that the curves of Fig. 1*F* plot the time-course of depolarization of the primary afferent fibres of the superficial radial nerve. When testing the excitability of the superficial radial fibres at various sites along their length, the greatest change occurred in the neighbourhood of their synaptic terminals in the cuneate nucleus. There was progressively less change with more caudal testing sites (Fig. 1*G*,) whether conditioning was by cutaneous volleys or by cortical stimulation.

As shown by the arrows in Fig. 1*G* a depolarization produced in the terminals of the presynaptic fibres of the cuneate nucleus would result in the extracellular flow of current indicated by the arrows so that this depolarization would spread electrotonically along the presynaptic fibres, and give the decrementing depolarization revealed by the excitability tests. Furthermore, this current would result in the observed field potentials—the negativity within the cuneate nucleus and the surface positivity that progressively decremented along the course of the cuneate tract (Fig. 1*E*). These tests indicate that the primary afferent terminals in the cuneate nucleus are subjected to the same depolarizing mechanism as in the spinal cord[8,9], where it has been shown to be responsible for presynaptic inhibition by depressing the output of transmitter[7,10,14]. The present investigation of the presynaptic inhibitory action on synaptic transmission through the cuneate nucleus has confirmed Marshall[1] and Towe and Jabbur[12] in showing that the postsynaptic spike response exhibits an increase in latency and a decrease in number of impulses that parallels the presynaptic depolarization, the maximum action being at about 20–30 msec. and the total duration of about 200 msec. The *P* wave produced by the testing volley exhibits a comparable depression, which was also observed by Therman[6] and which parallels that found in the spinal cord[10].

The present results further suggest that the cortical inhibition of the cuneate nucleus[12] is effected by presynaptic inhibition. The pathway has been shown to be from the sensori-motor cortex to the cuneate nucleus largely via the pyramidal tract[11,15–17]. On analogy with presynaptic inhibition in the spinal cord[9], it is likely that, in the cuneate nucleus or adjacent thereto, all the pathways giving presynaptic inhibition pass through several interneurones. From interaction experiments it seems that the cuneate tract fibres and the descending fibres from the cortex act through the same system of interneurones. Jabbur and Towe[17] found that some cuneate and gracile neurones were excited to discharge both from the sensori-motor area of the cortex and also from peripheral nerves. These neurones have properties that correspond with the interneurones in the spinal cord that are postulated to be on the presynpatic inhibitory pathway[9]. Alternatively, the interneurones of the presynaptic inhibitory pathway might be in the reticular substance subjacent to the cuneate and gracile nuclei[12,16].

Finally, it is postulated that these interneurones make synapses on the axons of the cuneate tract close to their synaptic terminals, and that activation of these axo-axonic synapses results in the observed depolarization of the cuneate tract fibres. Recently, axo-axonic synapses of the type postulated have been observed by Gray[18] and Szentagothai (personal communication) at other sites in the central nervous system.

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AN ABNORMAL HUMAN FŒTAL HÆMOGLOBIN WITH AN ABNORMAL ALPHA-POLYPEPTIDE CHAIN

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THE hypothesis that the α-chains of human fœtal hæmoglobin are identical with those of adult hæmoglobin[1] implies that cord blood obtained from an infant heterozygous for an α-chain abnormality would exhibit two different fœtal hæmoglobins[2,3]. These postulated fœtal hæmoglobins would result from the union of normal γ^F-chains with normal or abnormal α-chains. An example of such a cord blood constitutes the subject of this communication.

During an investigation of fœtal hæmoglobins in Burlington, Vermont, we encountered the cord blood of C. S., a male infant, in which, at *p*H 8·6, an electrophoretically 'rapid' abnormal component comprised 23 per cent of the hæmoglobin[4]. This component exhibited somewhat greater anodal mobility at *p*H 8·6 than did a sample of known hæmoglobin Bart's (γ_4^F).

Hæmatological investigations, including determinations of hæmoglobin F and A_2, were carried out on blood samples obtained from the mother, both maternal Italian-born grandparents, the father, O. S. (of northern European ancestry), and two siblings of the propositus. The only abnormality detected in these studies was the presence of an electrophoretically 'rapid' component amounting to about 20 per cent of the total pigment in the hæmoglobin of the father and of a two-year-old sister (Fig. 1). At *p*H 8·6 the 'rapid' component of the father's hæmoglobin exhibited slightly greater electrophoretic mobility than the 'rapid' cord component, and approximately the same mobility as hæmoglobin H. However, at *p*H 6·5, the father's abnormal component migrated toward the cathode. In accordance with recent recommendations concerning nomenclature of hæmoglobin[5], the 'rapid' component of O. S. was designated hæmoglobin $\text{I}_{\text{Burlington}}$.

In an attempt to delineate the relationship of the 'rapid' cord hæmoglobin to hæmoglobin $\text{I}_{\text{Burlington}}$, comparative studies were carried out on the hæmoglobin of O. S. (father) and of C. S. (cord blood) and on designated hæmoglobin components as isolated by starch block electrophoresis.

Determinations of alkali resistance were carried out on the total hæmoglobins[6], and on isolated components[7]. The results are shown in Table 1.

Table 1. Per cent Alkali Resistance of Total Hæmoglobins and of Isolated Components

C. S. cord hæmolysate			O. S. father's hæmolysate		
Total	Main F + A	'Rapid'	Total	Main (A)	'Rapid' (Hb.$\text{I}_{\text{Burl.}}$)
70	76	68	2	5	5

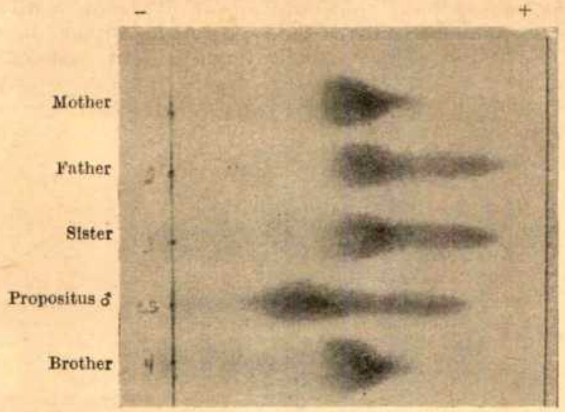

Fig. 1. Filter paper electrophoresis of C. S. family. Electrophoresis between glass plates for 6 hr. at 450 V. Bromphenol blue stain. Barbitol buffer, pH 8·6

In summary, the cord hæmoglobin and its components were about 70 per cent alkali resistant. Neither the total hæmoglobin of the father nor its A or I component exhibited significant alkali resistance.

Recombination experiments were carried out by a modification[8] of the method of Itano and Robinson[9]. Electrophoretically purified hæmoglobin $\text{I}_{\text{Burlington}}$ and hæmoglobin S were mixed, and half of the mixture acidified to pH 4·7. After the recombined and control (unacidified) mixtures had been dialysed against phosphate buffer at pH 6·8, electrophoretic analyses were carried out on cellulose acetate in veronal buffer pH 8·6 and on starch-gels, at 4° C., in borate buffer pH 8·8. In both cellulose acetate and starch-gel analyses, two new hæmoglobin components, both of electrophoretic mobility between S and I, were identified (Fig. 2).

Since new hæmoglobins were found after recombination of hæmoglobin S, which has an abnormality in the β chain, with hæmoglobin $\text{I}_{\text{Burlington}}$, the abnormality in the latter hæmoglobin must reside in the α chain. Thus:

$$\underset{\text{Hb. S}}{\alpha_2^A\,\beta_2^S} + \underset{\text{Hb.}\text{I}_{\text{Burl.}}}{\alpha_2^I\,\beta_2^A} \rightarrow \underset{\text{Hb. S}}{\alpha_2^A\,\beta_2^S} + \underset{\text{Hb. A}}{\alpha_2^A\,\beta_2^A} + \underset{\substack{\text{Doubly}\\ \text{Abn. Hb.}}}{\alpha_2^I\,\beta_2^S} + \underset{\text{Hb.}\text{I}_{\text{Burl.}}}{\alpha_2^I\,\beta_2^A}$$

The doubly abnormal hæmoglobin $\left(\alpha^I\beta_2^S\right)$ migrated between hæmoglobin A and hæmoglobin $\text{I}_{\text{Burlington}}$ in this work. Examples of hæmoglobin I examined by others have been found to yield two new components on recombination with hæmoglobin S[8,9]. In the sample of hæmoglobin I examined by Murayama[10], the abnormality was the substitution of aspartic acid for lysine in residue No. 16 of the α-chain.

Recombination experiments utilizing hæmoglobin S and the 'rapid' cord component were also carried out. While new hæmoglobin species were apparently formed, these components did not appear to be identical with the new components from the recombination of hæmoglobin S and hæmoglobin $\text{I}_{\text{Burlington}}$ (Fig. 2). Other investigators have reported incomplete hybridization in experiments involving hæmoglobin F (ref. 3). Moreover, evidence will be cited (*vide infra*) that the 'rapid' cord component of this work contained α-chains together with both γ- and β-chains. We have therefore not attempted to designate by formulæ the components resulting from recombinations of 'rapid' cord hæmoglobin and hæmoglobin S.

N-terminal amino-acid determinations were carried out on the 'rapid' cord component and on hæmoglobin $\text{I}_{\text{Burlington}}$ by the dinitrophenyl (DNP) method of Sanger. In hæmoglobin $\text{I}_{\text{Burlington}}$, only DNP-valine was found, as might be anticipated for $\alpha_2^I\beta_2^A$ composition. In contrast, both DNP-valine and DNP-glycine were identified in the 'rapid' cord component. The presence of DNP-glycine indicated the presence of γ-chains in the 'rapid' cord component[1].

A Beckman–Spinco amino-acid analyser was used for amino-acid analysis of a 22-hr. hydrolysate of globin prepared from the 'rapid' cord component. The results of this analysis were compared with published analyses of hæmoglobin F (ref. 11) and hæmoglobin A (ref. 12). A significant finding in the amino-acid analysis of the 'rapid' cord component was the presence of 6 isoleucine residues/mole of hæmoglobin. Since each γ^F chain contains 4 isoleucine residues, and isoleucine is absent in both α^A and β^A chains, the finding of 6 isoleucine residues is compatible with the presence of α-chain dimers with γ-dimers or β-dimers in a 3 : 1 ratio. This 3 : 1 ratio of γ- to β-chains would also fit the alkali resistance data (± 70 per cent) and the DNP–glycine data. While our recovery of total amino-acid residues was inexplicably high (106 per cent compared with hæmoglobin F (ref. 11)), the residues/mole of amino-acids other than isoleucine were also compatible with a theoretical 3 : 1 mixture of hæmoglobins F and A.

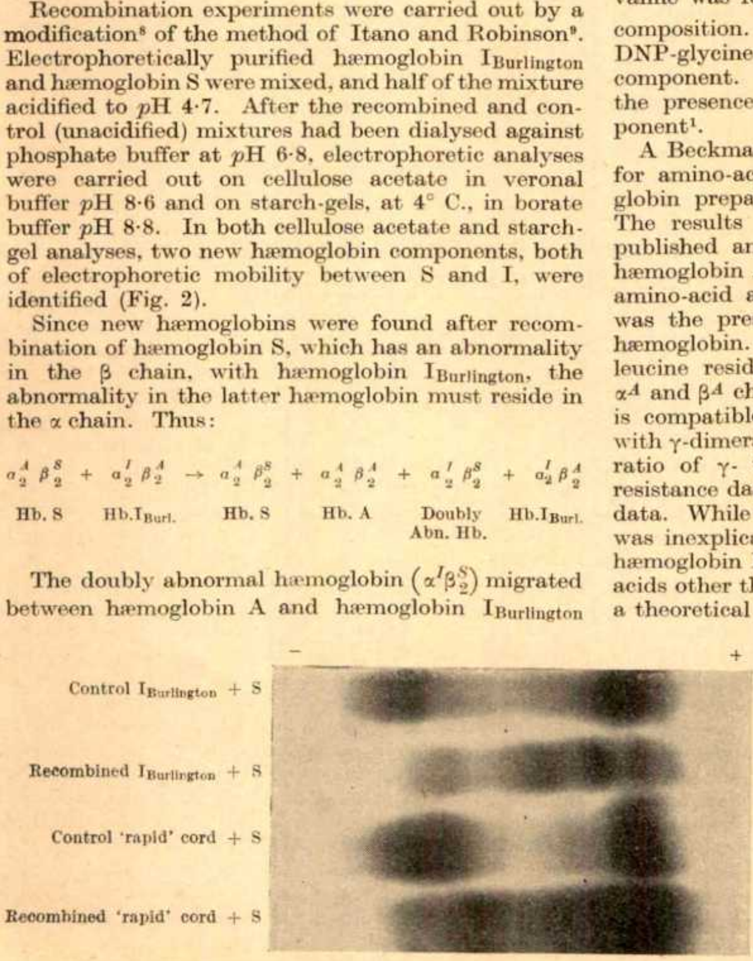

Fig. 2. Vertical starch-gel electrophoresis of hæmoglobin recombination studies in borate buffer pH 8·8 at 4° C. Migration time 16 hr., benzidine stain. In recombined hæmoglobin $\text{I}_{\text{Burlington}}$ + S, components had following mobilities (left to right): S, A, double abnormal, I. In recombined 'rapid' cord + S, the components are not designated by formulæ

Biochemical studies of fœtal hæmoglobin have provided evidence for the identity of the α-chains of hæmoglobins A and F. The differences between fœtal and adult hæmoglobin apparently reside in the remaining polypeptide chain: the β-chains of hæmoglobin A begin to replace the γ-chains of hæmoglobin F before birth. Thus, while β-chain abnormalities are grossly evident only some months after birth, an α-chain abnormality should be manifested by an abnormal fœtal hæmoglobin at birth. In the present study an infant, heterozygous for hæmoglobin I^a, had two normal hæmoglobins: F $(\alpha_2^A\gamma_2^F)$ and A

$(\alpha_2^A\beta_2^A)$, and two abnormal hæmoglobins: fœtal I $(\alpha_2^I\gamma_2^F)$ and adult I $(\alpha_2^I\beta_2^A)$, at birth. Five months later, the hæmoglobin of C. S. still contained the same amount (±20 per cent) of a rapid abnormal component. At this time, however, the electrophoretic mobility of this component was apparently the same as that of hæmoglobin $\text{I}_{\text{Burlington}}$, and the alkali resistance of the total hæmoglobin and of its components had declined from 70 to 12 per cent.

An example of another fœtal hæmoglobin with an α-chain abnormality $(\alpha_2^D\gamma_2^F)$ has been described by Minnich *et al.*[13].

These abnormal fœtal hæmoglobins provide biological evidence for the identity of the α-chains of hæmoglobins F and A. The change from fœtal to adult hæmoglobin is apparently the result of a switch over in synthesis from γ-chains to β-chains, which then combine with the normal or abnormal α-chains.

We thank Prof. Mary Jane Gray, who initiated the fœtal hæmoglobin survey in Burlington, Mr. Carl Franzblau for supervision of the amino-acid analyses, and Prof. V. Minnich for allowing us to read her unpublished manuscript.

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MOLECULAR WEIGHT OF RABBIT GAMMA-GLOBULIN

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ALTHOUGH rabbit γ-globulin is heterogeneous in electrophoretic behaviour, sedimentation velocity boundary spreading experiments indicate that the homogeneity in molecular size and shape is comparable with that for many other proteins of consistent and reproducible physical properties. Thus, though it may be anticipated that different fractionation procedures may result in samples of varying mean mobility, it is surprising to find such a wide range of molecular weight values (150,000–188,000) quoted[1] for different fractions studied in the past. Porter[1] has suggested that this variation arises from errors in the determination of S and D values inserted in the Svedberg equation, $M = RTS/D(1 - \bar{v}\rho)$. One theory of antibody formation due to Pauling[2] postulates that though an antigen may modify the shape of the γ-globulin molecule it can in no way alter its molecular weight, since it cannot interfere in the primary synthesis of polypeptide. It may therefore be of some importance to determine accurate molecular weights for γ-globulin and antibody.

Fractions examined in this work were isolated from batches of normal rabbit serum and a rabbit anti-ovalbumin serum obtained by short-term immunization over a 4-week period. The Nichol and Deutsch[3] low-temperature ethanol scheme was used for isolating the γ-globulin; the globulin was re-precipitated with 20 per cent w/w ethanol at – 5° under the conditions of Nichol and Deutsch to remove traces of serum albumin. Additionally, normal sera were fractionated by precipitation with sodium sulphate[4] and by chromatography on a DEAE cellulose (Whatman floc) column[5]. The partially immune globulin (batch 6/7) contained about 40 per cent antibody, determined under conditions for maximum precipitation with ovalbumin, and this agreed quite well with the figure deduced from combined precipitin and electrophoretic analyses of the original antiserum, providing good evidence that the protein had not been denatured by ethanol.

Sedimentation measurements were carried out in a Spinco model *E* ultracentrifuge, and rotor temperature was recorded at frequent intervals during the run. Diffusion measurements were made by the Gouy interferometric method[6] in an Ogston diffusion cell of optical depth 1·774 cm. All samples were dialysed against phosphate saline buffer (pH 7·00, I 0·045 sodium phosphates + I 0·125 sodium chloride). Physical homogeneity in the ultracentrifuge was tested by the value of the ratio $(\Delta n)uc/(\Delta n)dr$, where $(\Delta n)uc$ is the refractive increment across a velocity boundary corrected for sectorial dilution and $(\Delta n)dr$ is a direct measurement in the differential refractometer using dialysed solution and the buffer.

Each fraction gave a single velocity boundary in the ultracentrifuge. The $(\Delta n)uc/(\Delta n)dr$ ratios based on the initial sedimentation diagram photographed about 30 min. after attaining a maximum speed of 59,780 rev./min. were 0·94–0·99. Measurements on later photographs, taken at 16-min. intervals, revealed a steady decrease to values which were 6–10 per cent below the initial figures by the time the boundary had reached the bottom of the cell. Apparently minor components did resolve although the location of the base-line for the boundary is often difficult and introduces errors in $(\Delta n)uc$. Less drift was found in a double-sector cell where the base-line is accurately recorded under the same run conditions (Fig. 1), but as this cell is restricted to a centrifugal field of 100,000g compared with 250,000g in a standard cell the resolving power of the method is reduced. Electrophoresis in veronal buffer (pH 8·6, I 0·10 sodium veronal) showed boundaries quite typical of γ-globulin and no evidence for other serum com-

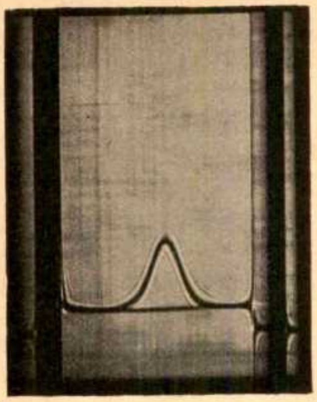

Fig. 1. Sedimentation diagram from the 'Spinco' model *E* ultracentrifuge. Material: rabbit γ-globulin (batch 6/7) isolated from anti-ovalbumin serum by fractional precipitation with ethanol. Solvent: *p*H 7·0, *I* 0·045 sodium phosphates + *I* 0·125 sodium chloride. Speed 37,020 rev./min. Sedimentation from right to left in double-sector cell. Initial $c = 0{\cdot}93$ per cent w/v. Phase plate angle 60°. Exposure 148 min. from reaching full speed

ponents. The electrophoretic mobilities showed variations from batch to batch ($-$ 1·50 to $-$ 1·68) × 10^{-5} cm.^{2} sec.$^{-1}$ V.$^{-1}$. After dialysis against unbuffered saline (*I* 0·145 sodium chloride) material not required for immediate use was stored at $-$ 20°. Six months later, after dialysing against neutral phosphate-saline, about 5 per cent of a new component, $S_{20,w}$ 9·0 was observed, which is probably a dimer of the normal molecule[7].

Sedimentation and diffusion coefficients for rabbit γ-globulins are plotted as a function of protein concentration in Figs. 2 and 3. The regression line for sedimentation data on partially immune γ-globulin (batch 6/7) may be expressed as $S_{20,w} = 6{\cdot}73 - 0{\cdot}236\ (\pm 0{\cdot}014)c$. Except for two diffusion results on the 'inert' γ-globulin fraction to be discussed later, all the diffusion data were combined in a common regression line given by $D_{20,w} = 4{\cdot}66 - 1{\cdot}21\ c$. From data extrapolated to zero concentration and a partial specific volume of $\bar{v} = 0{\cdot}745$, the molecular weight may be calculated as 137,000. The results for normal globulins isolated by various methods show that their molecular weights do not deviate markedly from this value. If concentration dependence of *S* and *D* is ignored the molecular weight calculated for 1 per cent protein is 179,100. An Archibald run performed on a 0·96 per cent solution of batch 6/7 gave a mean molecular weight of 172,700, again a high result but in fair agreement with an Archibald molecular weight of 187,600 ($\bar{v} = 0{\cdot}735$) determined quite independently by Charlwood[8] under similar conditions on a normal rabbit γ-globulin obtained by the sodium sulphate method. As expected, Archibald values for lower protein concentrations approached the true molecular weight, but the rapid deterioration in precision scarcely justified an extrapolation to zero concentration. The upper and lower figures (137,300–179,100) for the range of concentration examined are therefore comparable with results in the literature (150,000–188,000) and strongly suggest that the variation is due mainly to neglect of concentration-dependent diffusion.

A frictional ratio, $f/f_0 = 1{\cdot}34$, calculated from the revised figures is not much lower than earlier estimates, and, depending on what assumptions one may care to make regarding the effect of solvation on molecular configuration, various hydrodynamic models could be suggested which simulate the sedimentation and diffusion behaviour of γ-globulin, but without precise viscosity data the "effective hydrodynamic volume", as defined by Scheraga and Mandelkern[9], cannot be calculated.

An attempt to differentiate 'inert' from antibody globulin was made by first precipitating antibody from the 'partially immune' anti-ovalbumin fraction, batch 6/7, under conditions giving maximum precipitation of antibody, centrifuging off the specific precipitate, and then examining the 'inert' globulin left in the supernatant (cf. Porter[10]). Soluble antigen–antibody complexes may contaminate the 'inert' fraction and in detecting their presence the constancy of $(\Delta n)uc/(\Delta n)dr$ is very important. Previous measurements on the parent globulin, consisting of roughly equal proportions of 'inert' and antibody molecules, showed a drift of 0·99–0·94 during sedimentation, while the 'inert' globulin derived from the mixture showed an increasing drift from 0·97 to 0·84. This result indicated that not more than 5 per cent of the 'inert' fraction was in the form of soluble complexes with $S_{20,w} > 7{\cdot}0$. The slope of the regression line, $S_{20,w} = 6{\cdot}82 - 0{\cdot}389\ (\pm\ 0{\cdot}0138)c$, and $S^0_{20,w} = 6{\cdot}82$ (Fig. 2), were both significantly

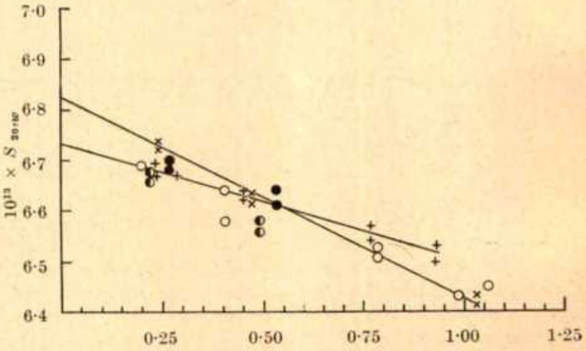

Fig. 2. Plots of $S_{20,w}$ against protein concentration for various rabbit γ-globulin fractions. +, batch 6/7, isolated from anti-ovalbumin serum by the ethanol method; ×, 'inert' fraction derived from batch 6/7. Regression lines are drawn for batch 6/7 fractions only. Normal globulins isolated by means of ○, precipitation with ethanol (results obtained on model *E* not fitted with RTIC unit); ●, precipitation with sodium sulphate; ◐, chromatography on DEAE cellulose. Solvent: *p*H 7·0, *I* 0·045 sodium phosphates + *I* 0·125 sodium chloride

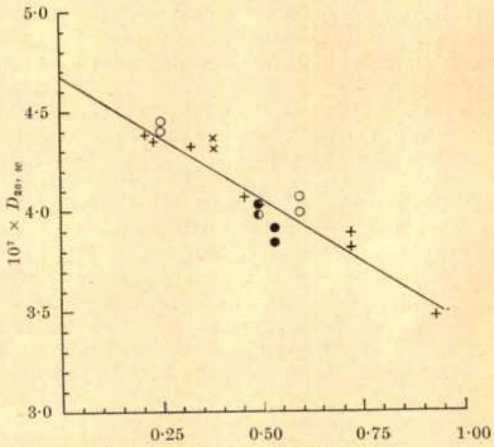

Fig. 3. Plots of $D_{20,w}$ against protein concentration for various rabbit γ-globulins using the same symbols as those in Fig. 2. The regression line is drawn for the pooled data omitting the 'inert' fraction derived from batch 6/7. Solvent: *p*H 7·0, *I* 0·045 sodium phosphates + *I* 0·125 sodium chloride

greater than those obtained for the original mixture. Nevertheless, these results, combined with a mean $D_{20,w} = 4\cdot33 \times 10^{-7}$ cm.^{2}sec.$^{-1}$ determined at $c = 0\cdot355$ per cent (Fig. 3), show that the molecular weights of both types of globulin did not differ by more than 3 per cent.

The results thus show that the molecular weights of various normal and one partially immune globulin differ by amounts which are comparable with present experimental error, and suggest that further precise work to test the validity of the Pauling theory may be justified. In particular, it would be of interest to confirm and to interpret the different sedimentation concentration dependencies which this work suggests may exist between 'inert' and antibody γ-globulins.

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DEVELOPMENT OF MOUSE EMBRYOS IN ORGAN CULTURES OF FALLOPIAN TUBES ON A CHEMICALLY DEFINED MEDIUM

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WHITTEN[1] demonstrated that 8-cell mouse embryos will develop into blastocysts when cultivated in a balanced salt solution containing glucose and bovine plasma albumin. Later, McLaren and Biggers[2] showed that blastocysts produced in this way would develop into normal mice after transfer to uterine foster mothers, and this fact has been amply confirmed and exploited by Tarkowski[3] for the production of mammalian chimeras. So far, however, no one has devised a medium which will regularly allow development *in vitro* of mouse embryos through the first three cleavage divisions.

As an indirect approach to this problem we have cultured mouse Fallopian tubes *in vitro* on a completely chemically defined medium, and have shown that mouse embryos will develop within these tubes from the uncleaved zygote through to advanced blastocyst stages. Furthermore, other preliminary experiments suggest that blastocysts produced in this way can develop within uterine foster mothers into normal embryos. Our results are summarized in this article.

The Fallopian tubes from super-ovulated, mated, 8–9-week-old female mice, from the randomly bred Swiss strain, were cultured on medium *BGJb* containing 5 mgm. glucose per ml. (Table 1). This medium is chemically defined, and was originally used in investigations of the growth and differentiation of embryonic cartilaginous long bones *in vitro*[4]. The vessel, used for the culture of one Fallopian tube, consisted of a Petri dish (6 cm. diam.) containing a 2 cm. × 2 cm. stainless steel grid, 3 mm. high. A disk of tea-bag paper (Grade No. 10–V–7–1/4; C. H. Dexter and Sons, Inc., Windsor Locks, Connecticut) was placed on the grid.

10 ml. medium were placed in each dish. Contact of the medium with the wire mesh and paper was maintained by surface tension, so that the explant was always exactly at the interface, ensuring, thereby, an adequate supply of oxygen to the tissues. The two Fallopian tubes were removed from each mouse by cutting through the ovarian bursi, as close to the ovaries as possible, and through the utero-tubal junctions. Each Fallopian tube was then uncoiled by carefully clipping the mesentery holding the coils together, and stretched out by gently drawing the tube across the paper. The dishes were incubated at 37° C. in a chamber through which humidified 5 per cent carbon dioxide in air continuously flowed.

The mice were superovulated by the injection at 12 noon of 5 I.U. pregnant mare serum gonadotrophin (Gestyl, Organon), followed 48 hr. later by 3 I.U. human chorionic gonadotrophin ('Pregnyl', Organon). Fertile males were placed with the females at the time of the second injection. This treatment results in ovulation and mating about 12 hr. after the second injection. Females with vaginal plugs were killed 10–12 hr. later, that is, 12–14 hr. before the expected time of the first cleavage division[5]. In several experiments we have always found that this schedule yields unsegmented ova at the top of the Fallopian tubes.

Fifty-four Fallopian tubes were cultured for either 3 or 4 days, and their contents examined. The results are summarized in Table 2. Forty-three tubes (80

Table 1. CHEMICALLY DEFINED MEDIUM *BGJb*

	mgm./100 ml.	m*M*
L-Lysine hydrochloride	24	1·31
L-Histidine hydrochloride H_2O	15	0·72
L-Arginine hydrochloride	7·5	0·36
L-Threonine	7·5	0·63
L-Valine	6·5	0·56
L-Leucine	5·0	0·38
L-Isoleucine	3·0	0·23
L-Methionine	5·0	0·34
L-Phenylalanine	5·0	0·30
L-Tryptophan	4·0	0·20
L-Tyrosine	4·0	0·22
L-Cysteine hydrochloride H_2O	9·0	0·51
L-Glutamine	20·0	1·37

	mgm./100 ml.
Nicotinamide	2·0
Thiamine hydrochloride	0·4
Calcium pantothenate	0·02
Riboflavin	0·02
Pyridoxal phosphate	0·02
Folic acid	0·02
Biotin	0·02
p-Aminobenzoic acid	0·2
α-Tocopherol phosphate (sodium salt)	0·1
Choline chloride	5·0
m-Inositol	0·02
Vitamin B_{12}	0·004
Calcium lactate	55·5
Potassium chloride	53·0
Potassium dihydrogen phosphate	16·0
Sodium chloride	800·0
Magnesium sulphate ($7H_2O$)	20·0
Sodium bicarbonate	350·0
Glucose	500·0
Streptomycin	5·0
Penicillin	10,000 units

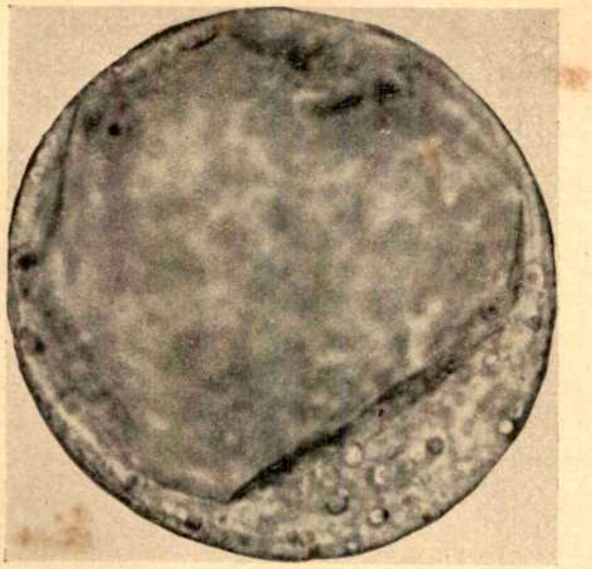

Fig. 1. Living mouse blastocyst produced in 4 days inside an organ culture of Fallopian tube (phase contrast, × 690)

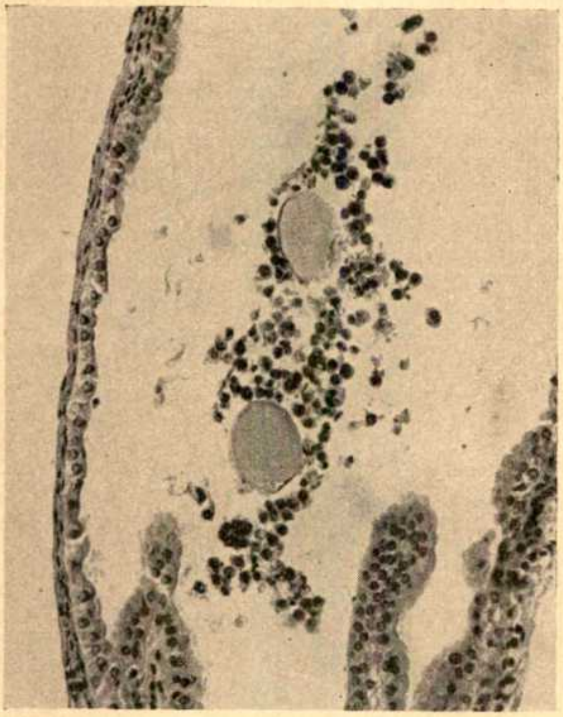

Fig. 2. Fallopian tube isolated from a superovulated mouse, approximately 12 hr. after the expected time of mating, containing two uncleaved ova (× *c*. 137)

per cent) contained developing eggs, and 159 of the 215 eggs (74 per cent) examined had developed at least into morulæ. However, the tubes examined on the third day contained only morulæ, with the exception of one early blastocyst, while the tubes examined on the fourth day contained a predominant number of blastocysts. According to Lewis and Wright[6] morulæs develop into blastocysts *in vivo* on the third to fourth day after fertilization, so the rate of development was unaffected by the conditions of culture. A typical living blastocyst produced in this way is shown in Fig. 1. A well-defined inner cell mass and a large central cavity has developed.

Table 2. DEVELOPMENT OF EARLY MOUSE EMBRYOS WITHIN FALLOPIAN TUBES MAINTAINED IN ORGAN CULTURE FOR 3 OR 4 DAYS ON MEDIUM *BGJb*

No. of days *in vitro*	No. of Fallopian tubes	No. of Fallopian tubes with developing eggs	No. of eggs removed	No. of morulæ	No. of blastocysts
3	29	22	109	75	1 (early)
4	25	21	106	14	69

Fig. 2 shows a section through a typical Fallopian tube at the time of explantation, containing two ova embedded in a mass of corona cells. Fig. 3 shows a section through a Fallopian tube which had been cultured for four days, containing a well-developed blastocyst. It is clear that the structure of the Fallopian tube is preserved throughout the period of cultivation. The cilia of the epithelial cells lining the Fallopian tube continued to beat throughout the culture period in almost all explants, causing the movement of particles within the lumen of the tube. During the first two days after explantation no muscular movement occurred in the tubes. On the third day, however, segmental movements occurred in a few explants and on the fourth day nearly all explants were undergoing similar movements. The significance of the onset of motility after an initial quiescent period is unknown. However, it may have no physiological significance, since muscle cells under adverse conditions may discharge action potentials spontaneously. If these potentials occur in the Fallopian tubes, therefore, the contractions may merely indicate early signs of degeneration of the cultures.

Several blastocysts which had developed in organ cultures of Fallopian tubes were transplanted into

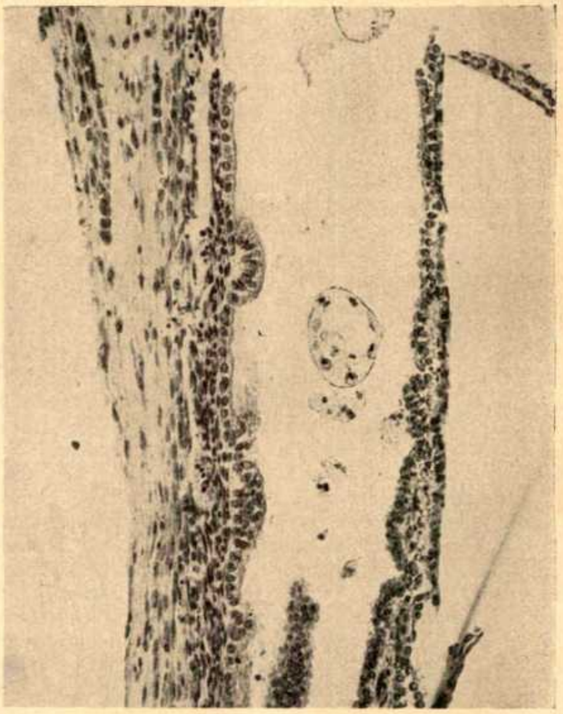

Fig. 3. Fallopian tube after 4 days cultivation on medium *BGJb*, containing a well-developed blastocyst (× *c*. 137)

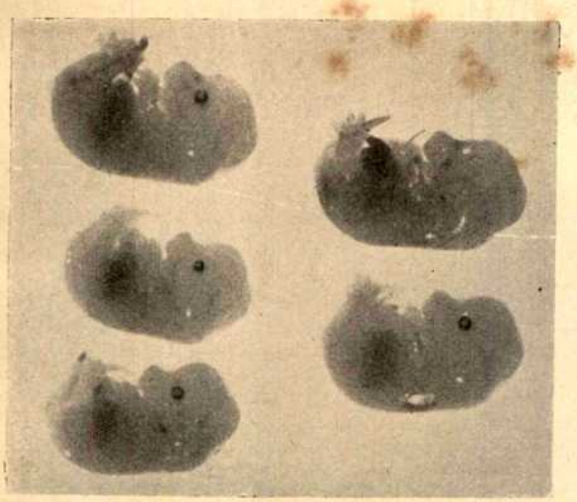

Fig. 4. Seventeen-day-old litter from a *C*57 uterine foster mother, containing 4 native embryos and 1 alien (cultured) embryo

the uterus of inbred *C*57 black mice which had been superovulated and mated to *C*57 males three days previously. On the seventeenth day of pregnancy the embryos inside the uterine foster mothers were examined for eye colour. Fig. 4 shows a litter of 5 mice in which 4 have black eyes (native fœtuses) and one is colourless (cultured fœtus). Although this result is very preliminary, it suggests strongly that the conditions of culture have little adverse affect on the early embryos. Further work on this problem is in progress.

Although the results in Table 2 shows that the ovary is not essential for the development of early mouse embryos in cultures of Fallopian tubes, we also did some experiments in which the ovary was left attached to the Fallopian tube. The presence of the ovary had no obvious influence on the development of the embryos.

The results described in this article show clearly that normal mouse embryos develop *in vitro* within Fallopian tubes maintained on a completely chemically defined medium. Such media do not allow the development of early stages outside the Fallopian tube, even if the media are supplemented with natural body fluids such as serum.

There are two possible explanations of these results. First, the Fallopian tubes may provide material which is essential for the first three cleavage divisions to occur. Secondly, the secretions of the Fallopian tube may exert a protective action on the embryos up to the 8-celled stage.

The results also demonstrate that early embryonic development inside the Fallopian tube does not depend on a continual supply of some hormone. If a hormone is required, then the amount present in the Fallopian tube at the time of explanation is sufficient for the 4-day period of development *in vitro*.

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INTERRELATIONS BETWEEN DIFFERENT MECHANISMS OF RELEASE OF BIOLOGICALLY ACTIVE PEPTIDES FROM BLOOD PLASMA

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IN recent years increasing attention has been given to a possible physiological role of a highly potent vasodilator substance which is released from blood plasma by various agents. According to the experimental system used, it has been called kallidin, bradykinin, pain-producing substance and plasma kinin[1–5]. All these names may well refer to the same substance, probably a nonapeptide[6], the formation of which presumably involves a proteolytic mechanism. Yet the process may be initiated by agents as varied as acetone, foreign surface, dilution, trypsin, snake venoms, saliva, urine, versene and ε-amino-caproic acid (EACA)[1–5,7,8]. As has been shown elsewhere[5,9], foreign surface acts by adsorbing and activating the Hageman factor (*HF*); this in turn activates a 'component *A*', which together with another factor ('component *B*') brings about the release of plasma kinin. In addition, plasma contains powerful inactivators which destroy the active products almost as soon as they are formed.

In order to find possible relations between the above and some of the other activation mechanisms we prepared human 'intact' plasma and its derivatives which were deficient in one or more of the essential precursors (*HF*, *A* and *B*)[9] and subjected them to various activation procedures. These samples were then tested on rat uteri by methods previously described[5,9], for plasma kinin and for the capacity to release kinin from intact substrate plasma. The latter ('indirect test') is a measure of activated component *A* (ac–*A*) probably identical with plasma kallikrein[1].

A summary of the findings as well as the probable equivalence of our terminology with that of the kallikrein–kallidin system is given in Table 1. In agreement with other authors[8,10,11], we have found that the factors required for the activation of plasma by surfaces are also necessary for the release of kinin by acetone, chloroform, versene and EACA (Table 1, tests 1, 2, 3), while urine and saliva do not require these factors but appear to act on the substrate (kallidinogen) directly. Acetone has a two-fold effect on plasma: it slowly activates *HF* and it destroys the inactivator of ac–*A*. Normally, ac–*A*

Table 1. Activation of Plasma and Derivatives by Various Agents

Test	Activator		Product tested for	Untreated	Heated 57° C. 30 min.	Heated 60° C. 30 min.	Deficient in *HF*	Depleted of comp. *A* and *B*	Depleted of comp. *B*
				Plasma and derivatives tested					
1	Foreign surface (glass, etc.)	*a*	Kinin (kallidin)	+	+	−	−	−	−
		b	ac-*A* (kallikrein)	+	+	−	−	−	+
2	Acetone, chloroform (→ plasma kallikrein)	*a*	Kinin (kallidin)	+/−	+/−	−	−	−	−
		b	ac-*A* (kallikrein)	+	+	−	−	−	+
3	Versene EACA	*a*	Kinin (kallidin)	+	+	−	−	−	−
		b	ac-*A* (kallikrein)	−*	−*	−	−	−	−*
4	Acetone + versene	*a*	Kinin (kallidin)	+	+	−	−	−	+†
		b	ac-*A* (kallikrein)	+	+	−	−	−	+
5	Saliva ('glandular kallikrein')	*a*	Kinin (kallidin)	+	+	+	+	+	+
6	Saliva, after 24 hr. hours with acetone‡	*a*	Kinin (kallidin)	−	−	+	+	+	−

* Presumably undetectable traces present. † After prolonged incubation. ‡ 'Kallidinogen consumption test'.

decays with extraordinary speed (half-life at 20° C. is 1–2 min.) but in the presence of acetone it is stable for days and so accumulates as the reaction progresses[12]. However, acetone has little, if any, effect on the plasma peptidase, which continues to destroy any kinin that is released (cf. test 2*a*). Versene arrests the decay of kinin, probably by immobilizing a metal (? zinc) on which the peptidase is dependent[12]. Therefore, when both acetone and versene are added to plasma, not only ac–*A* but also plasma kinin is preserved from destruction. Under these conditions we find that kinin can also be detected in plasma depleted of component *B* (test 4*a*). Webster and Ratnoff[11] raised the question whether plasma kallikrein could liberate kallidin (kinin) in the absence of component *B*. The foregoing results show that this can, indeed, take place but the process is so slow that normally it cannot outstrip the destruction of kinin. Thus, component *B* may be regarded primarily as an accelerator, but it becomes an essential factor under conditions when destruction of plasma kinin proceeds apace with its production as, for example, with glass beads.

EACA also prevents the decay of kinin in plasma, though not as efficiently as versene[12]. We were unable to find any evidence that EACA has any direct activating effect on the kinin-releasing system. As in the case of versene, the slow and variable appearance of plasma kinin under the influence of EACA[8] is probably simply due to cumulative effects of such marginal factors as contact with siliconed or plastic surfaces.

Up to now 'glandular' kallikreins like saliva could be regarded as acting quite independently of the components involved in surface or acetone activation. Both systems, however, converge on the same final substrate (kallidinogen). This is shown in the experiment in Fig. 1, which may be regarded as a 'kallidinogen consumption test'. Here plasma and derivatives were incubated with 20 per cent v/v acetone for 16–48 hr. and then tested for ac–*A* (kallikrein) (Fig. 1*a*) and also for the capacity to release kallidin with saliva (*b*). As could be expected, kallikrein was produced only in those samples which contained both *HF* and component *A*. This is shown most clearly in tracings 3 and 4 (see also Table 1, test 6). In tracing 3, in which *HF*-deficient and *A*-depleted plasmas were mixed first and then incubated with acetone, kallikrein was formed as in normal plasma; in test 4, where these samples were incubated with acetone separately and mixed immediately before addition to substrate plasma, kallikrein formation did not occur.

Activation with saliva gave exactly opposite results. All the samples which could be activated with acetone (tests 1, 3, 6, 7) no longer released kallidin when saliva was added next day, whereas those in which the conditions for the minimum reacting system (*HF*, component *A* and acetone) were not fulfilled (tests 2, 4, 5, 8) yielded normal amounts of kallidin with saliva. Similar observations were recorded using urine or trypsin instead of saliva. We regard these results as evidence that plasma kallikrein utilizes the same substrate as the 'glandular' kallikreins and, implicitly, as a further support for the hypothesis originally formulated to deal with plasma/surface reactions[9,13]. This concept can now be expanded to include some of the other kinin-releasing agents and also to indicate the role of inhibitors in the economy of these reactions.

It is true that in identifying such terms as, for example, component *A* and kallikreinogen there is a chance

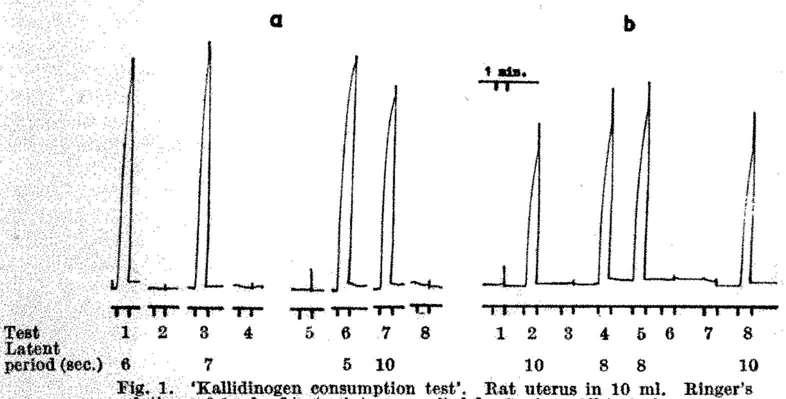

Fig. 1. 'Kallidinogen consumption test'. Rat uterus in 10 ml. Ringer's solution. 0·1 ml. of test mixtures applied for 1 min. All test plasmas were incubated with 20 per cent v/v of acetone for 24 hr. and tested for ac-*A* (kallikrein) by the 'indirect test' (Refs. 5 and 9) (*a*) and for formation of kinin (kallidin) after incubation with 1 part of saliva in 6 of plasma for 4 min. (*b*) *Test plasmas*: 1 and 6, normal; 2, depleted of comp. *A* and *B*; 5, deficient in *HF*; 3, plasmas (2) and (5) mixed first then incubated with acetone; 4, (2) and (5) incubated with acetone separately and mixed just before testing; 7, depleted of comp. *B*; 8, heated to 60° C. for 30 min. Note that in (*b*) the results are exactly opposite to those in (*a*)

that each may prove to be a complex system and not a single substance. None the less, we consider that at the present state of knowledge there is a need for a unifying concept even at the peril of some over-simplification.

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REVERSIBLE INACTIVATION OF BLOOD-GROUP A AND B SUBSTANCES FROM RED CELLS

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THE chemical nature of ABO blood-group substances from red cells still remains an unsolved problem, although several claims have been made that these substances are glycolipids composed of galactose, glucose, amino sugars, sphingosine and fatty acids[1–4]. The main difficulty in assessing the nature of these materials was the low blood-group activity of the preparations obtained so far which never exceeded more than 20 per cent of the blood-group active material isolated from human secretions. None of the preparations of red-cell substances can be regarded as homogeneous; even 'globoside', which was claimed to be a pure homogeneous substance[1], was later separated into several fractions by means of chromatography on silicic acid columns[5]. One of the most potent preparations of substance A obtained from red cells was that isolated by Kościelak and Zakrzewski[3]. This material was, however, a mixture of at least two compounds containing hexosamine[6]. Subsequent attempts to purify this material further yielded some unexpected results. Although it could be readily separated into two main fractions by means of starch-column and paper chromatography in butanol ethanol–water solvent, the hæmagglutination activity of the separated fractions was always 1–10 per cent only of the value of the original material[7]. Essentially the same results were obtained using chromatography on silicic acid columns. The hæmagglutination inhibition activity of the separated components amounted only to 1 per cent of the activity of the material before separation. Thus, the original substance behaved like a complex, since more than one component was required to exert full activity.

In the course of the present investigation, the blood-group active *A* material from red cells was isolated by a technique similar to one described previously[3,6]: the main difference was that instead of extracting whole cells with ethanol, stromata were used as a source of blood-group substance. Red cells were hæmolysed with 10 vol. of tap water; solid carbon dioxide was added together with a few ml. of capryl alcohol to prevent excessive foaming and the hæmolysate was centrifuged in a Sharples centrifuge. Absolute ethanol was then added to the stroma paste to give 83 per cent ethanol concentration, and the resulting stroma suspension allowed to stand at room temperature for a few days with occasional shaking. The ethanol extract was then filtered off and cooled to −8° when a white precipitate formed. The precipitate was collected by centrifugation at −8°, washed with one-tenth of its volume of cold 83 per cent ethanol then with a large volume of acetone and finally dried *in vacuo*. This 'crude' A substance had the same general composition as one obtained by extraction of whole cells. Its solubility properties were, however, somewhat different, and it could be largely dissolved by prolonged extraction with petroleum ether, whereas material from whole cells was practically insoluble in this solvent. The crude substance after brief extraction with acetone followed by ethyl ether was washed twice with petroleum ether and then subjected to hot methanol extraction and the material soluble in methanol (precipitate *M*) chromatographed on a cellulose column[3,6].

In contrast to the results with material isolated from intact cells, the decrease of activity was observed after separation of this precipitate *M* into fractions IA and IIA on a cellulose column (Table 1). The residual blood-group activity after separation was confined to fraction IIA. However, when these results were checked by means of quantitative precipitation test using anti-A rabbit serum it was found that the activity of fraction IIA had increased four times compared with the activity of precipitate *M*, whereas fraction IA was inactive in both tests. Later, an attempt was made to restore hæmagglutination activity of the isolated fractions by adding them together. This recombination experiment was simply done by dissolving fraction IA and fraction IIA in proportions of 3 : 1 in a mixture of chloroform–methanol and evaporating the solution to dryness *in vacuo*. The hæmagglutination activity was fully restored under these conditions (Table 2). This phenomenon is not due to some physicochemical effect of fraction IA on the red-cell membrane in the test system and true complex formation must occur as is illustrated by an experiment in which fraction IA and IIA were mixed together in aqueous solutions. Under these conditions no increase of activity was observed and the resulting activity of the mixture was due solely to its content of fraction IIA. Further confirmation of the formation of a complex between the two fractions is to be found in their solubility properties. Whereas precipitate *M* and fraction IIA are water-soluble, fraction IA is only slightly soluble in water and forms a heavy colloidal suspension. The possibility that fraction I contains some blood-group activity which is not detected owing to its

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Table 1. PROPERTIES OF THE FRACTIONS OBTAINED FROM A CELLULOSE COLUMN

	Precipitate *M*	Fraction IA	Fraction IIA
Hexosamine (per cent)	5·0	2·8	9·2
Reducing sugar (per cent)	20·5	17·2	42·7
Sialic acid (per cent)	1·3	0·6	4·1
Nitrogen (per cent)	2·8	2·6	1·9
Component sugars	glucose, galactose, glucosamine, galactosamine	glucose, galactose, galactosamine	glucose, galactose, glucosamine, galactosamine, traces of fucose
A activity in hæmagglutination inhibition test (dilution)	$2 \cdot 5 \times 10^{3}$	$< 2 \cdot 5 \times 10^{2}$	$1 \cdot 25 \times 10^{4}$
A activity in quantitative precipitation test (per cent of the antibody nitrogen precipitated by the standard cyst A substance)	15	nil	60

Table 2. RECOMBINATION EXPERIMENTS

Precipitate *MA*	20
Fraction IA	less than 0·005
Fraction IIA	1
Fraction IA + fraction IIA	20
Fraction IA + fraction IIA (recombined in aqueous solution)	0·25
Fraction IB + fraction IIA	20
Sphingomyelin from pig spleen + fraction IIA	1
Lecithin from pig kidney + fraction IIA	1

Activities (hæmagglutination inhibition) are expressed on the weight basis as the percentage of the standard preparation of cyst A substance 401.

insolubility in water was, however, excluded by an experiment with fraction IB isolated by essentially the same technique from B cells. In the case of substance B, decrease of hæmagglutination inhibition activity was also observed after separation on cellulose columns, and the activity could be restored by a recombination of the two fractions in organic solvent solution. Fraction IB, which was completely inactive in hæmagglutination tests, was then joined to fraction IIA. The full restoration of A activity occurred and the material did not exhibit any B activity. Obviously, fraction I has only carrier properties.

Some insight as to the nature of the phenomenon described is given by the attempt to restore blood-group activity of fraction II by combining it with simple lipids such as lecithin and sphingomyelin (kindly supplied by Dr. G. M. Gray). Some increase of activity (4 times, based on the amount of fraction IIA present) was observed with both these phosphatides, and the results suggest that the full blood-group activity of red-cell substances can be exerted only by combination with some 'carrier' material which produces possibly the favourable molecular shape and proper distance between chemical groupings of native blood-group substance which allows combination with antibody molecules. The linkage between blood-group substance and the 'carrier' must be a weak one and involves possibly the interaction between a sphingosine-fatty acid residue of the blood-group substance with large, predominantly lipid, micelles of the 'carrier', whereas hydrophilic carbohydrate groups of the active substance project outwards being available for combination with the antibody molecules. This complex formation is reminiscent of the results obtained with Shiga lipoprotein, which also functioned as a 'carrier' for blood-group substances isolated from human secretions, and thus brought about the enhancement of their antigenic properties[8]. The apparent discrepancy between hæmagglutination inhibition activity and precipitation activity of fraction II in the present study can be explained on the basis of qualitative difference between these tests. The hæmagglutination inhibition test is essentially a competitive one in which the inhibitor competes for antibody molecules with the native antigen on the red-cell surface. The firmness of this reaction depends on the correct molecular size and shape of the antigen. In precipitation tests only antigen and antibody are present in the reaction mixture and combination may occur even with an antigen more distantly related spatially towards the antibody.

The above results may explain the failure to obtain highly active and homogeneous blood-group ABH substances from red cells as well as antigens from other cells. They stress that caution is required in drawing conclusions from experiments involving treatment of intact cells or impure mixtures of biologically active substances with enzymes or 'specific' chemical reagents in order to ascertain the true chemical nature of the structures responsible for the biological activity under investigation. Any loss of activity which might occur under these conditions can be the result of a change of the specific spatial pattern of the receptor residues on the cell surface without any destruction of the receptor itself.

I thank Prof. W. T. J. Morgan for his interest and advice throughout this investigation.

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PERSISTENCE AND STABILITY OF SPECIES-SPECIFIC HÆMAGGLUTINOGENS IN CULTIVATED MAMMALIAN CELLS

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CULTIVATED mammalian cells when injected into laboratory animals induce hæmagglutinins[1] which react species-specifically[2,3]. This reaction has been used for classification of cultivated mammalian cells according to species[4]. Results have usually confirmed stated species origins of cell strains except for many 'transformed' cell strains, 17 of which were found to be of either human or mouse species although they were reportedly derived from other species[5]. At least two explanations have been suggested for the occurrence of cell transformation: (*a*) a genetic process involving mutation and selection under certain cell culture conditions[6]; (*b*) contamination and overgrowth of original cultures with cells of fast-growing established laboratory strains. If the former concept were true, lability and variability of cellular species-specificity should easily be demonstrable under controlled experimental conditions since cell transformation appeared a frequent event under 'normal' laboratory conditions. Experiments were therefore performed to examine the degree of stability and persistence of species-specific hæmagglutinogens in cultivated mammalian cells. Such experiments were of particular interest after it was found by Weiler[7] and Högman[8] that organ and blood group-specific cell antigens disappear during early stages of cell cultivation. Also these investigations were considered useful with regard to general classification of animal cells, since only if species antigens were shown to be stable could they serve as permanent labels of cells in continuous culture.

HeLa cells, cottontail rabbit epithelial cells, mouse *L* cells and cell lines reported to be variants of *L* cells[6,9,10] such as *MCN*, Lung To, *AMK*, *U*-12 and *FS* were used.

The basic procedures of cell cultivation using yeast extract medium have been described elsewhere[3,6]. Three sub-lines of HeLa were investigated, the culture media of which differed in serum supplements. *HuS*–HeLa had been kept for about 60 passages on yeast extract medium with 20 per cent human serum, *HoS*–HeLa for 75 passages on 20 per cent horse serum, *CaS*–HeLa for about 50 passages on 20 per cent calf serum. The cottontail rabbit epithelial cell strain established in this department on 20 per cent rabbit serum medium was adapted to and cultivated for about 15 passages on 20 per cent calf serum medium. The parent *L*-strain had been kept in this department for about 150 passages since 1951 on media with horse serum supplement. The other *L*-cell variants were received only shortly before use in these experiments[5]. They were carried through a few passages on 20 per cent horse serum (*MCN*, Lung To) or 20 per cent calf serum (*AMK*, *U*-12, *FS*).

Monolayers of *CaS*–HeLa cells were washed with buffered saline, mechanically dispersed and counted in a blood cell counting chamber. Aliquots were treated in the following ways: (*a*) heating for 5 min. at 45° C.; (*b*) heating for 5 min. at 80° C.; (*c*) 50 cycles of freezing (– 70° C.) and thawing (+37° C.); (*d*) enzymatic treatment of cell surfaces for 30 min. with 0·2 per cent trypsin; (*e*) no treatment (control cells). Four culture bottles of that same batch were X-irradiated with a total of 1,300 r. Cells of two culture bottles were washed, dispersed and counted. The remaining two cell cultures were maintained in yeast extract medium containing 20 per cent calf serum for 14 more days before the cells were prepared in the same way.

According to the original procedure[3] for demonstration of species-specific cellular hæmagglutinogens cultivated cells were washed free of medium, disrupted in phosphate-buffered saline (*p*H 7·2), and injected into guinea pigs. Pre- and post-immunization serums were titrated for hæmagglutinins against erythrocytes of various species.

In experiments with the above treated HeLa cells, cell-debris prepared from 10^2, 10^3 and 10^4 cells contained in 0·5 ml. were each injected intramuscularly into two guinea pigs. The immunization response of animals was determined in two ways: (*a*) Sera were checked two, three and four weeks after initial injection. If positive, this was considered a direct response; (*b*) if negative, animals received a hyperimmunization injection of 100,000 untreated HeLa cells intraperitoneally. Serum samples were taken on the fifth, tenth and fourteenth day after hyperimmunization injections. Immediate development of high-titred hæmagglutinins by the fifth–tenth day was considered to indicate preceding sensitization of animals from initial injections.

Table 1. INDUCTION OF SPECIES-SPECIFIC HÆMAGGLUTININS IN GUINEA PIGS BY CELLS OF VARIOUS SUB-LINES OF STRAINS HELA, *CRE*, AND *L*

Antisera against	Passage-number of cell lines in this laboratory	Increase of hæmagglutinins (by number of two-fold dilution steps) for erythrocytes from: Man	Monkey	Rabbit	Mouse	Others*
HuS–HeLa	60	555443 †	533322	------‡	------	------
CaS–HeLa	50	555543	543222	------	------	------
HoS–HeLa	75	555443	554333	------	------	------
CaS–CRE	15	2-----	------	43222-	------	------
L	150	2---	----	----	5554	----
MCN	<10	---	---	---	553	---
Lung To	<10	----	----	----	5432	----
AMK	<10	----	----	----	5444	----
U-12	<10	-----	-----	-----	55444	-----
FS	<10	------	------	------	555442	------

* Erythrocytes of other species tested included calf, horse, hamster, swine, sheep, cat, dog, chicken, duck. Antisera against control calf cell cultures agglutinated calf erythrocytes. Antisera against control horse cell cultures were not available.

† Each single figure expresses increase of hæmagglutinins by number of two-fold dilution steps in one individual guinea pig, for example, 3 = titres of 1 : 10 and 1 : 80, respectively, in corresponding pre- and post-immunization sera; 5 stands for 5 or more dilution steps.

‡, less than two dilution steps.

Table 2. SPECIES-SPECIFIC RESPONSES OF GUINEA PIGS TO INJECTION OF TREATED HELA CELLS

Treatment of cells	No. of cells injected (sub-groups of 2 animals)	No. of animals with positive response: Direct hæmagglutinin production	Sensitized only	Total per group of 6
No treatment (control)	10^2	0	0	3
	10^3	0	1	
	10^4	0	2	
Heated 5 min. 45° C.	10^2	0	0	2
	10^3	1	0	
	10^4	1	0	
Heated 5 min. 80° C.	10^2	0	0	3
	10^3	0	1	
	10^4	2	—	
50 × freezing-thawing	10^2	0	0	4
	10^3	0	2	
	10^4	1	1	
Trypsin 0·2 per cent 30 min.	10^2	0	1	5
	10^3	0	2	
	10^4	2	—	
X-irradiation, immediate injection	10^2	0	1	3
	10^3	0	0	
	10^4	0	2	
X-irradiation, injection after 2 weeks	10^2	0	1	5
	10^3	1	1	
	10^4	2	—	

In agreement with Westwood's[6] unsuccessful attempts to transmit a hypothetical cell-transforming principle, I was unable to demonstrate any change of species-specific antigenicity in human HeLa, cottontail rabbit epithelial and mouse *L*-cell cultures adapted to and cultivated for numerous passages on media with various heterologous serum supplements (Table 1). The mouse *L*-strain as the oldest established cell line in continuous culture was originated as early as 1943[11]. Treatment with carcinogens resulted in malignant and pronounced chromosomal changes. These characteristics were shown to vary considerably in individual sub-lines[9,10]. Yet, my results show no aberration from mouse-specific antigenicity in any of the *L*-cell lines tested (Table 1). Results with treated HeLa cells (Table 2) proved species-specific antigenicity to be remarkably stable under various 'normal' and experimental cell culture conditions. Deep freezing is used to preserve viable cell cultures. X-irradiated cells are used as 'feeder layer' cultures since X-irradiation blocks cell multiplication without inhibiting metabolic cell functions[12]. Trypsinization is a routine procedure for dispersing cells from monolayer cultures. This treatment has been shown to result frequently in chromosomal 'breaks' and is suspected to be one of the causative factors of progressive chromosomal alteration in continuous cell lines[13]. Cell strains investigated in this work had been routinely trypsinized during each preceding passage. Apart from this I thought, but could not verify, that trypsinization might have a direct effect on species-specific hæmagglutinogens if these were proteinaceous substances located only in the cell surface.

From these experiments the conclusion was drawn that cell 'transformation' with resultant species change is more likely due to laboratory cell contamination than to a genetic process. Methods based on species-specific antigenicity appear to be useful for permanent identification or classification of cell cultures.

This work was aided by research grants to the late Dr. J. T. Syverton, whose interest was invaluable, from the American Cancer Soc., Inc., the National Foundation and grant No. C–4559 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

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FOOD SELECTION BY SILKWORM LARVÆ

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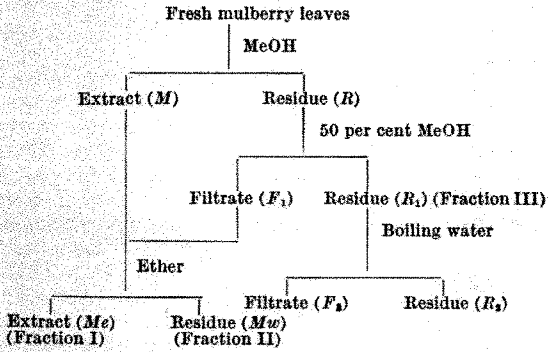
Water-Soluble Biting Factors

IN a previous paper[1] we reported the presence of three stimulants in mulberry leaves: attractants, biting factors and swallowing factor, in the preferential selection of food by silkworm larvæ (*Bombyx mori*). The attractants and one of the biting factors occur in methanol and ether-soluble fraction of the leaves (scheme 1), and water-soluble biting factor exists in methanol- and water-soluble but ether-insoluble fraction, and the swallowing factor which appears to stimulate continuous swallowing by the larvæ, is present in the residue insoluble to these solvents.

SCHEME 1



A combination of these factors is of importance for the positive acceptance of the food by the larvæ. This relation may be seen more clearly by the supplementary data (Table 1). Further, we reported that citral or some terpene[2] and β-sitosterol[3] which are isolated from ether extract of mulberry leaves, attract and stimulates the biting action[4], respectively, and these chemicals are used as substitutes for the methanol- and ether-soluble fraction for the food acceptance by silkworm larvæ.

This communication is concerned with the second fraction soluble in methanol and water. From the second fraction we isolated isoquercitrin (a flavonoid)[5], inositol[6], sucrose, fumaric acid, etc.[7]. Isoquercitrin stimulated the biting action of silkworm larvæ (Table 2). Morin[8] from *Morus tinctoria* also showed the same activity, and related substances such as rutin, quercetin belonging to flavonoids, showed little activity.

We call these substances water-soluble biting factors. Though the water-soluble biting factors cause lower activity than β-sitosterol, these flavonoids are of importance in the mechanism of food selection. But the second fraction cannot be substituted by them. To substitute the second fraction by chemically pure substances, inositol and sucrose in addition to isoquercitrin are necessary for complete acceptance of food by the silkworm. Inositol and sucrose do not stimulate the biting action, but seem to be concerned

Table 1

	No. of fæces after 18 hr.
Basal diet (B.d.) only	41
B.d. + leaf powder (5 gm.)	195
B.d. + *Mw*	86
B.d. + *Me*	55
B.d. + R_1	80
B.d. + *Mw* + *Me*	84
B.d. + *Mw* + R_1	131
B.d. + *Me* + R_1	102
B.d. + *Mw* + *Me* + R_1	200

Five silkworms at fifth instar were placed on the food. B.d. consisted of 50 c.c. of 2 per cent agar solution containing 1 per cent sucrose and 5 gm. cellulose powder.

Table 2

	Test of biting action
β-Sitosterol	+ + + + +
Isoquercitrin	+ + + − −
Morin	+ + + − −
Inositol	− − − − −
Sucrose	− − − − −

The test of biting action was done by the method described in the previous paper. + + + + + denotes that all the larvæ bite the agar thread, and − − − − − denotes none of the larvæ bite with the taste of the food[9]. We have temporarily named them co-factors (Table 3). In a previous paper[1], we used the basal substrate consisting of agar solution containing sucrose and cellulose powder to test for the continuous swallowing reaction. The addition of sucrose was to give the food a desirable taste.

Table 3

	No. of fæces after 32 hr.
Basal diet (B.d.) only	0
B.d. + leaf powder (0·4 gm.)	192
B.d. + β-sitosterol + R_1 (0·4 gm.)	89
B.d. + β-sitosterol + inositol + R_1	200
B.d. + β-sitosterol + morin + R_1	206
B.d. + β-sitosterol + inositol + morin + R_1	254
B.d. + β-sitosterol + inositol + isoquercitrin + R_1	226
(B.d. − sucrose) + β-sitosterol + inositol + morin + R_1	60

Ten newly hatched silkworms were placed on the food. B.d. consisted of 3 c.c. of 2 per cent agar solution containing 1 per cent sucrose and 0·7 gm. cellulose powder. When leaf powder or R_1 was added, 0·3 gm. cellulose powder was used.

Swallowing Factor

The third factor in the mechanism of food selection by the silkworm—the swallowing factor—is contained in a residue insoluble in ether, methanol and water. We have suggested the presence of some chemically active substances as the swallowing factor in hot-water extract of the residue, but importance is attached to cellulose itself. As reported previously, we have added cellulose powder to the basal substrate to test for the continuous swallowing reaction. The addition of this powder was to make the test quantitative, that is, to let the food pass through the alimentary canal and to make well-shaped fæces. It was made clear that cellulose powder which was added to the basal diet from the outset of our experiments is an indispensable factor in the mechanism of food selection. But the third fraction, the residue of mulberry leaves extracted with methanol and water, cannot be substituted so completely by chemically pure cellulose powder. The boiling-water extract or glacial acetic extract is necessary in addition to cellulose power for acceptance of food by silkworms. In the boiling-water extract of the residue we detected large amounts of silica and phosphate. It was observed that potassium phosphate and coated silica on cellulose are of importance, as shown in Table 4. The role satisfied by the residue was substituted by the use of phosphate and silica in addition to cellulose powder. Phosphate and silica are to be called as co-factors.

Table 4

	No. of fæces after 32 hr.
Basal diet (B.d.) only	0
B.d. + leaf powder (0·4 gm.)	192
B.d. + β-sitosterol + inositol + morin + *R–Ac* + R_2	160
B.d. + β-sitosterol + inositol + morin + phosphate + R_2	204
B.d. + β-sitosterol + inositol + morin + phosphate	127
(B.d.–*C.P.*) + β-sitosterol + inositol + morin + phosphate	68
(B.d.–*C.P.*) + β-sitosterol + inositol + morin + phosphate + *S–C.P.*	162

Ten newly hatched silkworms were placed on the food. B.d. consisted of 3 c.c. of 2 per cent agar solution containing 1 per cent sucrose and 0·7 gm. cellulose powder. When leaf powder of R_2 was added, 0·3 gm. cellulose powder was used. *S–C.P.*, cellulose powder coated with 5 per cent of silica sol; *R–Ac*, acetic acid soluble matter from R_1; (B.d.–*C.P.*), basal diet free of cellulose powder).

Thus, the principal members in the mechanism suggested in the previous communication are: *attractants*: citral, terpinyl acetate, linalyl acetate, linalol and β-γ-hexenol; *biting factors*: β-sitosterol and isoquercitrin or morin; *swallowing factor*: cellulose; and co-factors: sucrose, inositol, inorganic phosphate and silica.

Agar jelly containing these stimulants in the following proportion was eaten by the larvæ almost as readily as the mulberry leaf itself:

Principal factors	
Citral	1 c.c. (10 mgm. per cent ether solution)
β-Sitosterol	5 mgm. (spray on the surface)
Isoquercitrin or morin	3 mgm.
Cellulose powder	700 mgm.
Co-factors	
Sucrose	30 mgm.
Inositol	5 mgm.
Potassium phosphate	10 mgm.
Silica	40 mgm. (coated on cellulose)
2 per cent agar solution	3 c.c.

Note: Citral and other terpenes as attractants are not necessary for the larvæ placed on this food.

Investigations of the mechanism of food selection by silkworm may be concluded by the isolation of these stimulants from mulberry leaves. Work on the nutrients or other problems which are necessary for feeding of silkworm through succeeding generations are being investigated.

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A NEW VERTEBRATE FAUNA FROM THE EARLY JURASSIC OF THE DECCAN, INDIA

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ONE of the most serious gaps in our knowledge of the evolution of Mesozoic terrestrial vertebrates is due to the absence of a good land fauna of Lower Jurassic age. Wherever fossiliferous rocks of certain Lower Jurassic date occur they are marine deposits, yielding a great variety of invertebrates and a good fauna of fossil fishes, crocodilians, plesiosaurs and ichthyosaurs. In these rocks terrestrial vertebrates are represented by very occasional fragments washed into the Lower Jurassic seas, and by one good skeleton of a stegosaurian dinosaur from the British marine Lias. Two terrestrial vertebrates which may possibly be Lower Jurassic come from the continental deposits of the Kayenta formation (Welles[1] terminology) of North America, and consist of a crocodilian *Protosuchus richardsoni*, and a carnivorous saurischian dinosaur *Megalosaurus wetherilli*. Welles[1], in a preliminary study of the dinosaur, compared it with Upper Triassic, and Middle and Upper Jurassic megalosaurs, and concluded that the structure of *M. wetherilli* suggests a Lower or Middle Jurassic age for the Kayenta formation. Fragmentary remains of a large sauropod dinosaur, *Rhoetosaurus brownei*[2] from the Walloon Series of Queensland, and one or two incomplete sauropod bones from Argentina[3], may be Lower or Middle Jurassic in age. The tritylodont mammal-like reptile *Oligokyphus* was found in a fissure-filling of Lower Jurassic age in Somerset, England[4]. Although it was represented by dissociated bones these were so abundant that they allowed detailed knowledge and a full reconstruction. Thus, three good specimens of terrestrial vertebrates, and some fragments, represent our entire knowledge of early Jurassic land faunas.

Yet the evolution of land faunas was at a particularly interesting stage in Lower Jurassic times. The two orders of dinosaurs must then have been deploying in an adaptive radiation the results of which are seen in the excellent faunas from Upper Jurassic and Cretaceous rocks. Other kinds of reptiles, and early mammals, and possibly early birds, can be expected from Lower Jurassic land deposits, so that the meagre number of specimens actually known is disappointing. The purpose of this article is to make a brief preliminary report of the first discoveries of a land fauna from the Deccan, India, which we believe to be of Lower Jurassic age, and which we therefore hope may make a contribution towards filling the gap in our knowledge of the evolution of terrestrial Mesozoic vertebrates.

The River Godavari flows to the eastern seaboard of the Indian peninsula and receives, as one of its major tributaries, the River Pranhita. Along the valley of these two Rivers occur outcrops of the Gondwana Group of rocks. One member of the Gondwana Group, the Kota formation, contains thin bands of limestone, and during the nineteenth century fossil fishes were discovered near the village of Kota, on the bank of the River Pranhita, in one of the limestone bands. The fishes were recognized to be species of three genera, *Tetragonolepis*, *Dapedium* and *Lepidotes*[5]. All three genera are well-known members of European marine fish faunas of Lower Jurassic (Liassic) age, *Dapedium* ranging through the Lias, *Tetragonolepis* being confined to the Upper Lias, and *Lepidotes* including the Lias in its long time-range from Rhætic to Cenomanian. These fishes suggest that the age of the Kota formation is Lower Jurassic. But the Kota formation, like all the other members of the Gondwana Group in this part of the Pranbita–Godavari Valley, is a terrestrial deposit, consisting of fluviatile and lacustrine sands and clays, and thin limestones representing evaporite deposits formed in inland lakes. The attempt to find Lower Jurassic land vertebrates in the Kota formation was therefore one of the objects of expeditions made to the Pranhita–Godavari Valley during the past few years by members of the Geological Studies Unit, Indian Statistical Institute, Calcutta, and of University College, London.

In the field season 1958–59 a large number of fragmentary bones was discovered in the Kotas, as surface finds. The size and character of these remains suggested that they were dinosaurian. In 1960–61 an excavation of the site revealed an extraordinarily rich layer of bones, at the junction of a sandstone with a clay lens, and lying about twenty feet below a fish-bearing limestone band. About ten tons of bones were excavated from an area about 85 ft. × 35 ft., and transported to Calcutta, where preparation of the bones has begun. No complete associated skeletons were obtained, but partial associations were met with. The bone bed is reminiscent of a log jam in a river, and indeed large logs of wood, up to 10 ft. 6 in. long, occur with the bones. The directions of current-bedding in the sandstone suggest that excavation is proceeding 'upstream', and it is hoped that in future excavations corpses piled up behind the 'jam' may eventually be revealed as associated skeletons.

A proper discussion of the different kinds of dinosaurs represented in this new fauna, and their characters, will not be possible until preparation of the bones, and further excavations, have been carried out. As this will take some time, a few preliminary statements can be made, but must be regarded as very provisional. The majority of bones are those of a sauropod, the largest femora discovered being almost 5 ft. long, though smaller individuals are represented. Most of the bones of the skeleton of the sauropod are represented in the 1960–61 collection, except skull, feet and radius. In view of their early age the possibility of confusion of these bones with those of a prosauropod is a question which is bound to arise. However, the characters of the vertebræ, pubis, tibia, ulna and humerus, for example, are wholly sauropod. A peculiarity of the Kota sauropod lies in the proportions of its limb bones. An ulna almost 3 ft. long has a shaft which narrows to a diameter of 3 in. Femora are also elongate with a relatively small diameter, and tibiæ have a moderately slim 'waist' to their shafts. Fairly slender proportions are known as rather rare varieties of some sauropods, except *Barosaurus africanus*[6], where they are more common. If the present collection is a representative sample of the Kota sauropod, then this beast should prove even more generally slender-limbed than *B. africanus* (which has a comparable size-range) and may pose some interesting problems in functional anatomy and adaptation. A carnosaur is also present, but less well represented than the sauropod in this first collection. Judging by its pelvis, it should prove to be of about the same size as the well-known *Antrodemus valens* from the Upper Jurassic Morrison formation of North America; that is, more than 30 ft. long. One and possibly two other kinds of dinosaur are represented but diagnosis is not yet possible.

In considering the age of these dinosaurs the possibility arises that the fishes *Tetragonolepis* and *Dapedium* may have continued to exist in fresh waters after their extinction in a marine environment. It is unlikely, however, that they did so for long, for freshwater fish faunas of Upper Jurassic age are known, and are sufficiently different from those of the Lias to suggest that they are the results of a process of evolution and migration extending well back into Middle Jurassic times. It is improbable that the Kota formation is younger than early Middle Jurassic (Inferior Oolite) at latest. Fragmentary remains of a crocodilian have been found in the Kota limestone and if good specimens can be discovered these may be helpful in dating the Kotas, as Lower Jurassic crocodilians are well known from the European Lias.

Only a small part of the Kota formation has been prospected, and it is hoped that eventually discoveries may be made of the less-sizable members of its terrestrial fauna, to extend our knowledge of the evolution of land vertebrates in Lower Jurassic times.

We thank the authorities of the Indian Statistical Institute, Calcutta, for financing the expedition, and the Institute's director, Prof. P. C. Mahalanobis, and the head of its Research Division, Prof. C. R. Rao, for their encouragement of the work. We also thank the Royal Society for providing a grant to buy field equipment, and Prof. P. B. Medawar and Prof. Hollingworth, University College, London, for their interest and help. We are grateful to Mr. N. V. Raja Reddy, of Bhimaram, and to Dr. Bimalendu Raichaudhury, Mr. T. S. Kutty, and Mr. D. K. Rudra for their assistance in the field work.

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VARIATION OF YEAST RIBOSOMES WITH NUTRITIONAL STATE

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ALTHOUGH a great deal of research has been carried out in recent years on the physicochemical properties of bacterial and yeast ribosomes, relatively little work has been devoted to the discovery of variations in these properties concomitant with physiological changes in the cells of origin. Wolfe[1], and more recently Ashikawa[2], using ultracentrifugal methods, have found that old or starving yeast cells contain predominantly 80*S* particles and that several new components arise when cells are placed into nutrient. I shall present in this article more detailed information on these changes, including electron microscope as well as ultracentrifugal data.

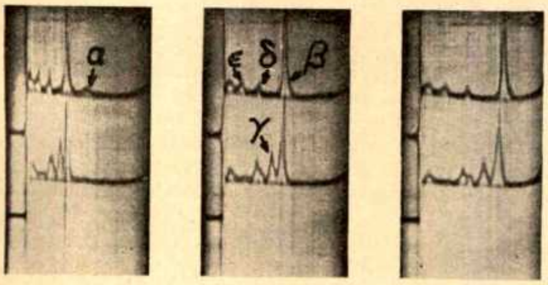

Fig. 1. Ultracentrifuge pattern of yeast ribosomes suspended in Chao and Schachman's buffer at *p*H 7·5. Schlieren photographs are taken 2 min. apart after reaching the speed of 62,500 r.p.m. Top pattern, ribosomes isolated from cells 72 hr. in starvation medium; bottom pattern, from cells 4 hr. in nutrient medium

Cells of baker's yeast (*S. cerevisae*) were cultured in nutrient medium containing 1 per cent yeast extract, 1 per cent peptone and 1 per cent dextrose. The cells were starved of nitrogen in a succinate medium buffered to *p*H 5·0 with potassium hydroxide. All cultures were continuously shaken in Erlenmeyer flasks at 30° C. Samples of cells (about 5 gm. wet) were taken at various times throughout the growth and starvation cycles and hand ground in a mortar with 'Carborundum'. The ribosomes were isolated utilizing the usual centrifugal procedures in the Mg^{++} buffer of Chao and Schachman[3]. Aliquots of each preparation were used for parallel electron microscopy and ultracentrifugal analysis.

Fig. 1 typifies the sedimentation patterns of the ribosomes obtained from physiologically differing cells. The top pattern represents ribosomes from cells after 72 hr. in succinate starvation medium, and the bottom pattern shows a ribosome preparation from cells incubated for 4 hr. in growth medium. Note the complete absence of the γ- or 65*S*-peak from the starved ribosome preparation. Sedimentation constants, corrected for concentration and water viscosity (20° C.),

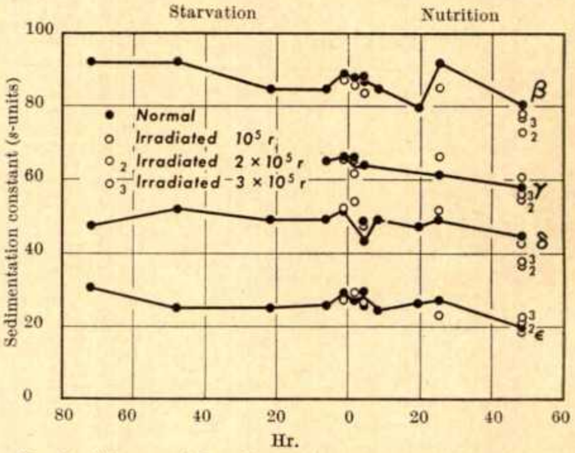

Fig. 2. The corrected sedimentation constants of the various ribosome components as a function of the nutritional state of the original cells

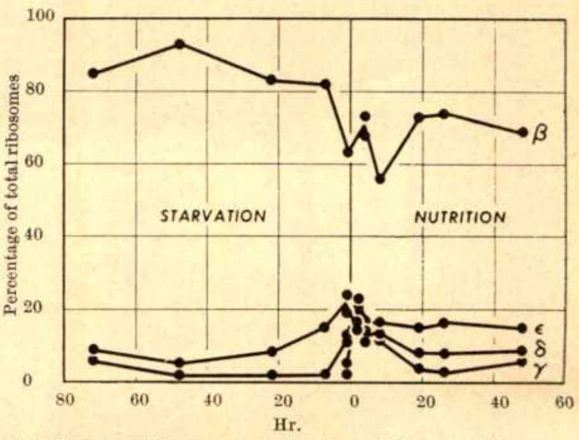

Fig. 3. The relative percentage abundance of the various ribosome components as a function of the nutritional state of the original cells

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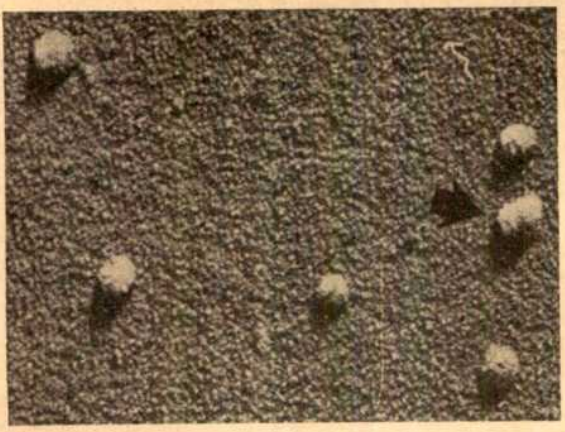

Fig. 4. Electron micrograph of nichrome-shadowed yeast ribosomes. (× *c.* 120,000)

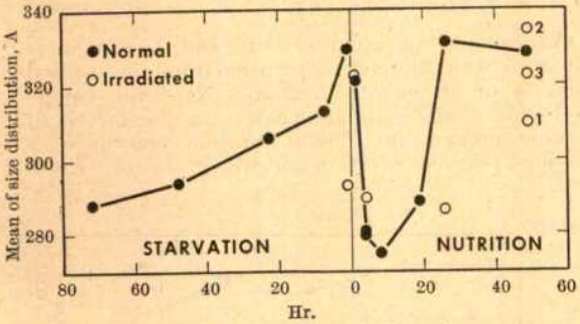

Fig. 5. The mean of size distributions of yeast ribosomes from various nutritional states as calculated from electron microscope observations

and the relative percentage abundances were calculated for the various components. The data are plotted so as to relate these parameters to the physiological state of the cells from which the ribosomes were isolated. Fig. 2 gives the sedimentation constant data for yeast ribosomes. It can be seen that there are four distinct size classes ranging from about 30*S* to 90*S* with the γ-ribosome existing only during the growth phase. Also present, particularly in starved preparations, is the α- or 120*S*- component which does not appear in this figure. Although there are apparent fluctuations of *S* with the nutritional state of the cells, essentially all the data can be fitted with straight lines having a slight negative slope ranging from 0·03 to 0·10*S* per hr. This would suggest that ribosomes isolated from starving cells are larger or denser than their counterparts isolated from cells suspended in nutrient. In the hope of splitting some of the ribosome components, certain samples were irradiated with X-rays. The open circles represent these samples and are seen to be little affected in terms of sedimentation behaviour. Fig. 3 shows the relative percentage of the various components as a function of cell nutrition. The 80*S* particles are always the majority component; however, during starvation these particles represent 85–90 per cent of the total, while during growth they average about 70 per cent. Concomitant with this decrease in 80*S* particles is the appearance of the γ- or 65*S*-ribosome. During early growth phase these particles represent about 20 per cent of the total and decay to just a few per cent within 20 hr. The 120*S* particles have again been omitted from this representation due to their minute abundance and the difficulty of making accurate measurements on the small, diffuse peaks.

Visualization of the various ribosome preparations in the electron microscope revealed no obvious differences in structure among the physiologically varying cells. This should be expected from the rather slight differences noted with the more sensitive ultracentrifugal analysis. Fig. 4 illustrates a typical ribosome preparation after shadowing with nichrome. Note the dimeric particle (arrow) and the clefts in some of the other particles. The sizes of ribosomes from each physiologically differing sample were carefully measured from enlarged electron micrographs and histograms were constructed. Fig. 5 gives the mean of each histogram as a function of cellular nutrition. Each point represents approximately 100 particle measurements. There is a decided drop in mean size at the onset of growth. This would indicate either a general shift of the entire population or the diminution of one or more of the larger-size classes with respect to the smaller classes. The hydrodynamic data indeed show that 65*S* particles appear and that 80*S* particles diminish in relative abundance at this time. The open circles again represent irradiated samples and vary in no interpretable manner from the controls.

The earlier studies of Wolfe[1] and Ashikawa[2] indicating that the hydrodynamic properties of yeast ribosomes are dependent on the physiological state of the cell of origin have been generally supported by this research. Both the sedimentation constants and relative quantity of the various components change in characteristic ways throughout growth and starvation. Histograms of sizes of ribosome measured

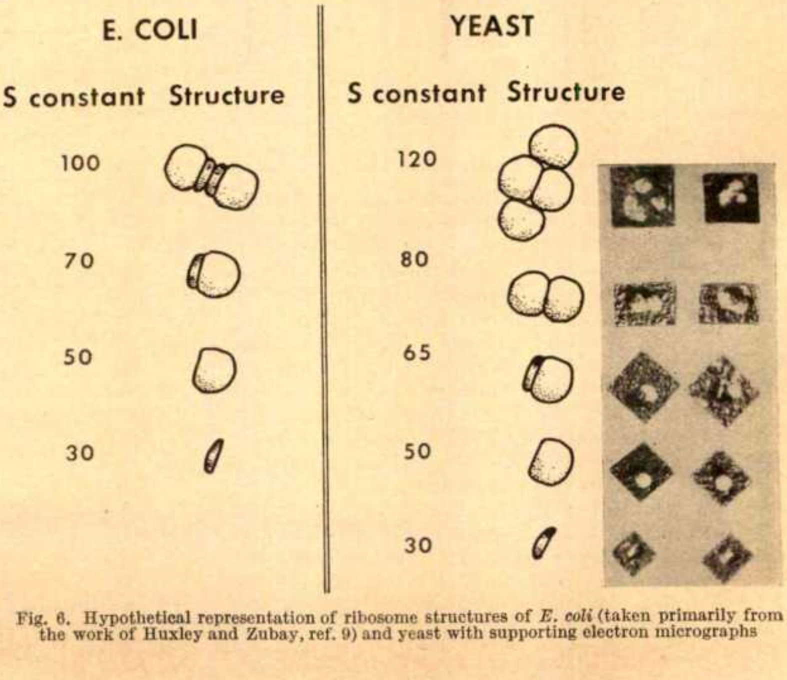

Fig. 6. Hypothetical representation of ribosome structures of *E. coli* (taken primarily from the work of Huxley and Zubay, ref. 9) and yeast with supporting electron micrographs

from electron microscope observations also reflect these changes. The most striking difference between starved and growing cells is the rapid appearance of a 65*S* ribosome at the onset of growth, whereas this component is completely absent in starving yeast cells. Peterman[4] has very recently reported the occurrence of a 65*S* ribosome in a mammalian cancer strain, again linking this particle to active growth processes.

Although the general consensus favours the theory that ribosomes represent the synthetic site for cellular proteins, several dissenting claims have been voiced. Osawa and Hotta[5], in their investigations of turnover procedures utilizing tagged amino-acids, suggest that, in growing yeast cells, "the RNA particles do not represent the precursors for soluble protein". Similar views have been expressed for the *E. coli* system by Roberts *et al.*[6], although a very recent report by McCarthy and Aronson[7], working in Roberts's group, suggests that small RNA (4–8*S*) builds up 20, 30 and 50*S* ribosomes which in turn produce 70*S* particles acknowledged to be active in protein synthesis. Recent work of Tissieres *et al.*[8] indicates that the incorporation of labelled amino-acids in *E. coli* is not uniform over the various ribosome size classes. A special kind of 70*S* ribosome called 'active 70*S*' was shown to incorporate 15–40 times more amino-acid than the 30 or 50*S* particles, and constitutes only 10 per cent of the total ribosome complement. It is possible that the 65*S* yeast ribosome plays a similar part and might help in explaining its rapid appearance during the early growth stage.

Huxley and Zubay[9], working with *E. coli* ribosomes, have shown by the use of positive uranyl and negative phosphotungstic staining techniques that the 100*S* particles constitute two 70*S* particles in dimeric form. The 70*S* units in turn contain two sub-units probably representing 50 and 30*S* particles. It was further shown that 50*S* units could combine under the proper conditions to form 80*S* particles. It is possible that the predominant 80*S* units of yeast are of this structure, and that the 65*S* units are actually the 70*S* units of Huxley and Zubay, the difference being of the order of magnitude of the errors involved in the ultracentrifugal determination. That there may be a significant difference between *E. coli* and yeast ribosomes is substantiated by the observation of a 120*S* component in yeast rather than 100*S*. This may represent the dimerization of 80*S* rather than 70*S* particles. Fig. 6 gives the comparative structures and sedimentation-rates for *E. coli*, derived primarily from the work of Huxley and Zubay, and yeast ribosomes as they are at present believed to exist. It must be stated that no definitive evidence has been obtained favouring the existence of the quadruple 50*S* particle representing the 120*S* ribosome.

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PREPARATION OF CRYSTALLINE *PSEUDOMONAS* CYTOCHROME OXIDASE AND SOME OF ITS PROPERTIES

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FROM the anaerobically cultivated cells of *Pseudomonas aeruginosa*, a cytochrome which has all the properties of a cytochrome oxidase has been purified in an ultracentrifugally homogeneous state, and it was named *Pseudomonas* (*P.*) cytochrome oxidase[1,2]. The *P.* cytochrome oxidase has two hæms, hæm a_2 and a *c*-type hæm, in the molecule, and consequently shows a complicated absorption spectrum. In addition, a preparation of this oxidase acts as a nitrite reductase, and it is very likely that the enzyme is responsible for the nitrite-reducing activity of the organism. The previously reported *P.* cytochrome oxidase preparation, although ultracentrifugally pure, contained some 30 per cent impurities judging from electrophoresis. We recently obtained the enzyme in a crystalline state as described here.

The acetone powder of the cells, cultivated in a submerged culture in the presence of potassium nitrate, was extracted with 0·1 *M* citrate at *p*H 7·0, and the cells were discarded by filtration. To the filtrate obtained, after treatment with rivanol, ammonium sulphate was added until saturation was reached, and the precipitate was collected. The precipitate, after dissolving in a minimum amount of water, was dialysed against water overnight at 5°, and further dialysis was performed overnight against 0·02 *M* ammonium phosphate at *p*H 6·0, at 5°. When the dialysate was passed through an 'Amberlite *CG*-50' column, which had been equilibrated at *p*H 6·0 with 0·02 *M* ammonium phosphate, *P.* cytochrome oxidase was adsorbed on the column, and formed a green band. The resin, on which *P.* cytochrome oxidase was adsorbed, was washed with 0·02 *M* ammonium phosphate (*p*H 6·0) until the eluate became colourless and clear. Then *P.* cytochrome oxidase was eluted by 0·3 *M* ammonium phosphate (*p*H 7·0). The first and last parts of the eluate were

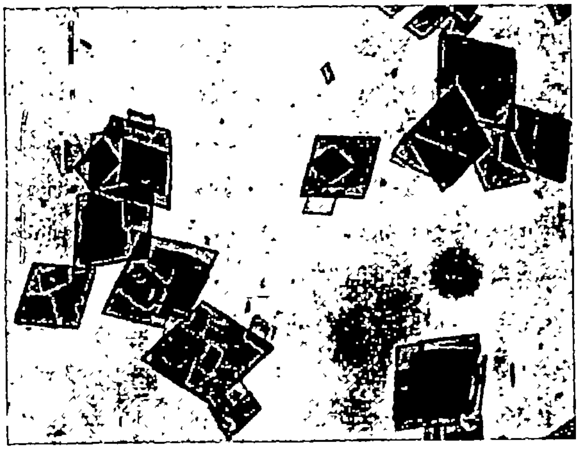

Fig. 1. Photograph of the crystals of *P.* cytochrome oxidase. Twice recrystallized crystals were washed with a small amount of water and then separated from the supernatant. (× 180)

discarded, and the middle part of the eluate, which was green in colour, was used in the subsequent procedure.

Ammonium sulphate was added to the green eluate until saturation was reached. The collected precipitate was dissolved in a small amount of 0·01 *M* phosphate buffer (*p*H 6·0), and followed by centrifugation to discard insoluble material. Solid ammonium sulphate was added to the supernatant until the solution became slightly turbid. The solution was centrifuged, and the precipitate was discarded. The supernatant was allowed to stand at room temperature for 30–60 min., and light-green, diamond-shaped crystals appeared. The collected crystals were dissolved in the 0·01 *M* phosphate buffer (*p*H 6·0), and the recrystallization was carried out by adding ammonium sulphate as described here. The crystals, twice recrystallized, were collected and washed with a very small amount of distilled water, and the crystals separated from the supernatant were used as a *P.* cytochrome oxidase preparation. Among *a*-type cytochromes, cytochrome *a* from bovine heart muscle has been obtained in a crystalline state, but it was found to contain a small amount of detergent[3]. The *P.* cytochrome oxidase, which may be classified as an *a*-type cytochrome on the basis of its absorption characteristics, was crystallized without any detergent as described above.

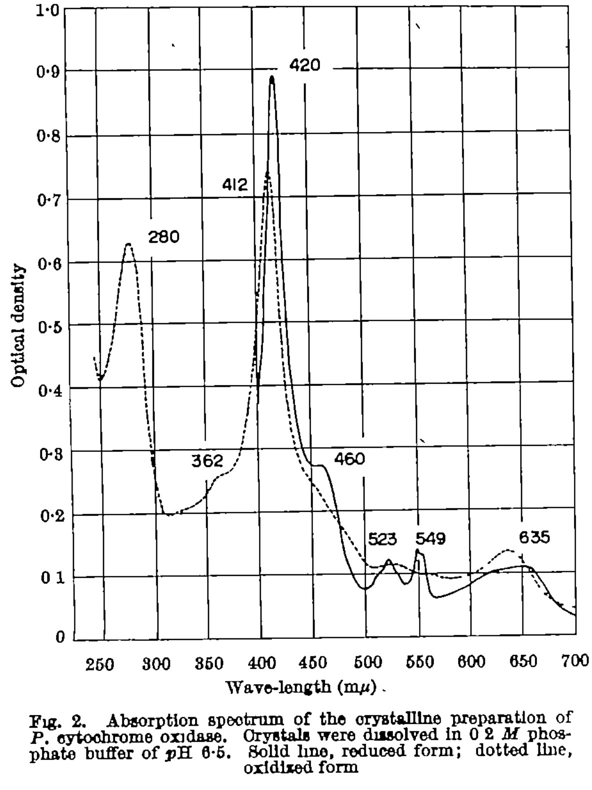

Fig. 2. Absorption spectrum of the crystalline preparation of *P.* cytochrome oxidase. Crystals were dissolved in 0 2 *M* phosphate buffer of *p*H 6·5. Solid line, reduced form; dotted line, oxidized form

The crystalline preparation of *P.* cytochrome oxidase showed a complicated absorption spectrum (Fig. 2). There were absorption peaks at 280, 412, 525 and 635 mμ, and humps at 362 and 562 mμ, in the oxidized form. In the reduced form, there were peaks at 420, 523 and 549 mμ, and humps or shoulders at 460 and 554 mμ, and a plateau between 620 and 660 mμ. The remarkable difference between the *P.* cytochrome oxidase preparation described previously and the one obtained here is that the previous preparation showed a peak at 625 mμ by reduction, while, in the crystalline preparation, the peak at 635 mμ in the oxidized form was depressed by reduction with sodium dithionite, and a plateau appeared between 620 mμ and 660 mμ in the reduced form. By the re-oxidation of the crystalline preparation with potassium ferricyanide, the absorption peak at 635 mμ appeared again. It was also noticeable that the peak at 554 mμ in the previous preparation became a shoulder in the crystalline preparation. In the present preparation, $E_{412\,m\mu}/E_{280\,m\mu}$ was very high (1·2), which is not comparable with the value for the previous preparation ($E_{408\,m\mu}/E_{278\,m\mu} = 0{\cdot}87$) (where E is the optical density at the indicated wave-length). This indicates that the crystalline preparation is very pure. By reduction, a hump appeared at 460 mμ, which may be the γ-band due to hæm a_2.

By treatment with acetone containing 0·12 *M* hydrochloride, the precipitated protein showed an absorption spectrum of a *c*-type cytochrome. The hæm extracted in the acetone layer was confirmed as hæm a_2 by the formation of its pyridine hæmochromogen. These facts confirm that *P.* cytochrome oxidase itself has hæm a_2 and a *c*-type hæm in the molecule, as reported previously.

It was also reported previously that *P.* cytochrome oxidase had general properties of a cytochrome oxidase, and acted also as a nitrite reductase[4,5]. These properties were confirmed by using the crystalline preparation. Therefore, these enzymatic properties were for the single enzyme, *P.* cytochrome oxidase (= *P.* cytochrome *c*–551 : oxygen or nitrite oxidoreductase[6]). Thus, it is very likely that the *P.* cytochrome oxidase is responsible for the nitrite reductase activity of the organism.

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LETTERS TO THE EDITOR

GEOPHYSICS

Atmospheric Blow-up at the Auroral Zone

ELSEWHERE[1,2] I have examined joule heating of the upper atmosphere by those ionospheric electric currents which cause geomagnetic disturbance. Some further consequences were recently reported[3]. It is the purpose of the present communication to point out that in regions of the auroral ionosphere above about 150 km. altitude the scale height may increase 5- to 10-fold during geomagnetic disturbance. This would cause population of the exosphere to great heights with ionospheric gases and even loss of a portion of such gases to the interplanetary medium.

Joule heating in the auroral ionosphere is rapid. In the event of no loss of heat by conduction or convection it would cause the scale height at 160 km. altitude to double in a matter of seconds[1,2], whereas the electric currents responsible flow for periods of order 0·5 hr. Let us estimate the effect of convection and conduction on the process of increasing the scale height. Suppose an auroral electrojet is 100 km. wide in latitude cross-section. Winds of the order of 100 msec.$^{-1}$ are associated with the likely gradients of pressure[2]. Such winds would take 10^3 sec. to replenish the air in the electrojet. Thus convection would not inhibit the process of increase of scale height in an electrojet of this size. Now conduction proceeds better at greater heights[4]. Temperature equilibrium through conduction may be established to distances of the order of 1,000 km. in 10^3 sec. at 1,000 km. altitude but only to distances of the order of 10 km. at 200 km. altitude in the same time. Thus a steady model of the dimensions in space and time considered here yields a good approximation to the actual magnetic disturbance conditions in the auroral zones.

It has been shown elsewhere[1,2] that during such steady disturbance the pressure at 200 km. altitude in the auroral ionosphere can be equal to that at 140 km. in temperate latitudes. This represents an increase in scale height at 150 km. by a factor of about five. This calculation was based on only moderate disturbance. At times of increased disturbance the scale height could change by a factor 10. At these times the temperatures at 200 km. altitude would be increased by amounts of order 1,000° K. Such increases in temperature are sufficient to cause the ejection of hot neutral particles upwards and outwards to populate the magnetosphere, where they could be afterwards ionized by solar radiation to modulate the density of the whistler medium. These density fluctuations would in turn cause variations in the low-frequency phenomena in the magnetosphere such as whistler and dawn chorus propagation. Such variations in density of the background medium would modulate the rate of scatter and of degradation of energy of geomagnetically trapped radiation.

The escape of gases from the auroral zone to interplanetary space is also inevitable with such heating[5], the lighter molecules hydrogen and helium having the greatest probability of escape. This would explain the representations of earth gases in the spectrum of the counter glow (Gegenshein)[6] and also their enhancement with geomagnetic disturbance. Conceivably joule heating in the auroral zones plays an important part in the problem[4] of the helium balance in the atmosphere.

The theory suggests that static models of the ionosphere above 100 km. and the exosphere may only be adequate for an average picture. This region is dynamically dependent on geomagnetic disturbance and at a given instant its structure may differ significantly from the average. A bulge must develop in the ionosphere above disturbance electric currents. Moreover, since the movement of ionization across the geomagnetic field is inhibited in and above the F region of the ionosphere, field aligned inhomogeneities in electron density would project upwards from the parent electric current.

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PHYSICS

Optical Refractivity of Free Electrons

A NUMBER of experiments have been reported recently in which electron densities have been determined from the refractivity of free electrons in the visible region of the spectrum[1-3]. In none of these, however, has an independent check been made on the values obtained. We wish to report some recent measurements of the optical refractivity of free electrons in a hydrogen plasma in which simultaneous determinations of electron density are obtained from the Stark effect broadening of H_β and H_γ (ref. 4) and from a measurement of the absolute continuum intensity. A measurement of the electron temperature from the ratio of the total line intensity of H_β to the continuum[5] is used to confirm the high degree of ionization obtained and the dominant role of the free electrons in determining the refractive index.

The plasma was produced in an electromagnetic T-tube[6] and the refractivity measured using a Mach–Zehnder interferometer. A commercial xenon flash-lamp was used to produce the fringes which were recorded on a streak camera[7], with a time resolution of $\sim$ 0·1 μsec. The fringe system was perpendicular to the slit of the streak camera and the fringe shift (Fig. 1) recorded as a function of time as the shock passed the plane of observation. An interference filter ($\lambda \sim 5530$ Å.) in front of the slit served to overcome the self-luminosity of the plasma.

The refractive index of free electrons is given by classical dispersion theory. The frequency of light is much greater than the plasma frequency under the

Table 1

Fringe	Interferometer data		N_e ($\times 10^{16}$ cm.$^{-3}$)	Continuum measurement N_e ($\times 10^{16}$ cm.$^{-3}$)		Line width data N_e ($\times 10^{16}$ cm.$^{-3}$)		Electron temperature (eV.)	Degree of ionization
	Shift	Mean		λ 5,200	λ 4,530	H_β	H_γ		
2	0·34 ± 0·03	0·33 ± 0·03	4·5 ± 0·4	5·6 ± 0·7	5·4 ± 0·7	4·8 ± 0·6	4·8 ± 0·7	1·8	~ 100%
3	0·33 ± 0·03								
4	0·32 ± 0·03								

conditions of the experiment, and the refractivity $(n - 1)$ is given by:

$$(n - 1) = 4\cdot46\ 10^{-14}\ \lambda^2 N_e$$

where N_e is the electron density. Collisions of electrons with heavy particles and the effect of magnetic fields can be neglected. At the high degree of ionization achieved the contribution of atomic and molecular hydrogen to the refractivity is less than 1 per cent. The contribution of the protons is negligible, and the fringe shift shown in Fig. 1 is almost entirely due to the free electrons.

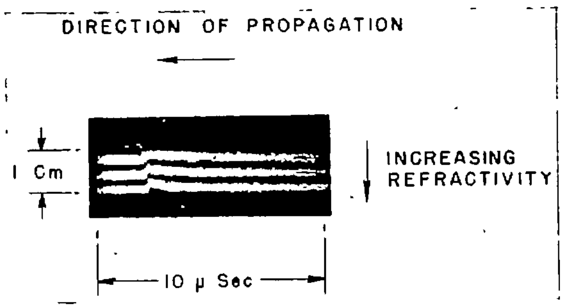

Fig. 1. Monochromatic interferogram (λ = 553 mμ) for a Mach 20 shock into hydrogen at an initial pressure of 0·5 mm. mercury

In Table 1 the values of electron density obtained from the refractive index of the free electrons are compared with values obtained from Stark effect broadening and absolute continuum intensity, agreement being found to within the accuracy of the measurements.

This work was carried out while one of us (S. A. R.) was on leave at the U.S. Naval Research Laboratory, Washington, D.C.

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Electron-Hole Scattering at High Injection-Levels in Germanium

THE principal scattering mechanism which determines the mobilities of the charge carriers in pure semiconductors over a wide range of temperature is the interaction with lattice vibrations. According to the calculations of Fletcher[1], interaction between the Coulomb fields of electrons and holes as they drift in opposite directions leads to a progressive reduction in their conductivity mobilities as the injected carrier density increases above 10^{16} cm.$^{-3}$ in germanium at room temperature. Experiments in this laboratory have demonstrated this effect, and a value $m_n m_p/(m_n + m_p) = 0\cdot15\ m_0$ has been obtained for a combined effective mass for electron-hole scattering (m_0 is the mass of an electron in free space).

In my experiments the injected carrier density and conductivity for the central lightly doped (L) region of a p^+–L–n^+ structure are calculated from the p.d. across the structure during and after a current pulse in the forward direction; the technique utilized is similar to that of Lederhandler and Giacoletto[2]. From each measurement a value for the sum of electron and hole mobilities, $\bar{\mu} = \mu_n + \mu_p$, is obtained; the results for one sample are shown in Fig. 1 as a function of the injected carrier density n. Departures from uniformity of the excess carrier distribution in the L-region are taken into account.

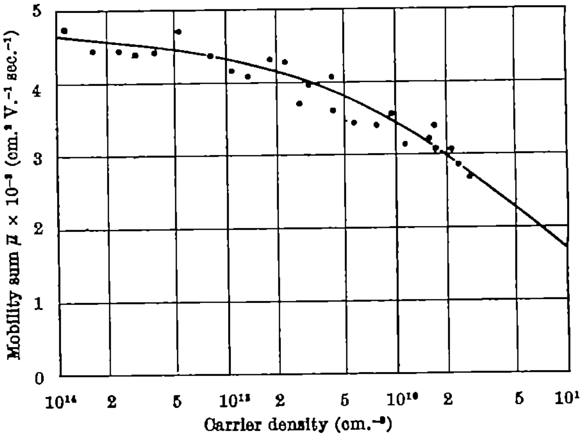

Fig. 1. The sum of electron and hole conductivity mobilities as a function of injected carrier density in germanium at 300° K.

The observed decrease in $\bar{\mu}$ with increasing carrier density is attributed to the effects of electron-hole scattering. In order to separate contributions to the mobility sum by scattering due to lattice vibrations and other imperfections on one hand ($\bar{\mu}_0 = \mu_{n0} + \mu_{p0}$), and by electron-hole scattering on the other hand (μ_{np}), it was assumed that the mobilities may be compounded, as are those for lattice scattering and ionized impurity scattering[3]. The values of μ_{np} calculated in this way are found to be inversely proportional to the carrier density n if $\bar{\mu}_0 = 4{,}800$ cm.^{2} V.$^{-1}$ sec.$^{-1}$, a value consistent with the alloying technique used in the fabrication of the p^+–L–n^+ diode samples. From a series of observations on three samples of varying dimensions and carrier lifetimes we find the empirical relationship:

$$\mu_{np} = 2\cdot5 \times 10^{20}\ n^{-1}\ \text{cm.}^2\text{V.}^{-1}\ \text{sec.}^{-1}$$

for germanium at 300° K., where n is in units of cm.$^{-3}$.

Chapman's classical analysis[4] of the mutual diffusion of two groups of oppositely charged particles has been applied[1] to the interaction of electrons and holes in a semiconductor; in this theoretical treatment, a cut-off approximation was made to the Coulomb fields of the scattering charge carriers. If the screening[5] of distant scattering charges by electrons and holes is taken into account, we obtain the theoretical expression:

$$\mu_{np} = \frac{3K^2(kT)^{3/2}}{\pi^{1/2}2^{5/2}nq^3}\left(\frac{m_n m_p}{m_n + m_p}\right)^{-1/2} \left[\ln \frac{12\pi K(kT)^2}{h^2q^2n}\left(\frac{m_n m_p}{m_n + m_p}\right)\right]^{-1}$$

where K is the dielectric constant, and m_n, m_p are the effective masses of electrons and holes, assumed isotropic; q is the electronic charge.

My experimental result is in agreement with theory if we take the combined effective mass term $m_n m_p/(m_n + m_p) = 0{\cdot}15\ m_0$, and from this reasonable value we conclude that the interpretation of our measurements of the mobility sum μ in terms of electron-hole scattering is justified. The continuous line in Fig. 1 is obtained from the resultant theoretical expression for μ_{np}, compounded with the chosen value of $\overline{\mu}_0$.

The above results are relevant to theories of the current-voltage characteristics of semiconductor rectifiers; for example, the measurements presented in Fig. 1 extend to current densities of 100 amp. cm.$^{-2}$ in the diode, at which stage the mobility sum $\overline{\mu}$ has fallen to 58 per cent of its value at low injection-levels. The experiments are being continued over a range of temperatures with both germanium and silicon, with the aim of further verifying theory and the determination of the combined effective mass term.

I thank W. M. Eades for the fabrication of samples and for assistance with the measurements.

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CRYSTALLOGRAPHY

Crystal Growth and Cell of Cellotriose

Examination of the detailed crystal structure of the lower oligosaccharides is of importance in elucidating the structure of cellulose. The single glucose ring exists in three forms, α-glucose, β-glucose and α-glucose monohydrate. The detailed crystal structure of these compounds has already been given[1-3]. The second member of the series is cellobiose in which two β-glucose rings are linked 1 to 4; the detailed crystal structure has recently been given by Jacobson, Wunderlich and Lipscomb[4]. The third member of the series is cellotriose in which three β-glucose rings are joined with two 1 to 4 linkages.

The study of cellotriose is complicated by the extreme difficulty in producing single crystals which are big enough to be used for X-ray diffraction. The two accepted factors influencing the production of single crystals are the rates of nucleation and growth. An extensive study of the growth behaviour of the lower oligosaccharides shows that the nucleation-rate is of primary importance. These substances are soluble in water, the solubility decreasing as the series is ascended. Standard methods of crystal growth were used, namely, precipitation using different solvents, for example, acetone and various alcohols, slow cooling with varying degrees of saturation and evaporation from assorted shapes of vessel under various conditions. In each case and with all materials a high nucleation-rate was observed. The effect was more pronounced as the series was ascended, but even glucose shows a significantly high nucleation-rate and stable saturated solutions such as can be obtained with sucrose are not easily produced. With cellotriose the very high nucleation results in large numbers of very small crystals.

It is a reasonable assumption that the high nucleation results from comparatively large-sized aggregates of molecules in the solution. These would provide a large number of nuclei on which growth could readily take place once the solutions became saturated. It has been pointed out by many observers, for example, Egli and Zerfoss[5], that superheating a solution does reduce the nucleation. The effect of superheating the solution is to reduce the size of molecular aggregates and probably in many cases to disperse them altogether.

Solutions of cellotriose in water (various concentrations) were sealed in thick-walled 'Pyrex' capillary tubes, which were then heated under oil to temperatures approaching 200° C. After this curing process the solutions were treated in the normal way to produce crystals. No observable improvement resulted from slow cooling or evaporation methods possibly because the aggregates had time to reform. However, with solutions immediately treated with acetone, small crystals in the form of curved plates were precipitated. The plates were rectangular in section of dimensions approximately $\frac{1}{2}$ mm. × $\frac{1}{4}$ mm. and of extremely small thickness estimated to be of the order of μ. Their curvature, which gives them the appearance of propellers under the microscope, must arise from growth conditions and is probably due to solution pressure resulting from comparatively fast growth.

It was found possible to mount these crystals in the normal way for X-ray diffraction. Oscillation pictures through 10° were taken about each crystal axis in turn using copper $K\alpha$ radiation. Very long exposures (about 100 hr.) were necessary to produce a reasonable number of detectable reflexions, and the quality of the pictures was poor because of large background and extension of reflexions due to the misorientation produced by their curvature (fibre effect). It was found possible to identify each crystal axis as consistent with a crystallographic axis in an orthorhombic unit cell:

$$a = 29{\cdot}6\ (\pm\ 0{\cdot}1)\ \text{Å.}, b = 15{\cdot}3\ (\pm\ 0{\cdot}1)\ \text{Å.}, \quad c = 4{\cdot}91\ (\pm\ 0{\cdot}03)\ \text{Å.}$$

This gives four molecules in the unit cell and a calculated density of 1·50 (± 0·05) gm. cm.$^{-3}$ compared with an experimental value of 1·56 gm. cm.$^{-3}$.

An examination of the absences is complicated by the large background, and it is only possible to say that a particular reflexion is definitely present. The (001) is observed to be present, and it seems likely that $(0k0)$, k odd and $(h00)$, h odd are absent with no other systematic absences. If these conclusions are correct there is only one possible space group $P2_12_12$. Assuming the size of the cellotriose molecule as approximately 15 Å. × 5 Å., a very reasonable packing arrangement can be obtained with this space group.

It is hoped to produce better single crystals of cellotriose either by an improvement of the present method or by some other method and proceed to a detailed crystal analysis.

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Optical Fourier Synthesis for Non-centrosymmetric Projections of Crystal Structures

THE production of an X-ray image showing details of the atomic arrangement in a crystal is not possible because X-radiation cannot be focused; the possibility of representing the diffracted X-ray beams by beams of visible light which can be focused has therefore always been attractive. The first step is, of course, to obtain complete information about the X-ray spectra; it is a fairly simple matter to measure their amplitudes, but it is not possible to record their relative phases experimentally. The determination of the phases is, therefore, the major problem in X-ray diffraction investigations of crystal structures. Once the phases have been determined by one of the many techniques available the production of an 'image' by Fourier synthesis is possible.

The summation process is most often carried out with the help of a digital computer; but several alternative methods have been suggested, for example, the special electronic computer designed by Pepinsky[1], which presents the result as a contoured electron-density map on a cathode-ray tube. The use of optical methods has been described by several workers[2-4]; but these were restricted in practice to projections of structures involving only phase of 0 and π, that is, to centrosymmetrical projections.

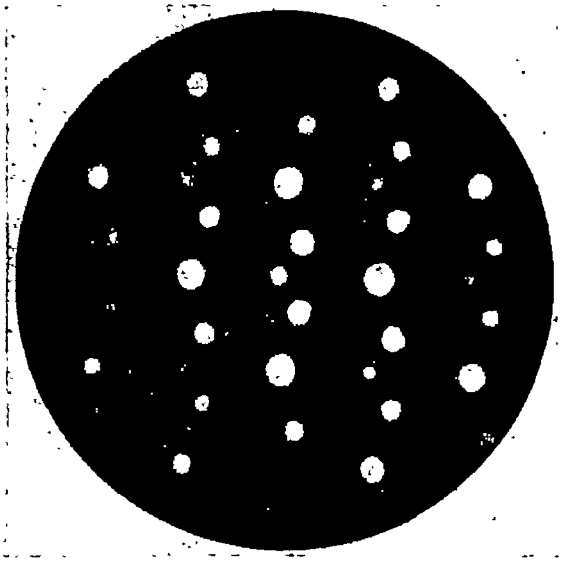

Fig. 1. Optical Fourier synthesis of sodium nitrite Na = N$<^{O}_{O}$

Recently, in extending optical-transform methods to three dimensions, it was necessary to devise a technique providing continuous phase control of light beams[5]. The essential feature of the method is that phase differences in the diffracting mask are obtained by the relative orientations of half-wave plates in a beam of circularly polarized light. At the same time a very satisfactory method for controlling the amplitude of light beams—using gauzes of different transmission factors etched from copper foil—was developed. With both amplitude and phase-control available it is a relatively simple matter to carry out optical Fourier syntheses for structures having non-centrosymmetric projections and the possibilities have been demonstrated by applying the technique to a simple known structure.

Crystals of sodium nitrite ($NaNO_2$) belong to the space group *Imm*; there are two molecules in the unit cell which has dimensions $a = 3{\cdot}55$ Å., $b = 5{\cdot}38$ Å., $c = 5{\cdot}56$ Å. A diffracting mask, representing the $0kl$ reflexions in amplitude and phase, was made according to Carpenter's results[6], and Fig. 1 shows the optical Fourier summation which was obtained. Once a mask representing the observed X-ray amplitudes for an unknown structure has been made any postulated set of signs may be tested very quickly. However, for structures having larger unit cells containing more atoms, some of them perhaps overlapping in projection, the optical technique becomes less favourable and the photographs obtained are not so easy to interpret. In view of these difficulties and because of the general availability of electronic computing facilities the optical method of performing Fourier summation is unlikely to be of great practical value; it remains, however, as an elegant demonstration of the basic physics of Fourier synthesis.

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Structure of Calcium Aluminate, $12CaO.7Al_2O_3$

Bussem and Eitel[1] determined the unit cell, space group, and approximate structure of calcium aluminate. From the unit cell (body-centred cubic, $a = 11{\cdot}95$ Å.) and density ($\simeq 2{\cdot}69$ gm./c.c.) the cell contents were fixed as $Ca_{24}Al_{28}O_{66}$. This gives a CaO : Al_2O_3 ratio of 12 : 7 and differs only slightly (0·71 wt. per cent Al_2O_3) from the 5 : 3 ratio proposed on chemical grounds[2]. The most probable space-group appeared to be T_d^6, or $I\bar{4}3d$. Only 64 of the 66 oxygen atoms in the unit cell could be placed in this space-group; the remaining two were assumed to be distributed statistically. Because of this, the structure has been accepted with reservation, and it seemed worthwhile to check it, especially as more powerful methods of structure analysis are now available.

A $12CaO.7Al_2O_3$ preparation (see below) showed only one phase under the petrographic microscope and gave a powder pattern agreeing with that in the literature[1,3]. An equant single crystal was selected for the structure analysis; its rotation photograph matched the powder photograph of the bulk material taken on the same camera. *hk*0, *hk*1, *hk*2 and *hk*3 Weissenberg photographs gave $a = 11 \cdot 98$ Å., in good agreement with Bussem and Eitel's value, and confirmed their space-group.

A series of graduated intensity *hk*0 Weissenberg photographs were taken using unfiltered molybdenum radiation; the intensities of four *h*00 and fifty-two *hk*0 reflexions were measured by visual comparison with an intensity strip. Structure factors calculated from these agreed well ($R \simeq 25$ per cent) with those calculated from Büssem and Eitel's structure, which is, therefore, essentially correct.

$12CaO.7Al_2O_3$ has recently been found to contain 'zeolitic' water[4,5], which is retained up to very high temperatures (1,000–1,100° C.). Crystals prepared at higher temperatures and cooled rapidly in air are anhydrous and do not hydrate readily in air at room temperature. They will, however, hydrate on heating at 400–500° C. This unusual behaviour might be caused by unsatisfactory co-ordination of some of the cations, possibly connected with the presence of the two 'surplus' oxygen atoms, the location of which is still uncertain.

The crystals used in this work were prepared by heating an intimate mechanical mixture of calcium carbonate and alumina (in the ratio 12 : 7) to 1,200° C. for 12 hr., cooling and grinding the sintered cake, and reheating to 1,390° ± 10° C. for several days. The crucible was cooled quickly. Dynamic weight-loss curves showed that the product was essentially anhydrous. It is proposed to refine the structure, and also to collect data for the hydrated material in the hope of locating the 'surplus' oxygen atoms and the water. Independent chemical investigations to establish the CaO/Al_2O_3 ratio and check the melting behaviour of the compound are also being undertaken.

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METALLURGY

Dendritic Growth of Graphite from Melts

Observations on the dendritic growth of graphite from nickel–carbon melts have recently been made in this laboratory. Nickel shot containing 0·1 per cent iron and 0·005 per cent cobalt was melted in graphite crucibles. Temperature was held at 1,450° C. to saturate the melt and attain hypereutectic composition. Samples were cooled in each case at the rate of 15° C./min.

A section taken vertically through a solidified button (Fig. 1) displays a micro-structure comparable with that shown by Nipper[1] for cast iron. Extremely large discontinuous plates of primary graphite are observed, separated from eutectic graphite by the α-phase.

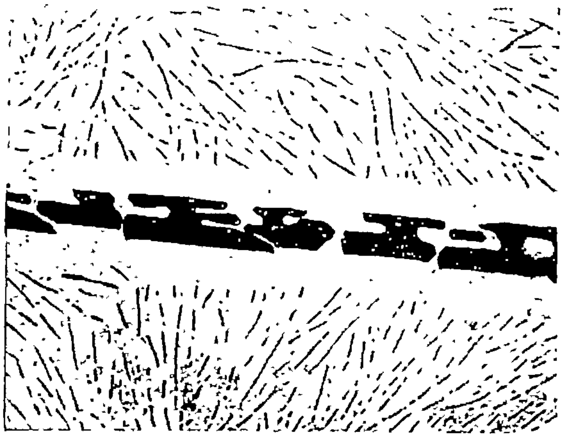

Fig. 1. Unetched. (× c. 75)

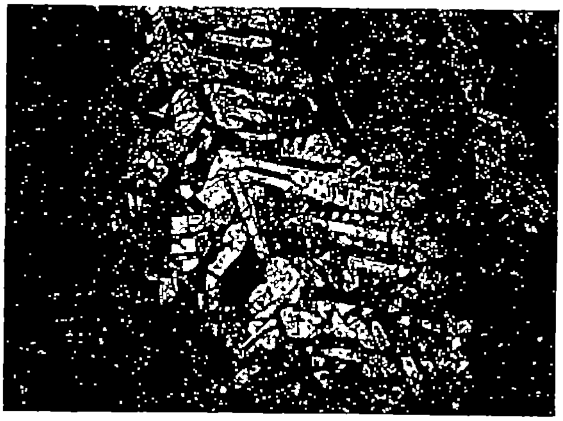

Fig. 2. (× 45)

Crystals of graphite from several of the solidified buttons were extracted by dissolving the specimens in a solution of 1 : 1 nitric acid and by filtering. The large primary flakes of graphite were separable from the fern-like eutectic graphite. These flakes were approximately 2–5 mm. in cross-section and 0·5 mm. thick and exhibited a variety of growth markings on their surfaces. Observations on the manner of growth of the crystals were readily made by removing successive layers of the crystals with 'Scotch' tape.

Fig. 2 shows a characteristic dendritic interior of a crystal with branching occurring in the close-packed plane according to the trigonal symmetry of graphite. In some places, remnants of growth layers from neighbouring sections adhere to the dendrite arms. In Fig. 3, which shows part of the crystal of Fig. 2 at higher magnification, large growth steps are clearly visible. Flat two-dimensional dendritic branching of the steps is observed, the dendritic arms of each step growing independently over the underlying step.

The average step height in the crystal interior was 0·01 mm. while at the surface smaller steps of 0·001 mm height were measured. At the advancing face of the main arm of the crystal, small steps which form the macro-steps were visible.

Careful observation of the upper surfaces of the crystals by light microscopy and by phase-contrast microscopy has so far failed to reveal any growth features characteristic of screw dislocations. Instead, systems of concentric circular steps or hexagonal steps have been observed.

Our observations differ from those summarized by Saratovkin[2], who classified primary graphite crystals grown from melts as "foliated crystals". These were

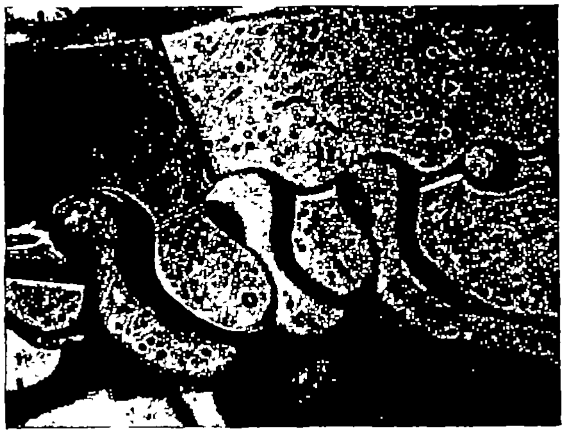

Fig 3. (× 450)

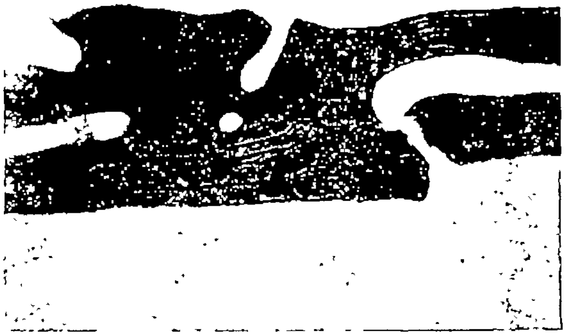

Fig. 4. (× c. 260)

considered as being built up of plates in which screw dislocations play a part in the growth mechanism. Impurity blocking of the screw front would lead to formation of a branch while plates growing from the branch would complete the layered dendritic appearance.

While growth patterns due to screw dislocations have been observed on graphite found in the natural state[3] and on graphite formed by solid state graphitization[4], no direct evidence is available for participation of screw dislocations in the growth of graphite from the melt.

The dendritic graphite observed in the present experiments appears to grow by the repeated nucleation of steps which advance as a series of macro-steps into the liquid. The aggregation of small steps into macro-steps suggests kinetic factors associated with the flow of steps. The layered structure of the crystal is related to the macro-steps and may be observed on etching a prepared microsection in hydrogen peroxide (Fig. 4).

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RADIATION CHEMISTRY

Radiation Decomposition of Sodium Chlorate

THE decomposition of alkali chlorates by ionizing radiation has already been examined. In 1959, Heal[1] identified chloride, chlorite, hypochlorite, oxygen, dichlorine hexoxide and chlorine dioxide as the decomposition products from solid potassium chlorate subjected to X-rays. Baberkin[2] reported only chloride, oxygen and chlorite as products of the decomposition of solid potassium chlorate by cobalt-60 γ-rays. The yield of chlorite was 1·2 molecules formed/100 eV. of energy absorbed. In samples afterwards heated to 200° C. the yield of chlorite decreased to 0·8 molecule/100 eV. The decrease in the yield of chlorite was attributed to its decomposition into chloride and oxygen. An investigation of the decomposition of solid sodium, potassium and barium chlorates by cobalt-60 γ-rays has been carried out by Burchill[3]. Oxygen, chlorite and chloride were identified as major products in solutions of the irradiated salts, with smaller amounts of chlorine dioxide, hypochlorite and perchlorate being formed.

The work reported here concerns a more detailed investigation of the effects of annealing previously irradiated sodium chlorate. Irradiations were carried out at room temperature in a commercial cobalt-60 irradiation unit. It was found by chemical analysis of solutions of the irradiated salt that $G_{O_2} = 1·95$, $G_{ClO_2} = 1·34$, $G_{Cl^-} = 0·95$, $G_{ClO_2} = 0·23$, $G_{ClO^-} = 0·21$, and $G_{ClO_4^-} = 0·22$.

Irradiated crystals were heated for varying times at a given temperature, and the effect of this thermal annealing on the G values of all the products was studied. The most striking change was observed to occur in the chloride yield. The variation in G_{Cl^-} is shown in the figure. On annealing at 50°, G_{Cl^-} reaches a maximum, $G_{Cl^-} = 1·38$, then falls, levelling off at a plateau value $G_{Cl^-} = 0·55$. At the higher temperatures of 100°, 185° and 210° C., the yield of chloride increases, but the later decrease is not observed.

The results may be interpreted in terms of the formation of a high-energy lattice defect structure produced by irradiation, which normally yields chlorate ion when the crystals are dissolved. How-

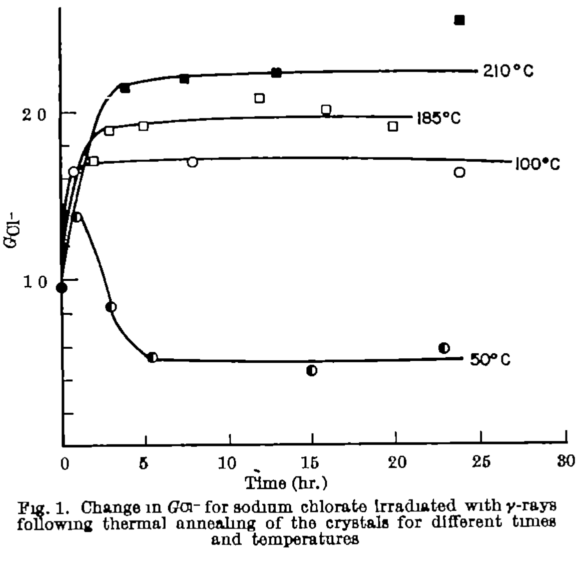

Fig. 1. Change in G_{Cl^-} for sodium chlorate irradiated with γ-rays following thermal annealing of the crystals for different times and temperatures

ever, when the solid is heated, sufficient energy is provided for the defect structure to decompose to chloride on heating. At 50° C. the later decrease in chloride is presumably due to recombination of chloride with the oxygen atoms to reform chlorate. At higher temperatures, this recombination is less complete, probably because of competing reactions such as diffusion of oxygen atoms away from the damage site or by combination of oxygen atoms to form oxygen molecules.

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Electron Spin Resonance in γ-Irradiated Deoxyribonucleic Acid

DURING the past five years radiation-induced free radicals in deoxyribonucleic acid (DNA) have been the object of studies using electron spin resonance[1-5]. In particular, some work in this field was undertaken in our laboratory[6] with the main result that the yield of free radicals from γ-rays depends on the dose-rate.

The present communication summarizes some new results obtained using DNA from trout sperm which, irradiated in air at a temperature of 30° C. with γ-rays of cobalt-60, gave rise to a remarkable structure of the resonance line. The sample contained $0{\cdot}1$ per cent by weight of protein, approximately 20 per cent of water and a trace of sodium acetate. A conventional X-band electron spin resonance spectrometer with reflexion cavity was used. The external magnetic field was modulated at 416 c./s. and the first derivatives of the absorption curves were recorded after selective amplification and phase sensitive detection.

Fig. 1 shows a typical spectrum recorded 2 hr. after irradiation at a rate of 5×10^{5} r /hr. to a dose of 3×10^{7} r It consists of a central peak and two less-intense non-symmetrical side peaks. The central peak has a width at maximum slope of 10 œrsteds and its *G* value is $2{\cdot}003 \pm 0{\cdot}003$. The number of free radicals per gram measured from the total area under the curve is $3{\cdot}6 \times 10^{15}$. The radiation yield *G* is 2×10^{-4} as compared with 9×10^{-4} obtained previously by us[6] with commercial samples of DNA from herring sperm irradiated in air at the same rate. It should be mentioned that yields of 9×10^{-4} and 2×10^{-1} have been obtained with γ-rays by Blumenfeld[3] and Alexander[5] respectively on DNA's of different origins. However in these cases, the irradiations were performed *in vacuo* so that only doubtful comparisons may be drawn. We have indeed shown that the *G* values obtained even with identical samples of DNA from herring sperm irradiated by example at a rate of $2{\cdot}7 \times 10^{4}$ r./hr. to a dosage of 34×10^{6} r. are greatly affected by the external conditions[7] and are in fact much greater *in vacuo* (24×10^{-4}) than in air ($1{\cdot}9 \times 10^{-4}$).

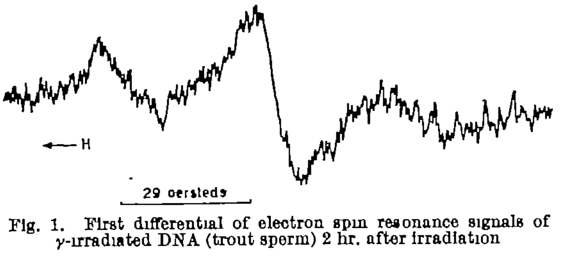

Fig. 1. First differential of electron spin resonance signals of γ-irradiated DNA (trout sperm) 2 hr. after irradiation

In the present experiments, both the shape and the intensity of the resonance line was found to vary with time. Four hours after the end of irradiation the central line was practically unchanged, though the heights of the derivatives of the side lines had decreased significantly. But, after 24 hr., the central line width had become 14 œrsteds whereas the side lines were so smeared out and the signal-to-noise was so bad that they could not be measured with any accuracy. In these conditions, the total number of unpaired spins may only be tentatively estimated as being 45 per cent less than in the initial state.

We thank Prof. J. Duchesne for helpful discussions, Prof. J. A. V. Butler and Dr. P. Alexander for samples of DNA, and the Centre d'Etude de l'Energie Nucléaire, Mol, for providing irradiation facilities.

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CHEMISTRY

Catastrophic Failure of Stressed Polyethylene Terephthalate

IT has recently been reported[1] that nylon and 'Plexiglas' plates, if subjected to a bending stress in a vice, will give rise to catastrophic failure if the point of greatest tension is treated with a drop of some suitable liquid. It was concluded that the cohesive forces in the plane of greatest tensile strain collapse via the action of wetting and adsorption processes.

Similar behaviour occurs with amorphous polyethylene terephthalate sheet; but here a somewhat different mechanism for the cohesive breakdown is suggested.

If a strip of sheet (approximately $0{\cdot}1 \times 1 \times 5$ cm.) is given a bending stress by wrapping round a steel rod ($1{\cdot}5$ cm. diam.) and then held firmly in a vice, it will initially become opaque in the area of treatment with a spot of acetone, and ultimately fracture.

It has been shown[2,3] that a series of organic liquids will induce crystallization in amorphous polyethylene terephthalate, particularly if the solubility parameters of the liquids lie within a certain critical range. This process of crystallization is accompanied by an increase in opacity. It is therefore suggested that in the case of the stress-cracking described above, the acetone, or indeed presumably any liquid the solubility parameter of which lies within the permitted range, induces local crystallization. This creates a tendency to closer packing of polymer chains on the outer surface of the strip which sets up further stresses in addition to the already substantial bending stresses, which in turn may lead to fracture of the sample at the point of greatest tensile strain. Presumably the thicker the strip the more catastrophic will appear the breakdown. Although a similar explanation may be valid for certain nylons where some latent amorphous but potentially crystallizable material may exist, it is difficult to see how such an explanation would apply to an essentially amorphous polymer with no crystallization tendencies.

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A Total Resolution of Aspartic Acid Copper Complex by Inoculation

RESOLUTION by inoculation of a supersaturated solution with one enantiomorph has been discussed by Greenstein[1]. More recently, the resolution of glutamic acid by inoculation has been perfected for industrial use[2,3].

Attempts in this laboratory to resolve aspartic acid by preferential inoculation have been unsuccessful, but a supersaturated solution of the copper complex prepared from the acetate[4] gave a virtually complete resolution in yields of 70–75 per cent of each of the optically pure aspartic acids. The mother liquor can be used for repeated resolutions.

DL-Aspartic acid (10·0 gm.) was dissolved in 750 ml. of 4 per cent acetic acid by warming at 60°. To this was added 16·0 gm. of Baker's cupric acetate monohydrate while warm and the resulting solution was filtered. When the filtrate cooled to approximately 50°, it was inoculated with about 2 mgm. pure L-aspartic acid copper complex. After the L-complex precipitated (precipitation was complete in a period of 10 min.–3 hr.) the crystals were filtered. This filtrate was inoculated with the pure D-complex and the precipitate of D-complex collected in the same way. By inoculation with alternate forms, successive crops were obtained. Six crops each of L-isomer and of D-isomer were obtained in a yield of 7·7 gm. total of L- and 7·6 gm. total of D-isomers.

To the mother liquor warmed to 60°, 10·0 gm. of DL-aspartic acid and 15·0 gm. cupric acetate were added; the resultant solution was filtered. From this supersaturated solution was obtained by seeding 8·3 gm. L-complex and 8·5 gm. D-complex. By addition of 19·5 gm. sodium hydroxide to the mother liquor, 1·57 gm. of L-complex and 1·66 gm. of D-complex were obtained. The total recovery of L-copper complex was 17·6 gm. (85 per cent) and 17·8 gm. (85 per cent) of D-complex.

The isolated copper complex salts were each recrystallized from 1·5 litre 3 per cent acetic acid containing 3·0 gm. cupric acetate. The recoveries were 14·2 and 14·1 gm. of L- and D-forms respectively. Each of these was dissolved in 1 N hydrochloric acid and the solution saturated with hydrogen sulphide. The aspartic acid isomers were isolated by adjusting a 200-ml. volume of aqueous solution to pH 3. Thus was obtained 7·4 gm. (74 per cent) L-aspartic acid and 7·4 gm. (74 per cent) D-aspartic acid The respective $[\alpha]_D^{26}$ values in 6 N hydrochloric acid were $+ 24{\cdot}4° \pm 0{\cdot}0°$ and $- 24{\cdot}6° \pm 0{\cdot}0°$. Nitrogen analyses agreed with the theoretical value.

Although the copper salt may be an unlikely kind of material for spontaneous resolutions in the primordial era, the result obtained illustrates in principle processes of a type which may have contributed to the origin of optical activity on the primitive Earth. Many plausible explanations for the origin of optical activity are now at hand[5-12]. The demonstration with the copper complex suggests that the chance crystallization of one enantiomorph of a key biochemical substance could trigger sequences of reactions favouring one optical type in those compounds having an asymmetric carbon atom. (In a personal communication, Dr. Henry Gilman has pointed out that chemists who might observe spontaneous resolution would be loathe to report such observation, much as explained by him some years ago[13].)

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Thin-Layer Chromatography of Nucleotides on Layers of Cellulose Ion-Exchangers

As has recently been shown[1], thin-layer chromatography[2] on ECTEOLA cellulose layers is a rapid and simple method for separating nucleic acid derivatives.

Improved and standardized cellulose ion-exchangers for thin-layer chromatography are now obtainable (Serva-Entwicklungslabor, Heidelberg, Germany). These materials have a low substitution grade and are prepared from a special wood cellulose. On these layers the complete separation of nucleoside diphosphates (for example, ADP) from their corresponding triphosphates (for example, ATP) can be achieved within 5–10 min. The separation of CTP, ATP, GTP and UTP requires 60–100 min.

Preparation of ECTEOLA and DEAE cellulose layers: A slurry is made by mixing 10 gm of the ion-exchanger powder with 60–70 ml. distilled water and shaking vigorously for 30–45 sec. The suspension obtained is spread in the usual way[2] over the glass plates, which must be thoroughly clean. (Previous treatment with a detergent, followed by water, is recommended.) 7–10 plates, size 10 × 20 cm., are obtained. The layers are allowed to dry overnight at room temperature.

Table 1. R_F VALUES OF NUCLEOSIDE DI- AND TRI-PHOSPHATES

Compound	Solvent 0·02 *N* HCl	 0·03 *N* HCl	 0·04 *N* HCl
ADP	0·48	0·68	
ATP	0·11	0·20	0·56
GDP	0·27	0·51	
GTP	0·07	0·14	0·41
CDP	0·53		
CTP	0·18	0·31	0·64
UDP	0·15	0·25	
UTP	0·04	0·08	0·18

Solutions of nucleotides (in general 10^{-3}–10^{-1} µmole of each compound) are applied from a pointed micropipette (10 µl.) at a line 3·0 cm. from the edge of the plate. The plates are placed in an open beaker filled with an appropriate solvent to a height of about 1·2 cm. A covered jar is not necessary. The chromatography can be observed by means of a short-wave ultra-violet lamp. It is stopped after the solvent has travelled an appropriate distance. The chromatograms can be traced on 'Cellophane' sheets under an ultra-violet lamp.

For many separations dilute hydrochloric acid is a sufficient solvent. Table 1 lists the R_F values of ribonucleoside di- and tri-phosphates obtained on DEAE cellulose layers. ATP, GTP and UTP or CTP, GTP and UTP can be completely separated with 0·04 *N* hydrochloric acid. ATP and CTP, however, are only partially resolved. These compounds can be separated by chromatography with 0·1 *M* sodium chloride on the DEAE cellulose layer.

The differences of the R_F values of the corresponding di- and tri-phosphates are very remarkable. As a consequence of this, analytical quantities of these compounds (10^{-3} µmole) can be completely separated on ECTEOLA or DEAE cellulose layers within 5–10 min. These layers might also be useful for the chromatography of oligonucleotides, polynucleotides and proteins.

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Melting Point of *p*-Bromophenacyl Formate

THE recent note by Crosbie[1] has given the melting point of *p*-bromophenacyl formate as 98°. This is in good agreement with the melting points of 99° and 99–100° given by Grove and Suter[2], and Kubota and Matsuura[3] respectively. Attention is directed to these two earlier papers[2,3] which were overlooked by Neish and Lemieux[4] and have not been mentioned directly by Crosbie[1]. A sample of *p*-bromophenacyl formate (m.p. 99°) was sent to me by Dr. Grove. A mixture of this with our sample (m.p. 92°) melted in an intermediate range. It was concluded that our sample was impure and the true melting point of this compound is about 99°.

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BIOCHEMISTRY

Structure of Ferroverdin

IN a previous communication from this laboratory the purification and preliminary characterization of ferroverdin, a green iron-containing pigment with formula $C_{30}H_{24}FeN_2O_8$ produced by a new *Streptomyces* species, were reported[1]. The iron atom was shown by magnetic measurements to be bound in the divalent form[2].

Chemical investigations, to be briefly described in the present communication, show that ferroverdin is built up of two identical C_{15} moieties held together by iron, each being the *p*-vinylphenylester of 3-nitroso-4-hydroxybenzoic acid. The previously reported formula for ferroverdin must therefore be replaced by $C_{30}H_{20}FeN_2O_8$, which is in good agreement with new elementary analyses made on a thoroughly dried crystalline sample (found: C, 59·07, 59·16, H, 3·52, 3·71; Fe, 9·07; N, 4·26, 4·62; calc. for $C_{30}H_{20}FeN_2O_8$ - $1H_2O$: C, 59·03; H, 3·63; Fe, 9·15; N, 4·59).

As mentioned previously, ferroverdin is readily reduced by hydrogen in the presence of a palladium catalyst. The iron-free compound so formed has been converted into a crystalline triacetyl-derivative, which can also be obtained by reductive acetylation of ferroverdin with zinc, followed by catalytic hydrogenation on a palladium catalyst. It melts at 125°–7° C. and gives analytical results in good agreement with the formula $C_{21}H_{21}NO_6$ (found: C, 65·95, 65·66; H, 5·86, 5·49; N, 3·69, 3·72; CH_3–C, 28·22, 28·35; mol. wt. 335,337; calculated: C, 65·78; H, 5·52; N, 3·65; $4CH_3$–C, 28·20; mol. wt., 383).

Its ultra-violet spectrum in 55 per cent ethanolic solution shows a single maximum at 238 mµ (ε = 18,500) and an inflexion at 270 mµ (ε = 4,000); on addition of alkali two maxima appear at 250 mµ (ε = 19,100) and 314 mµ (ε = 28,600), which are shifted to 243 and 288 mµ respectively when the solution is kept for 27 hr. at room temperature.

The infra-red spectrum (in potassium bromide disk) shows strong bands for *O*-acetyl (1,770 cm.$^{-1}$), *N*-diacetyl and ester (1,720 cm.$^{-1}$, broad) groups. The nuclear magnetic resonance spectrum obtained with a $CDCl_3$ solution (at 60 Mc./sec.; τ-values relative to tetramethylsilane as internal standard) clearly indicates an ethyl group attached to an aromatic ring (quadruplet centred at 7·31 τ, and triplet centred at 8·74 τ) and three acetyl groups (7·65 τ, partially resolved into two peaks).

By working up a 1 *N* aqueous methanolic sodium hydroxide solution of this triacetyl compound kept for 27 hr. at room temperature, two known substances have been isolated, namely, *p*-ethylphenol in 91 per cent yield, and 3-acetylamino-4-hydroxybenzoic acid in 64 per cent yield. Acid hydrolysis (6 *N* hydrochloric acid, 3 hr. at 100° C.) of the same compound gave *p*-ethylphenol and 3-amino-4-hydroxybenzoic acid in more than 90 per cent yield.

A compound with chemical and physical properties identical with those of the above triacetyl compound was synthesized by reacting *p*-ethylphenol with 3-acetylamino-4-acetoxybenzoyl chloride, followed by further acetylation with acetic anhydride and sodium acetate, thus proving its structure as the *p*-ethylphenylester of 3-diacetylamino-4-acetoxybenzoic acid. Elementary analysis, ultra-violet, and nuclear magnetic resonance spectra of the compound obtained by reductive acetylation of ferroverdin, without further catalytic hydrogenation, clearly showed it to have the formula $C_{11}H_9NO_6$ and to contain a vinyl substituent in place of the ethyl substituent found in the fully reduced triacetyl compound. Alkaline degradation of the vinyl substituted triacetyl compound gave again 3-acetylamino-4-hydroxybenzoic acid; but *p*-vinylphenol instead of *p*-ethylphenol was formed, thus suggesting that the compound is the *p*-vinylphenylester of 3-diacetylamino-4-acetoxybenzoic acid.

As in the case of the two triacetylated compounds referred to here, ferroverdin undergoes a rapid spectral change in the ultra-violet in alkaline solution though its green colour is retained. From the alkaline solution *p*-vinylphenol and a green iron-containing acid were isolated. The latter was shown to be identical by infra-red, ultra-violet and visible spectrum, paper chromatography and paper electrophoresis with the ferrous complex of 3-nitroso-4-hydroxybenzoic acid, synthesized by mild alkaline hydrolysis of the corresponding methylester, which in turn was synthesized from the methylester of 4-hydroxybenzoic acid by the method of Cronheim[3]. The methylester of the green iron-containing product of mild alkaline hydrolysis of ferroverdin was also shown by the same methods to be identical with the synthetic methylester.

It is well known that iron forms deep green co-ordination compounds with *o*-nitrosophenols[3,4].

The exact configuration of the iron complex has yet to be elucidated; several possibilities exist, including that involving the quinone-oxime tautomer and the nitroso dimer.

Ferroverdin is a new member of the rapidly growing group of natural iron-containing compounds, but unique in its structure as an *o*-nitrosophenol; it would, in fact, appear to be the first *C*-nitroso compound encountered in nature.

We thank Prof. K. Wiesner and Prof. L. Paoloni for valuable discussions. We also thank Prof. K. Wiesner for providing us with the nuclear magnetic resonance spectra.

The ferroverdin used for this work was produced in the fermentation pilot plant of this Department under the supervision of Dr. A. Tonolo.

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* Aided by a grant from the Chemische Fabrik Dr. R. Pfleger, Germany.

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Inhibition of Hyaluronidase by Urinary Kallikrein

INVESTIGATIONS have shown that crude kallikrein prepared from human urine and human serum possesses *in vivo* spreading properties which cannot be attributed to hyaluronidase activity[1-3]. Turbidimetric[4], mucin clot[5] and viscosimetric[6] procedures reveal that urinary kallikrein unlike hyaluronidase does not depolymerize hyaluronic acid. On the contrary, a marked inhibition of testicular hyaluronidase occurs when kallikrein is combined with the enzyme. In order to define further the properties of kallikrein, we investigated *in vitro* the effect of a urinary preparation on hyaluronidase.

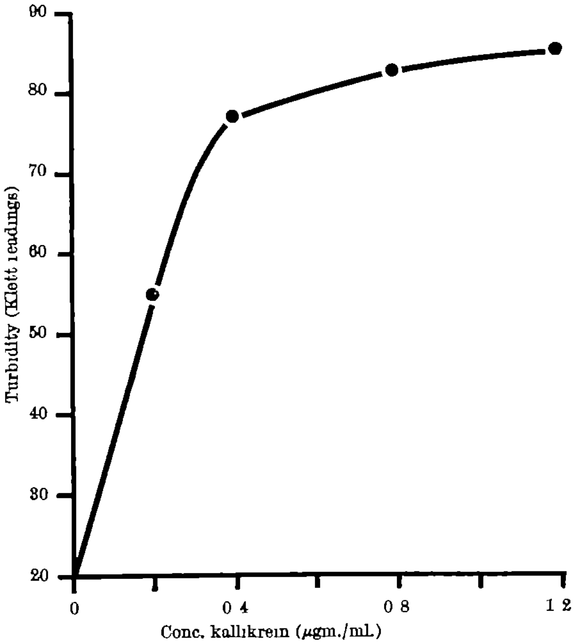

Fig. 1. Inhibition of hyaluronidase by various concentrations of kallikrein

The hyaluronidase-inhibiting properties of a crude preparation of human kallikrein was assayed turbidimetrically. Several dilutions of inhibitor were prepared in volumes of 0·4 ml. in 0·1 *M* acetate buffer at *p*H 6. Substrate for the system consisted of partially purified bovine testicular hyaluronidase (691 U.S.P. units/mgm.) and purified vitreous humour potassium hyaluronate. The solution of enzyme in 0·1 *M* acetate buffer at *p*H 6 was added in a volume of 0·2 ml. The tubes were shaken in a rack and then incubated at room temperature for 30 min. Residual hyaluronidase activity which remained after incubation of the inhibitor dilution and hyaluronidase mixture was determined as follows: To each tube was added 0·4 ml. of potassium hyaluronate (0·15 mgm.) dissolved in 0·1 *M* acetate buffer (*p*H 6) and sodium chloride to a molarity of 0·05. The tubes were incubated in a water bath at 37° C. for 30 min. To each tube 4 ml. of a 1 : 40 dilution of horse serum (*p*H 3·1) was added. After another 30 min. incubation at room temperature, the turbidity of the mixture was determined in a Klett-Summerson colorimeter using a No. 66 filter.

The potency of kallikrein as an inhibitor is determined in terms of decrease or loss of hyaluronidase activity. By comparing the turbidity produced by the enzyme-inhibitor-substrate mixture to that of inhibitor-substrate control a linear relationship is demonstrated between the concentration of the enzyme and depolymerization of substrate. Loss of activity due to the presence of inhibitor is expressed as percentage reduction in enzyme activity. No effect on the turbidity of substrate occurs within the range of kallikrein concentrations used when no hyaluronidase is present.

Fig. 1 shows the effect of various concentrations of kallikrein on the activity of 2·5 μgm./ml. hyaluronidase. This demonstrates that the inhibition of hyaluronidase occurs rapidly with the first small increments of kallikrein and reaches a maximum at

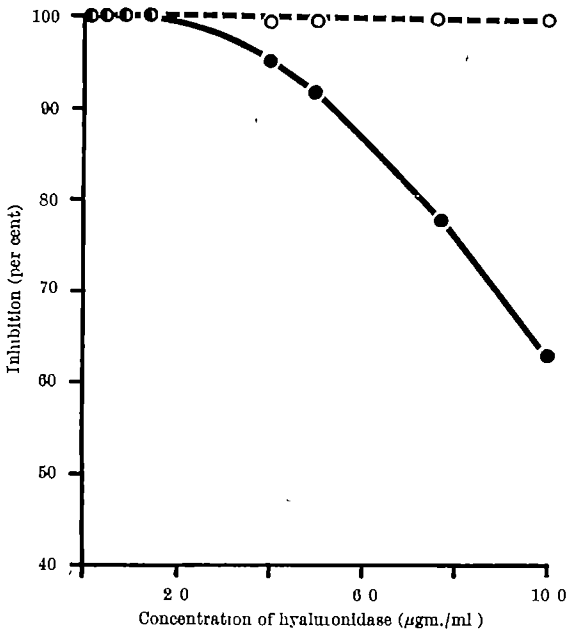

Fig. 2. Effect of kallikrein on the depolymerization of potassium hyaluronate by hyaluronidase; ●, 2 μgm./ml kallikrein, ○, 20 μgm./ml. kallikrein

1·2 μgm./ml. Confirmation is readily obtained by both the mucin clot prevention test and viscosimetric procedures. The curves in Fig. 2 show the extent of depolymerization of a constant amount of substrate by 2 and 20 μgm./ml. inhibitor. Thus, as the hyaluronidase concentration increases, the inhibition by small amount of kallikrein decreases. Large amounts such as demonstrated by 20 μgm./ml. kallikrein are completely inhibitory.

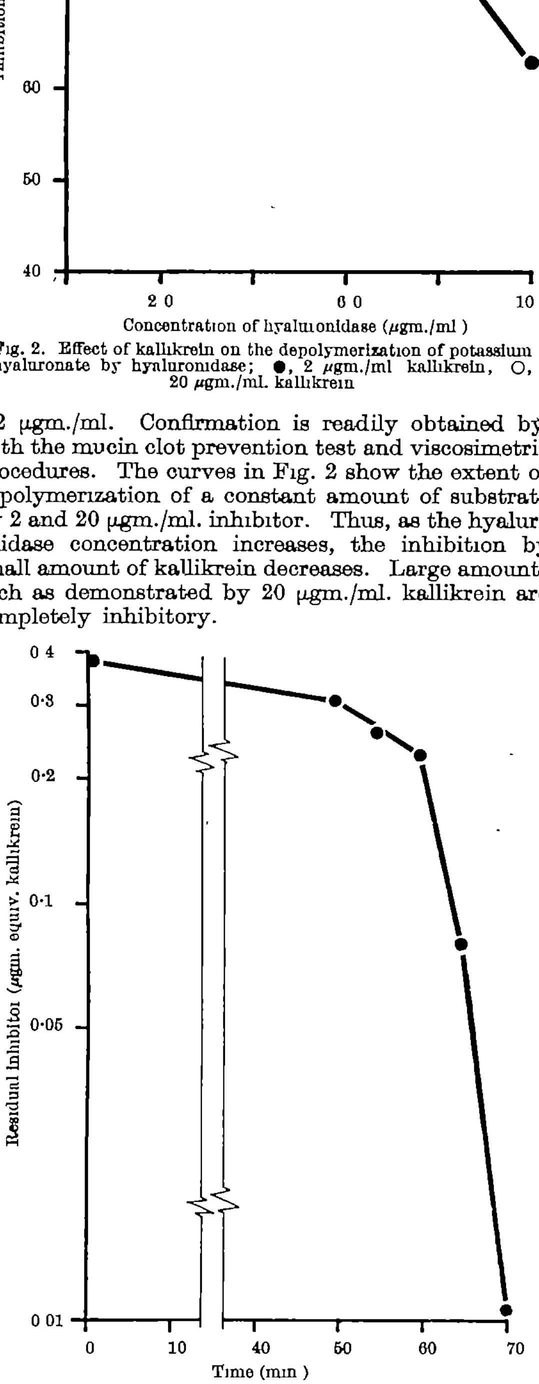

Fig. 3 Rate of inactivation of antihyaluronidase activity of kallikrein as a result of exposure to ultra-violet light

The inactivation of kallikrein by ultra-violet light was demonstrated by Werle[7]. This reaction is therefore one of the characteristic properties of the material. In order to determine that the results we obtained are due to kallikrein 0·1 μgm./ml. kallikrein was exposed to a Westinghouse $G15T8$ ultra-violet bulb at a distance of 5 in. The inactivation as measured by the rate of destruction of hyaluronidase inhibitory activity is shown in Fig. 3 and confirms, in part, our initial assumption.

Although kallikrein is found in urine, blood and certain organs and glands, its biological role is obscure. It has been suggested that kallikrein circulates in the blood in an inactive form and may be activated by disease or changes in blood *p*H. Recently Ginetzinsky and Berlyne[8] demonstrated a relationship between urinary hyaluronidase and various diuretic conditions. Our results suggest that the hyaluronidase-inhibitory properties of kallikrein may have significance from the standpoint of regulating the activity of this enzyme *in vivo*. Since no one has been able to obtain a purified kallikrein, the spreading activity as described by Christensen is most likely due to unspecific factors.

We thank Drs. M. E. Webster and J. V. Pierce, National Heart Institute, Bethesda, Md., for the sample of human urinary kallikrein (6 Frey units/mgm.).

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Separation of Lecithin and Phosphatidyl Ethanolamine by Chromatography on Paper impregnated with Zinc Salts

THE separation of phospholipids by paper chromatography has generally been performed on modified papers. Marinetti and Stotz[1] and Lea *et al.*[2] report the use of silicic acid-impregnated paper, and Brown *et al.*[3] have used silicic acid-impregnated glass-fibre 'paper'. Rouser *et al.*[4] investigated the possibility of using paper impregnated with mineral oil, rubber, silicone, and formamide, and paper oxidized by periodic acid, but found all these to be unsuitable. Recently, however, in this laboratory[5] a method has been evolved for the separation and differential staining of phospholipids on untreated paper. During work on this method difficulty was encountered with the separation of lecithin from phosphatidyl ethanolamine, and a procedure for separating them on paper impregnated with zinc salts is described here.

Since cadmium chloride forms a complex more readily with lecithin than with phosphatidyl ethanolamine the use of this salt during chromatography was examined. Formation of the phospholipid–cadmium chloride complex in a solution of lecithin and phosphatidyl ethanolamine, followed by application of the solution to Whatman No. 1 paper, and chromatography in di-*n*-butyl ether/propionic acid (4 : 3 v/v)

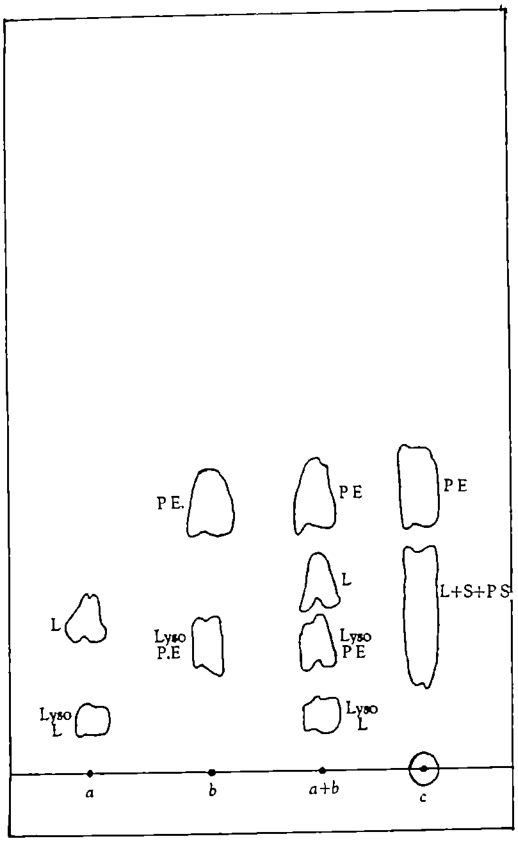

Fig. 1. Line drawing of ascending one-dimensional chromatogram impregnated with zinc salts, and run in di-*n*-butyl ether/propionic acid (2 . 1 v/v) saturated with zinc chloride. *a*, Sample of lecithin; *b*, sample of phosphatidyl ethanolamine, *c*, extract of liver of mouse (ref. 6). L, Lecithin, P.E., phosphatidyl ethanolamine; lysoL, lysolecithin; lysoP.E., lysophosphatidyl ethanolamine; S, sphingomyelin; and P.S., phosphatidyl serine

saturated with water (a solvent system suitable for phospholipids), was unsuccessful, since the acid solvent disrupted the complex. Formation of the complex on the paper after application of the lipids, by soaking it in an aqueous solution of cadmium chloride, washing out of excess cadmium chloride, and drying, did not give separation after chromatography. If, however, the paper was not washed after soaking in the cadmium chloride solution but merely dried so that the paper was impregnated with cadmium chloride, which acted as an adsorbant, some small separation was achieved. It was also necessary to saturate the solvent system with cadmium chloride.

Salts of aluminium, magnesium, barium, and zinc were also investigated. Of these, zinc chloride gave the best separation of lecithin and phosphatidyl ethanolamine. A solvent system without water was found to be most efficient: di-*n*-butyl ether/propionic acid (2 : 1 v/v) saturated with zinc chloride (B.D.H., fused) was used. There was some difficulty with zinc chloride impregnation of the paper, since in solution this salt is a cellulose solvent, and variation was found between different batches of zinc chloride. This was overcome by the use of a solution of zinc oxide in a 3·5 per cent aqueous solution of hydrochloric acid (a 10 per cent solution of concentrated hydrochloric acid, sp. gr. 1·18, in water). The acid solution was saturated with zinc oxide at about 60° C., allowed to cool to room temperature, and filtered. It seems likely that under these conditions of excess oxide and weak acid, followed by evaporation to dryness, the oxychlorides would also be formed. Zinc chloride forms several hydrates, and the paper is thus impregnated by a zinc chloride–zinc oxychloride–hydrate system. Since the solvent does not contain water the usual partition of the phospholipids between water and the organic solvents is impossible, and separation must therefore be effected by differential adsorption.

The procedure for the separation of lecithin from phosphatidyl ethanolamine is as follows: (1) The phospholipids (about 40 μgm.) are applied to the base of the paper (Whatman No. 1). (2) The paper is soaked in a solution of zinc oxide in 3·5 per cent hydrochloric acid for 5 min. (Steps 1 and 2 may be reversed.) (3) The paper is removed from the solution, laid momentarily on dry filter paper, and dried under a stream of hot air. If some of the excess solution is not blotted off the paper the values R_F are increased and separation improves. However, the distribution of the zinc salts is usually uneven: it is therefore better to lay the paper on dry filter paper for two or three seconds before drying it. (4) The paper is chromatographed by the ascending method in di-*n*-butyl ether/propionic acid (2 : 1 v/v) saturated with zinc chloride. The solvent travels about 27 cm. in 4 hr. (5) The paper is then dried under a stream of hot air and stained by the 'Solochrome' technique for phospholipids[5].

It was found that this technique also separated lysolecithin from lysophosphatidyl ethanolamine: from a mixture of partially lysed lecithin and partially lysed phosphatidyl ethanolamine four spots were obtained—phosphatidyl ethanolamine, lecithin, lysophosphatidyl ethanolamine, and lysolecithin (Table 1). Lecithin and phosphatidyl ethanolamine can be separated not only from an artificial mixture but also from a tissue extract (Fig. 1).

Table 1

Phospholipid	R_F value
Phosphatidyl enthanolamine	0 32–0·40
Lecithin	0 20–0·25
Lysophosphatidyl ethanolamine	0 13–0·18
Lysolecithin	0 05–0 08

This method has, as yet, been applied only to the separation of lecithin and phosphatidyl ethanolamine, and the separation of other phospholipids by impregnation of the paper with inorganic salts has not been studied. It seems likely, however, that by the choice of suitable inorganic compounds, other than the troublesome and unreproducible silicic acid, and by the use of appropriate solvent systems all phospholipids could be separated. The use of paper impregnated with inorganic salts for the separation of organic compounds seems to have been a neglected aspect of chromatography and should repay further investigation.

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L-Glutamate Dehydrogenase Isoenzymes

SEVERAL dehydrogenases have been resolved into isoenzymes by electrophoresis in starch gel. Attempts to fractionate L-glutamate dehydrogenase in this way, however, have so far failed. Markert and Moller[1] found that this enzyme was electrophoretically immobile in starch gel, and Tsao[2] obtained negative results in attempts to fractionate and render glutamate dehydrogenase visible.

Using a technique developed for determination of lactate dehydrogenase isoenzymes in clinical routine[3,4], however, it proved possible to demonstrate multiple forms of glutamate dehydrogenase in homogenates of human organs.

Fractionation was effected by electrophoresis in agar gel, and the dehydrogenase activity was made visible by incubation of the gel in a solution containing sodium glutamate, diphosphopyridine nucleotide, phenazine methosulphate and a tetrazolium salt.

Human tissues were obtained by autopsy less than 12 hr. after death. The tissues were stored at − 20° C. for one night. Tissue extracts were prepared by homogenizing 1 gm. of tissue with 1 ml water by means of a motor-driven Potter–Elvehjem tissue grinder. The extracts were clarified by centrifugation. Electrophoresis was carried out with 8 μl. of the extract, using the technique on microscope slides of Wieme[5]. The slide was then incubated for 1 hr. at 37° C. in the following medium: sodium-DL-glutamate (M) adjusted to pH 7·4 (1 ml.); diphosphopyridine nucleotide (10 mgm./ml.) (1 ml.); sodium cyanide (0·1 M) (1 ml.); phosphate buffer (0·5 M, pH 7·4) (2·5 ml.); nitroblue-tetrazolium (1 mgm./ml.) (2·5 ml.); phenazine methosulphate (1 mgm /ml.) (0·25 ml.).

At the sites of dehydrogenase activity purple deposits of formazan are formed during incubation. When glutamate is omitted from the incubation fluid, no formazan bands are formed. After incubation the slide is washed with water and, if desired, densitometry can be carried out with the wet slide. The proteins can also be fixed in the following fluid: ethanol 70 vol., acetic acid 5 vol., distilled water up to 100 vol. The plate on which the protein fractions become visible during fixation is afterwards dried while covered by filter paper. Densitometry can then be carried out on the dry plate. The slides must be stored protected from daylight; in the light, a background slowly develops. The distance between human albumin and dextran, run on the same plate, was taken as unit of measurement for the determination of relative mobilities.

The result obtained by this technique with an extract of human heart muscle is shown in Fig. 1. Five glutamate dehydrogenase fractions are visible, and some activity is retained in the application slit. The results of electrophoretic separation of glutamate dehydrogenase isoenzymes of other tissues are given in Table 1. A total of six isoenzymes has been found. Also it appears that there are differences in the isoenzyme contents of the different tissues.

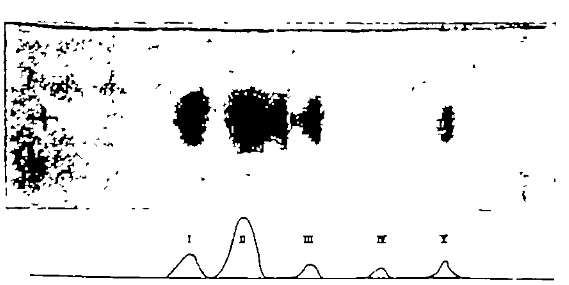

Fig 1 Distribution of glutamate dehydrogenase isoenzymes in the myocardium

Table 1. DISTRIBUTION OF GLUTAMATE DEHYDROGENASE ISOENZYMES IN HUMAN TISSUES

Fraction	I	II	III	IV	V	VI
Relative mobility	0 95	0 74	0 46	0·20	0·00	−0 12
Liver	+	+ + +	+	+	+ +	+
Heart	+ +	+ + +	+	+	+	
Kidney	+ + +	+ + +	+	+	+	
Spleen	+ + +	+ + +	+	+ +	+ +	

+, Weak, + +, medium, + + +, strong band.

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Heat-Coagulation of Edestin

PROTEINS in aqueous solution are denatured by heating; few separate proteins resist the temperature of boiling water for more than a few minutes. It is therefore surprising to find that under certain conditions a protein molecule as large as that of edestin can be heated for a long time at 100° C. without causing any change. This was observed during experiments being carried out to establish the best conditions for complete heat-coagulation of edestin.

For this purpose we selected conditions similar to those used by M. and S. P. L. Sørensen[1] for the heat-coagulation of ovalbumin, namely, an acidity very near the isoelectric point and a high ionic strength (1/3 saturated sodium sulphate). The isoelectric point of edestin being unknown, it was necessary to establish the optimal pH under the conditions which were as follows: to one volume of a solution in 10 per cent sodium chloride and 0·1 M sodium bicarbonate of a carefully purified edestin was added one volume of a buffer mixture and one of a saturated solution of sodium sulphate. The mixture was heated in a boiling water bath (98° C.) for 20 min. and filtered hot on steam-heated funnels. The amount of uncoagulated protein in the filtrate was determined by the Kjeldahl procedure. Measurements of pH were made at room temperature before heating.

The results (Fig. 1) show that coagulation is practically complete at pH 4·6 and lower. It may be remarked that at these low pH values the protein precipitates before heating. Only an insignificant amount of clot is formed at pH 5·8–7·8, 95 per cent or more remaining in solution. At higher pH values partial coagulation occurs with a maximum around pH 10.

The interesting pH-range is that where practically no coagulation occurs. At first, we thought that denaturation did occur but that coagulation had been prevented by the high temperature. Cooling of the filtrates did show indeed the formation of a precipitate, but only at pH values of less than 6·3, as indicated by the broken curve in Fig. 1. It is interesting to note that at pH 5·5 the protein is soluble when heated but precipitates when cooled, and this may be repeated at will, imitating the behaviour of Bence-Jones protein.

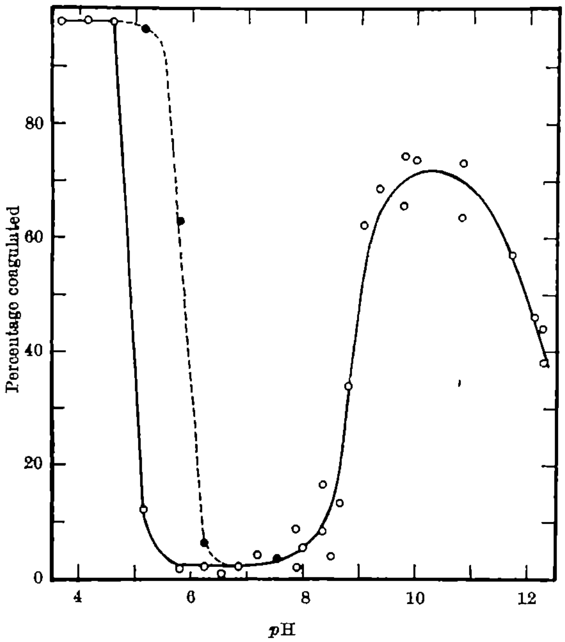

Fig. 1. Heat coagulation of edestin. Full line and open circles, filtered hot; broken line and black circles, filtered after cooling

Between pH 6·3 and 7·8, there is a small region where no coagulation could be observed by heating and no precipitate appeared by cooling afterwards. In order to decide if, at this critical range, the protein had undergone denaturation or not, a large sample of edestin was heated for 20 min. under the same conditions as described, at pH 6·3. This solution was submitted to the following tests. Light-scattering revealed the same molecular weight as the original (338,000). There was no increase in the light-scattering after two days, indicating absence of any slow aggregation or tendency to coagulate. The ultraviolet absorption spectrum was quantitatively identical with the spectrum of the unheated solution. After precipitation by half-saturation with ammonium sulphate, the protein again dissolved in 10 per cent sodium chloride and 0·1 M sodium bicarbonate, giving a perfectly clear solution. Finally, by dialysis against a phosphate buffer at pH 7·0 (Sørensen) crystals were formed identical with those of the original protein.

These observations show, at the short pH-range 6·3–7·8, edestin was not denatured by heating at 100° C. for 20 min. and perhaps even longer, since after 40 and even 60 min. no clot was formed.

This unexpected resistance to heat denaturation of edestin in a short pH-range suggests that denaturation is only possible through the exchange of protons. According to this hypothesis, heat, as such, is not a denaturing agent but a factor influencing the proton exchange reaction.

We thank the Institut pour la Recherche Scientifique appliquée à l'Industrie et l'Agriculture (Brussels) for the grant of a fellowship to one of us (L. M.).

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Presence of Indole Compound in Nematode Galls

Multi-nucleate structures or giant cells in plants infected by root-knot nematodes are believed to arise in response to substances emanating from the parasite[1]. DuCharme[2] stated that the resulting tumours and galls are due to reactions of the host plant and might be considered as a kind of defence mechanism. According to him, growth-stimulating substances, presumably released by the nematodes, lead to hyperplasia of the pericycle and root tip in citrus. Krusberg[3] found that tryptophan, the precursor of indole, is present in the nematodes infected or nematode-free callus tissues and also in the galls caused by *Ditylenchus dipsaci* (Kuhn) Filipjev on alfalfa roots. According to Goodey[4], Wallace suggested that formation of giant cells in roots attacked by *Heterodera* spp. and *Meloidogyne* spp. might possibly be due to the presence of growth hormones. The experiment reported here was undertaken to examine whether any indole compound is present in the galled tissues of nematode-infected plants.

Meloidogyne javanica (Trueb) Chitwood, isolated from tomato root galls, was inoculated into healthy roots of *Abelmoschus esculentus* Moench, grown in sterilized soil, following the method used in the previous studies in this laboratory[5] and the root galls were obtained after 45 days. The roots were washed thoroughly in running water and then in sterilized water and examined for the presence of indole compounds. 25 gm. of galled tissues were removed from the roots and kept in a refrigerator at 8° C. for 24 hr. Healthy roots of the same age from uninoculated control plants were also prepared in a similar manner. The frozen roots were macerated separately and extracted with ether, using 5 ml./gm. of the tissue. The extracts were assayed chromatographically following the method recommended by Wright[6]. Known samples of indolyl-3-acetic acid (IAA) and indolyl butyric acid (IBA) and samples of the extracts from the galls and healthy roots were spotted on circular chromatographic paper (Whatman No. 1)

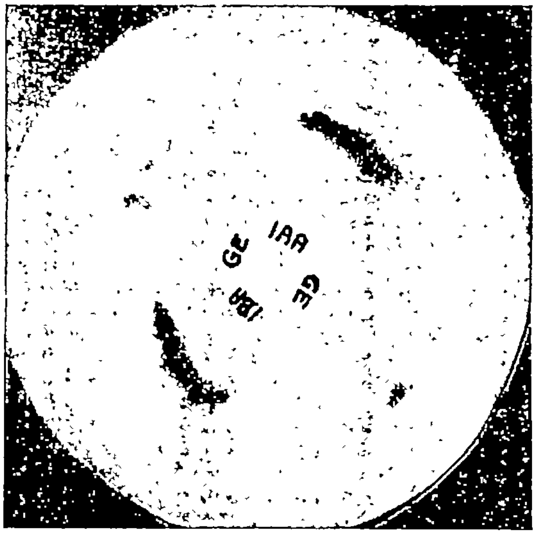

Fig. 1. Circular chromatogram run with ether extracts of nematode root galls of *Abelmoschus esculentus*, developed in butanol/ammonia/water (100 100 · 8), at 28°–29° C. in complete darkness and differentiated with Salkowski reagent. IAA, Indolyl-3-acetic acid; IBA, indolylbutyric acid; GE, gall extract

and developed with butanol/ammonia/water (100 : 100 : 8) at room temperature (28°–29° C.) in complete darkness for 12 hr. Salkowski reagent was used to differentiate the indole compounds on the paper. The extract from the gall and the known IAA gave spots with similar R_F values (0·56) and pink colour, the colour in the latter case being more intense (Fig. 1). Both spots were ash coloured when viewed through ultra-violet light. According to Sen and Leopold[7] the pink colour in daylight and ash colour in ultra-violet light of the spots indicate IAA. IBA gave a brown spot with an R_F of 0·42 (Fig. 1). No spots developed when the extract from healthy roots was similarly assayed. These chromatographic studies were repeated several times and the same results were obtained each time. Thus there are indications of the presence of an indole compound in the extracts of galled tissues of *A. esculentus*. It is quite probable that the compound plays a part in gall formation.

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Tryptophan and Indole Compounds in Banana Ovaries

THE ovary of an edible banana, *Musa paradisiaca* Linn., develops into a vegetatively parthenocarpic fruit and at maturity is filled with starchy parenchymatous tissues. Simmonds[1] reported the autonomous production of auxin, suspected as being indolyl-3-acetic acid (IAA), in the young ovaries of banana. The presence of 'coconut milk factor' in the banana ovaries has also been reported[2,3]. The results reported here are from investigations carried out in this laboratory during 1960–61 on the type of auxin and amino-acids present in the ovaries of banana.

A popular culinary banana variety, Monthan, was selected for the purpose. The ovaries were obtained from the hearts of bananas cultivated in the Annamalai University Experimental Farm. The methods of extraction of the ovaries and chromatographic differentiations to detect the auxins were the same as those described by Wright[4]. Extracts of ovaries at emergence, 48 hr. after emergence and later at weekly intervals, were taken and assayed chromatographically to detect indole compounds. Ascending chromatograms were run for 12 hr. in complete darkness at room temperature (28°–29° C.) with Whatman No. 1 paper strips, using butanol/ammonia/water (100 : 100 : 8) as the medium. Salkowski reagent was sprayed on the chromatograms to differentiate the spots.

In Fig. 1 is shown a comparison of the spots developed in the ascending chromatogram run with the extract of ovaries taken at the emergence of the inflorescence (1) and 48 hr. after emergence (2) with a sample of IAA (British Drug House, Bombay). The spots developed with the first extract were orange with a red tinge in daylight and green in ultra-violet light, with an R_F of 0·61, whereas there were two spots from the second extract, one pink coloured with an R_F of 0·23 and the other orange with red tinge with an R_F of 0·60, the latter turning pale green in the ultra-violet light. The two spots obtained with the known IAA were almost similar to those obtained with the second extract. According to Sen and Leopold[5], the orange spots with red tinge obtained with this chromatographic system refer to indole compounds. In the present investigations the orange spots with red tinge and an R_F of 0·60–0·61 obtained with the first and second extracts would seem to represent an indole compound similar to IAA.

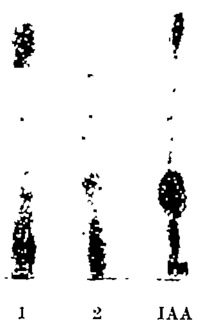

Fig. 1

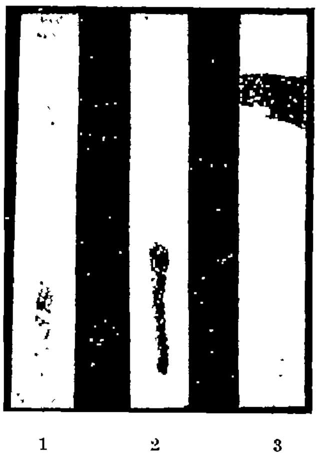

Fig. 2

Fig. 1. Ascending chromatogram of banana ovary extracts at emergence (1) and 48 hr. after emergence (2) as compared with known IAA

Fig. 2. Ascending chromatogram of banana ovary extracts at emergence (1), as compared with known tryptophan (2) and the extract of three-week-old ovaries (3)

When the ovaries were extracted 8 and 15 days after emergence and assayed chromatographically for indole compounds, the characteristic orange with red tinge spots of indole compounds were found and these spots turned pale green in ultra-violet light. Their R_F value was, however, 0·84 as compared with 0·61 for the spots from the extracts obtained from the ovaries at the early stages of development. The extract of three-week-old ovaries when spotted on chromatogram showed a green spot with bluish tinge and an R_F of 0·82 (Figs. 2–3). This spot resembled that of indolyl acetonitrile (IAN), which according to

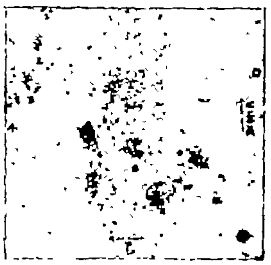

Fig 3

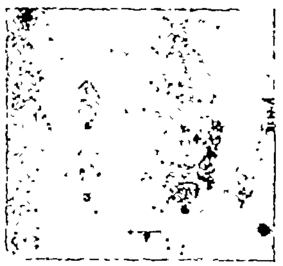

Fig 4

Fig. 3. Two-dimensional chromatogram showing the amino-acids in the extracts of ovaries at the time of emergence. leucine (1), valine (2), methionine (3), proline (4), tryptophan (5), alanine (6), histidine (7), threonine (8), glutamic acid (9), serine (10), hydroxy-proline (11) and aspartic acid (12)

Fig. 4. Two-dimensional chromatogram showing the amino-acids in the extracts of ovaries 48 hr. after emergence leucine (1) alanine (2), histidine (3), glutamic acid (4), serine (5), hydroxy-proline (6) and aspartic acid (7)

Sen and Leopold[5] turned greenish-blue in ultra-violet light. Jones *et al.*[6] discovered the presence of IAN in cabbage leaves which, according to them, was more active than IAA. It is probable that the IAN-like compound in the later stages of development of the banana ovary has a similar activity.

When the ether extract of the ovary at the time of emergence of the inflorescence was assayed chromatographically for the presence of indole compounds, indications for the presence of a tryptophan-like compound were obtained. In Fig. 2 is shown a comparison of the known tryptophan (2) with an R_F of 0·37 and the unknown (1) with an R_F of 0·40 from the extract of the ovaries. It seemed advisable, therefore, to examine the extracts for the presence of amino-acids. When the ovaries were extracted in a similar manner as described above and two-dimensional chromatograms run to detect the presence of amino-acids, on applying the method recommended by Giri and Rao[7] (using butanol/acetic acid/water (4 : 1 : 1) and phenol as solvents and ninhydrin as the colour-developing reagent) indications of the presence of 12 amino-acids, inclusive of tryptophan, were obtained in the extracts of ovaries at the time of the emergence of the inflorescence (Fig. 3), whereas in the extracts obtained 48 hr. after emergence indications for only 7 amino-acids were apparent and tryptophan could not be detected (Fig. 4). Perhaps the presence of tryptophan in the early stages indicates that it is a precursor of the IAA-like substance in the ovaries.

According to Simmonds[8], in bananas, as in other plants, two systems of stimuli are responsible for initiating and maintaining fruit development. It is also possible that the IAA and IAN-like compounds observed in the present investigation are the factors involved and the pathway of their biosynthesis is by means of tryptophan.

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PHYSIOLOGY

Afferent Cutaneous C-fibre Reactivity to Repeated Thermal Stimuli

SINGLE afferent *C*-fibres from the skin of the rat's tail were isolated by the method described by Hensel, Iggo and Witt[1]. These fibres were non-specific; activity was recorded in them both to mechanical and strong thermal stimuli applied to the skin. There was in this respect a strong similarity between these fibres and a few *C*-fibres in the cat's skin described by Iruichijima and Zotterman[2] which responded to mechanical stimuli, to heating the skin above 50° C. and to cooling the skin below 10° C. Non-specific fibres responding to all kinds of noxious and non-noxious thermal and mechanical stimuli have been found recently by Witt[3] and fibres, responding to extreme thermal stimuli only by Iggo[4]. It has been suggested[2-4] that these fibres might have a "nociceptive function". The present investigations, which concern the behaviour of non-specific *C*-fibres to repeated stimulation, would appear to confirm the suggested "nociceptive function" of these afferents.

Identical thermal stimuli were repeatedly applied to the skin using a close-fitting water-filled copper thermode, which completely enclosed the rat's tail; the temperature of the water flowing through the thermode could be rapidly changed from 31° C. to 46° C. to 4° C. During each period of cooling there was a constant rate of fall of skin temperature from 31° C. to 4° C., and during each period of heating there was a constant rate of change of temperature from 31° C. to 46° C., the period of time for which the skin was heated at 46° C. or cooled at 4° C. was 10 min., and between periods of thermal stimulation the skin temperature was returned to 31° C. for 10 min. The pattern of firing recorded in the single fibre preparations during cooling was of two types, first those fibres which fired phasically, only during the fall of skin temperature, and secondly those fibres which fired continuously throughout the 10-min. period of cooling at 4° C. In the latter case the highest frequencies of firing (max. 75 imp./sec.) achieved during the fall of skin temperature, and the frequency of firing gradually fall off to a steady level during frequency. The fibres in which a phasic activity was recorded during the fall of skin temperature to 4° C. showed a constant and repeatable activity to consecutive (up to 10) periods of identical cooling. Repeatable activity was also observed in some of the fibres with a continuous discharge at 4° C., others showed evidence of fluctuations of activity.

A continuous, but irregular, discharge of fairly high frequency was observed in all these non-specific *C*-fibres during periods of heating the skin at 46° C. Contrary to the rather repeatable activity recorded from these fibres during successive cooling periods quite large and apparently random fluctuations of response were consistently observed during periods of repeated identical heating. These changes of activity sometimes took the form of potentiation; but diminution of activity or complete changes in the pattern of firing from single impulses to high-frequency bursts of impulses has also been recorded.

It was found that fluctuations of response to identical heating occurred in the same fibres which responded uniformly with phasic responses to cooling the skin at 4° C. There was also some evidence that similar fluctuations, though less marked, might also occur during repeated periods of identical cooling at 4° C. in some of the fibres that fired continuously while the skin was at 4° C. The fluctuations of response to repeated cooling at 4° C. was more marked if the skin had been previously subjected to repeated periods of heating at 46° C.

The nature of these changes of response is unknown, but we can consider two possible mechanisms that could account for this effect:

(1) It might be similar to the potentiation or depression taking place after previous activity in the Pacinian corpuscles described by Loewenstein and Cohen[5,6]. These changes increase with increasing previous activity of the corpuscle. In our experiments a change in *C*-fibre activity was only found after stimuli that gave long-lasting firing of rather high frequency.

(2) Due to sustained high temperature of the skin to some degree possibly tissue damage begins. Witt[3] has shown that, after repetition of noxious stimuli

which do not cause irreversible destruction of skin receptors, the sensitivity of non-specific *C*-fibres increases in response to all kinds of stimuli. Chemical reactions, that is, local production of special substances, may be responsible for this effect.

Because of the non-specificity and the fluctuation in activity of the fibres observed in our experiments, we suggest that they might have a nociceptive function rather than a function as thermoreceptors. The sensation of pain may therefore be mediated by the stimulation of these non-specific afferents, rather than by excessive stimulation of specialized thermoreceptors or mechano-receptors.

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Potentiation by Anæsthetics of Brain Damage due to breathing High-Pressure Oxygen in Mammals

THERE have been several reports that barbiturates administered to animals before their exposure to high-pressure oxygen reduce the incidence and severity of convulsions and delay death[1-4]. We have confirmed this protective action for barbiturates in mice and rats and have also shown that lung damage in rats due to exposure to high-pressure oxygen is decreased[5]. However, when rats were exposed for shorter periods to high-pressure oxygen, which did not result in acute death during pressurization, and were allowed to recover, it was found that pretreatment with anæsthetic doses of pentobarbital sodium (37·5 mgm./kgm. body-weight, intraperitoneally) immediately before exposure to high-pressure oxygen resulted in a very marked increase in delayed damage to the central nervous system.

In Table 1 representative results are shown for male rats, weighing 160 gm., exposed to 45-lb./sq. in. gauge pressure of pure oxygen for varying periods. All rats were compressed after flushing of the pressure vessel at a rate of 15 lb./sq. in./min. and decompressed at 9 lb./sq. in./min. The treatment periods refer to the actual times during which 45-lb./sq. in. gauge pressure was maintained in the chamber. The incidence and times of convulsions and death were recorded, and 24 hr. after pressurization the incidence and severity of damage to the central nervous system were assessed.

Among unanæsthetized animals no deaths or obvious residual brain damage resulted from exposure to oxygen at 45 lb./sq. in., for as long as 45 min. In rats previously given pentobarbital sodium, the incidence of deaths was increased, and animals showed residual brain damage following exposures of only 15 min. to oxygen at a pressure of 45 lb./sq. in. The delayed lesions to the central nervous system that we observed follow closely the pattern described by Bean and Siegfried[6], although their classification of acute and chronic phases was less apparent. Some affected animals showed either hyperexcitability or lethargy (classified as mild damage) and a number showed unsteadiness of gait and postural disturbances (moderate damage). However, most of the affected rats featured gross motor dysfunction (severe damage), particularly flexor paralysis of the fore limbs resulting in a kangaroo-like locomotion. A more extensive paresis was also common and resulted in spastic deformities with opisthotonus. Some rats showed catatonic behaviour while others were comatose. In the latter, hypothermia was usually severe and commonly preceded delayed death.

Table 1. EFFECT OF BARBITURATE ANÆSTHESIA ON LETHALITY AND BRAIN DAMAGE IN RATS EXPOSED TO PURE OXYGEN AT 45 LB./SQ. IN. GAUGE PRESSURE

	Treatment	Fraction of animals dead within 24 hr. after exposure	Fraction of animals showing residual brain damage 24 hr. after exposure
Exposure for 15 min.	Unanæsthetized	0/3	0/3
	Pentobarbital Na (37·5 mgm./kgm.)	0/3	2/3
Exposure for 25 min.	Unanæsthetized	0/12	0/12
	Pentobarbital Na (37·5 mgm./kgm.)	3/9	6/6
	ββmethylethyl glutaramide (40 mgm/kgm.) and pentobarbital Na (37·5 mgm./kgm.) both before exposure	0/3	0/3
	Pentobarbital Na (37·5 mgm./kgm.) before exposure, ββ-methylethyl glutaramide (40 mgm./kgm.) after decompression	0/3	3/3
	ββmethylethyl glutaramide before exposure	3/3*	—
Exposure for 45 min.	Unanæsthetized	0/6	0/6
	Pentobarbital Na (37·5 mgm./kgm.)	0/6	6/6
	Pentobarbital Na (37·5 mgm./kgm.) before exposure, ββ-methylethyl glutaramide (40 mgm./kgm.) after decompression	1/3	3/3
Exposure for 60 min.	Unanæsthetized	3/3*	—
	Pentobarbital Na (37·5 mgm./kgm.)	1/3*	2/2

* Deaths in chamber occurred during exposure to high-pressure oxygen.

It was also found that a barbiturate antagonist, ββmethylethyl glutaramide (megimide), in large doses protected against this potentiation of damage to the central nervous system by barbiturates, only if given with the barbiturate before pressurization of the rats. We have since found that anæsthetic doses of paraldehyde results in a similar potentiation of the central nervous system after effects of oxygen poisoning. Barbiturate anæsthesia also potentiated delayed damage to the central nervous system in mice exposed to high-pressure oxygen. However, large doses of chlorpromazine or reserpine, sufficient to cause severe hypothermia, failed to increase damage to the central nervous system after exposure to high-pressure oxygen.

This paradoxical action of anæsthesia in accentuating delayed damage to the central nervous system due to oxygen poisoning, while protecting against acute damage, has important practical implications and is at present the subject of further investigations in this laboratory. It would seem that chemical protection against the acute effects of high-pressure oxygen poisoning is largely due to a lowering of metabolic rate[7,8] or possibly a reversal in tissue redox potential changes which may result from exposure to high-pressure oxygen. However, organic nervous system damage due to exposure to high-pressure oxygen appears refractory in this regard and possibly is related to raised cerebral carbon dioxide tension and its effect on cerebral vascular calibre and cerebral oxygen tension[9]. Preliminary investigations have shown that if respired carbon dioxide tensions are maintained at higher levels during exposure to high-pressure oxygen, delayed effects in the central nervous system are increased in unanæsthetized rats.

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Effect of Mucopolysaccharides and Colloid on the Function of the Thyroid

In previous experiments we have shown that the serum mucopolysaccharide (hexosamine) content of hyperthyroid patients was considerably less than the normal level. On the other hand, the mucopolysaccharide (hexosamine) content of the blood of hypothyroidic patients was found to be higher than normal and considerably higher than the mucopolysaccharide-level of hyperthyroid patients[1].

It has also been shown that experimental thyroidectomy and methylthiouracyl treatment caused a significant raising of the mucopolysaccharide (hexosamine)-level of the serum of dogs, whereas thyroxine treatment brought about a significant decrease of the serum mucopolysaccharide-level[2]. We have reported too that experiments on rats applying heat and cold stress failed to give tangible results.

In the work reported here carried out on 30 male rats (series 1), we administered thyroxine instead of that of methylthiouracyl over a long period. In contrast to the former experiments in which the changes of the mucopolysaccharide content of the serum were registered within 24 hr., these changes were examined after 25–28 days treatment.

The rats of the control group (10 animals) received a daily injection of physiological saline. The rats of the thyroxine-treated group (10 animals) were given thyroxine (Roche) in increasing daily doses (0·07–0·5 mgm.). The 10 rats of the third group received 75 mgm. methylthiouracyl mixed up in their food daily.

The values of the three groups (average: 90·3, 107·7, 125·7 mgm. per cent) are given in Fig. 1. The average weights of the thyroid glands were: control group, 22 mgm.; thyroxine-treated group, 16 mgm.; methylthiouracyl-treated group, 89 mgm.

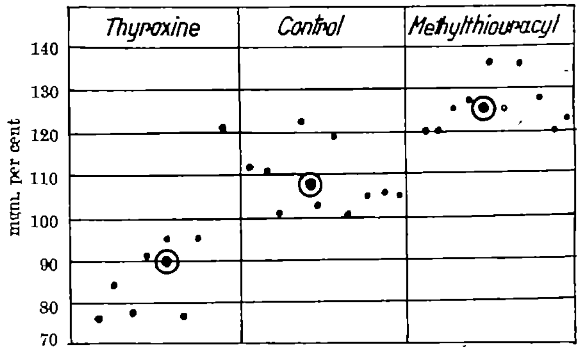

Fig. 1. Serum hexosamine content after 25–28 days treatment

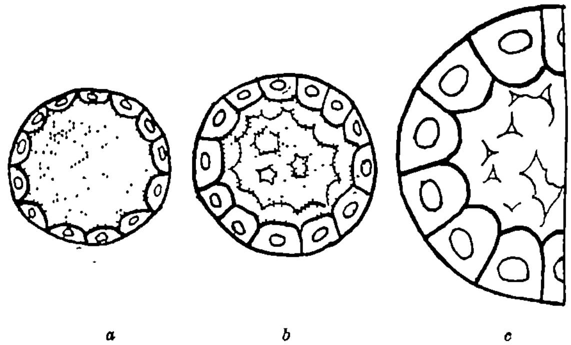

Fig. 2. Thyroid follicle. (*a*) After 27 days of thyroxine treatment flattened cells, accumulated colloid, intense periodic acid – Schiff positivity, hexosamine content, 1,372 mgm. per cent. (*b*) Control: cuboidal cells, normal colloid content; periodic acid – Schiff positivity, hexosamine content, 1,129 mgm. per cent. (*c*) After 27 days of methylthiouracyl treatment. tall columnar cells, scarce remnants of colloid, weak periodic acid – Schiff positivity, hexosamine content, 659 mgm. per cent

In contrast to the histological picture of the normal thyroid, the thyroids of the thyroxine-treated animals showed a flattened epithelium, and large amounts of colloid accumulated in the follicles. On the other hand, animals treated with methylthiouracyl showed a tall columnar lining and only small amounts of colloid. Histochemical investigations (periodic acid – Schiff staining, acetylation, mucase digestion according to Banga and Baló[3], etc.) showed that the colloid of the thyroids of the thyroxine-treated animals contains larger amounts of periodic acid – Schiff positive material than the normal glands, whereas the methylthiouracyl-treated groups show only a very weak positive reaction to periodic acid – Schiff reagent.

Repeating the experiment on 60 female rats (series 2), the average values of serum hexosamine were as follows: 77·1 (thyroxine), 96·4 (control) and 111·7 (methylthiouracyl). In this series we have made parallel biochemical measurements of the hexosamine content of the tissues on the total amount of the glands of the three groups according to Boas's method[4]. The average tissue hexosamine content of 20 normal thyroids was 1,126 mgm. per cent (1,120–1,132); that of 30 thyroxine-treated glands with flattened epithelial cells and much colloid; 1,372 mgm. per cent (1,314–1,430) and the average of 10 methylthiouracyl-treated thyroids with tall columnar cells and only remnants of colloid, 659 mgm. per cent (658–660).

It is thus evident that the hexosamine content is not dependent on the cells but on the colloid (Fig. 2).

These results confirm the assumption based on experiences with man and dogs that thyroid function has an important role in the regulation of the mucopolysaccharide (hexosamine)-level of the serum. In addition to this regulating role of the thyroid, the experiments seem to demonstrate the histological, histochemical, enzymological and biochemical correlation existing between the colloid and hexosamine content.

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Effect of Additions of Sodium and Potassium and of Peritoneal Dialysis with a Hypotonic Solution of Sodium Chloride on the Sodium and Potassium Contents of the Maternal and Fœtal Serum and the Amniotic Fluid of Rats

Electrolytic changes undergone by the mother will lead, both in man and animals, to similar changes in the fœtus within a few hours[1,2]. We have been able to establish that when gravid rats and guinea pigs are subjected to a 2–3-day period of thirsting there is serious damage of the fœtus at the end of pregnancy—a large number of stillbirths or of non-viable young[3]. In order to study the effects of short-term additions, Wistar rats, weighing about 200 gm., were given, on the nineteenth day of pregnancy, 1·7 m.equiv./100 gm. body-weight of sodium in a 20 per cent sodium chloride solution, or 0·8–1·5 m.equiv./100 gm. body-weight of sodium was removed by peritoneal dialysis with 0·5 per cent sodium chloride solution. Another series of animals was given 1·5 m.equiv. potassium in isotonic solution of potassium chloride, intraperitoneally. As there was rapid renal elimination of potassium, 1·0 m.equiv. potassium was administered intraperitoneally to a further series of animals after bilateral nephrectomy. At fixed periods samples of blood from the vena cava inf., of amniotic fluid and of fœtal blood from the umbilical vessels were taken from the animals, which had been anæsthetized with ether, and the sodium and potassium in the serum and amniotic fluid were determined by photometric flashes. We used lithium as a standard and utilized the emission-increasing effect of methanol[4] when determining the potassium content. The animals were given neither food nor drink during this time.

Fig. 1 (upper part) shows the changes observed after addition of sodium and (lower part) after dialysis with a hypotonic solution of sodium chloride, as against 10 control animals (maternal serum: 136 ± 3·6 m.equiv./l., fœtal serum: 146 ± 7·9 m.equiv./l., amniotic fluid: 136 ± 4·3 m.equiv./l.). Figs. 2 and 3 show the changes, after the addition of potassium, in potassium content of the three above-mentioned fluids. The control values for Fig. 2 are:

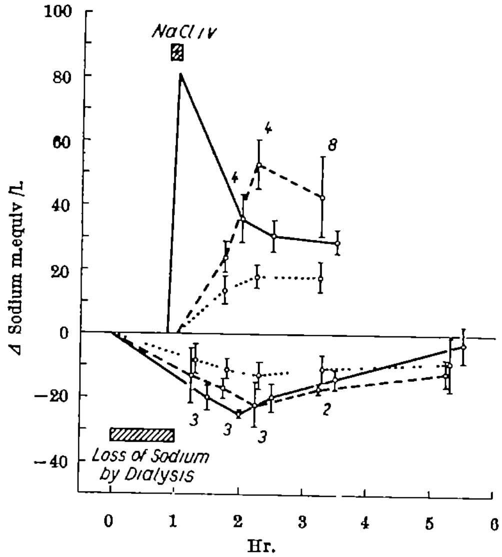

Fig. 1. Differences in the sodium concentration with standard deviations from the control values (= 0) after administration of hypotonic solution of sodium chloride (upper part of diagram) and (lower part) peritoneal dialysis with hypotonic solution. ——, Maternal serum; – – –, fœtal serum; · · · ·, amniotic fluid

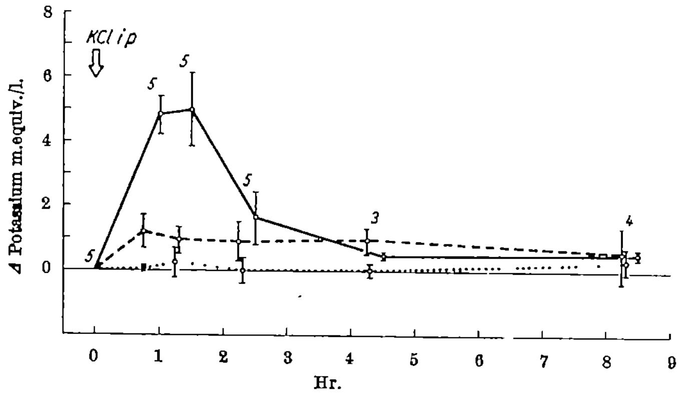

Fig. 2. Differences in the potassium concentration as against the control values (= 0) after intraperitoneal injection of isotonic solution of potassium chloride. ——, Maternal serum; – – –, fœtal serum; · · · ·, amniotic fluid

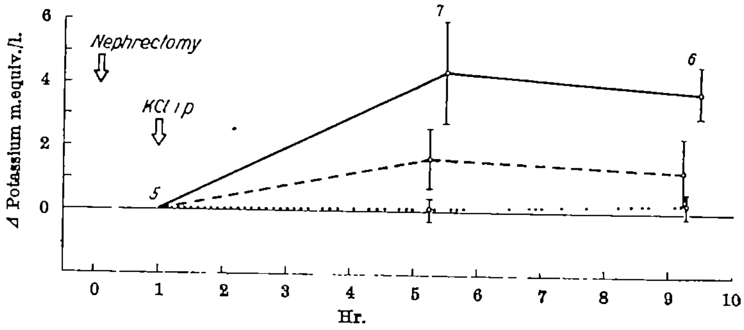

Fig. 3. Differences in the potassium concentration as against the control values (= 0) after intraperitoneal injection of isotonic solution of potassium chloride with bilateral nephrectomy. ——, Maternal serum, – – –, fœtal serum; · · · ·, amniotic fluid

maternal serum, 3·55 ± 0·35 m.equiv./l.; fœtal serum, 4·98 ± 0·38 m.equiv./l.; and amniotic fluid, 4·79 ± 0·30 m.equiv./l. For Fig. 3: maternal serum, 4·26 ± 0·33 m.equiv./l.; fœtal serum, 5·90 ± 0·63 m.equiv./l.; and amniotic fluid, 5·42 ± 0·40 m.equiv./l.

All tests showed abnormal behaviour (apathy and cramp) of the mother animals, and in the case of the fœtus noticeably slight mobility, when the osmotic balance was disrupted.

The tests show that changes in the mother animal lead to protracted, usually lesser but more lasting, changes in the fœtus, but to much slighter changes in the fœtal water. The high sodium values where sodium had been given, and the low ones for the case of dialysis, may be attributed primarily to a disruption of the water supply. Work by Flexner[5-7] indicates that there is a much greater exchange of water than of sodium between the maternal and fœtal fluid-spaces. Similarly, the inconsiderable changes in the fœtal water accord well with investigations by Flexner[8]. Potassium is eliminated so rapidly through the maternal kidneys that the potassium content of the fœtal serum increases only slightly. In the case of animals with nephrectomy a gradual increase is more in evidence. Unless the high potassium-level in the case of the animals without nephrectomy is, likewise, to be attributed to a loss of fœtal water, potassium is retained by the fœtus. This conclusion would agree with recently published results by Stewart and Welt[9], who found that a diet deficient in potassium and dialysis with solution of sodium bicarbonate resulted in a much more considerable loss of potassium in the maternal than in the fœtal tissues.

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PHARMACOLOGY

An Antigen in Malignant and in Embryonic Tissues

A NUMBER of human tumours have been found to grow in cortisone or hydrocortisone-treated weanling rats and in hamsters. Growth cannot be obtained without the use of cortisone or adrenocorticotrophic hormone. It is well known that these animals develop an immunity against the implanted tumour.

We have been interested in trying to determine the basis of this antigenic activity and, to this end, have injected human sera or homogenates of various human tumours or other human tissues into weanling rats. Seven days later these animals were challenged with the transplantable human tumour *HS*1, and at the same time, and again later, injected with cortisone.

The antigenic response was assessed by comparing the growth of this tumour *HS*1 in control rats and in the rats thus 'immunized'.

Potent antigenic activity, producing almost complete growth inhibition, has been found in homogenates of tumour *HS*1, and of rapidly growing sarcomata recently removed from patients as well as in a variety of fœtal tissues, for example, spleen, and to a lesser extent skeletal muscle. On the other hand normal human plasma or serum is practically inactive and a homogenate of normal human adult muscle has little effect. So far as can be determined this effect is not related to the blood group or the *Rh* factors.

Table 1. INHIBITION OF GROWTH OF SARCOMA *HS*1 IN RATS PREVIOUSLY TREATED WITH NORMAL AND MALIGNANT HUMAN TISSUE

No. of samples tested, each on a group of 6 'immunized' rats	Tissue injected as antigen	Tumour size in arbitrary units after 14 days growth		Per cent of inhibition of tumour growth
		Control rats	'Immunized'* rats	
	(*A*) *Normal tissue*			
29	Adult serum	1·34±0·10	1·5±0·14	None
12	Adult muscle	1·8±0·28	1·87±0·40	24
9	Fœtal muscle	1·77±0·20	0·38±0·10	79
4	Fœtal spleen	1·77±0·13	0·07±0·05	96
	(*B*) *Malignant tissue*			
190	*HS*1	2·0±0·17	0·15±0·17	92
2	Melanotic sarcomata *M* and *G*	2·2	0·1	96
1	Chondroma *R*	2·3	0·05	98
1	Synovial sarcoma *J*	2·0	0·1	95

* Immunized rats are injected with antigen 7 days before challenge with tumour and cortisone treatment.

The antigen appears to be very labile in that it is destroyed by freezing and thawing and disappears on prolonged storage. M. J. How has found that it may be included in the methanol-chloroform fraction of the tumour extract.

Embryonic tissue is very active as an antigen and thus resembles malignant tissue: it appears possible that antigenic activity is related to the high rate of cellular division in these tissues which may accentuate the potency of an antigen present in normal human tissues.

The high incidence of carcinoma of the lung in Cushing's disease patients with their increased hydrocortisone blood-level is possibly a pointer to the importance of cortisone in allowing the implantation and growth of cancer cells in man.

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Rapid Hepatic Shift of Worms in Mice infected with *Schistosoma mansoni* after a Single Injection of Tartar Emetic

MUCH work has already been carried out on the treatment of mice infected with schistosomes. It has been observed that the worms which normally inhabit the mesenteric veins of animals infected with *Schistosoma mansoni* are forced to migrate to the liver after treatment with active schistosomicidal drugs[1-6]. This hepatic shift has usually been observed after a full course of treatment, and where the course had been inadequate, relapses were found to occur owing to the re-migration of the worms back to the mesenteric veins. Standen[6] observed a hepatic shift as early as one day following two oral doses of 42 mgm./kgm. sodium antimonyl III gluconate, but after 7 days the worms were completely restored to their normal distribution in the hepatic portal system. In the work reported here we followed up the effect of a single dose of tartar emetic on the worm migration to show that the hepatic shift actually occurs much earlier than had been previously expected.

Five groups of mice infected with *Schistosoma mansoni* were used 9 weeks after infection. Each group consisted of 10 mice: one group was used for controls and each of the remaining four groups were given an intraperitoneal injection of tartar emetic 25 mgm./kgm. and autopsied at intervals of 30 min., 2 hr., 8 hr. and 32 hr. respectively. After ligaturing the portal vein immediately after death, the number of worms in the mesenteric veins, portal vein and liver were counted separately. In the latter procedure, the liver-crush technique described by Standen[6] was used.

Table 1. DISTRIBUTION OF SCHISTOSOMES IN THE HEPATIC-PORTAL SYSTEM OF THE MOUSE FOLLOWING AN INTRAPERITONEAL INJECTION OF TARTAR EMETIC

Group (10 mice per group)	Post-treatment period (hr.)	Average No. of worms/ mouse	Distribution of worms (per cent)		
			M	*PV*	*L*
1	½	13	10	12	78
2	2	13	2	2	96
3	8	12	0	10	90
4	32	15	35	31	34
Controls	—	13	75	21	4

M, mesenteric veins, *PV*, portal vein; *L*, liver.

The results are summarized in Table 1, which shows the percentage of worms in the mesentery, in the portal vein and in the liver at the various time intervals investigated.

It is evident that the worms start migrating from the mesentery to the liver in even less than half an hour after the injection of tartar emetic, and that by the end of 2 hr. this hepatic shift is virtually complete and practically all the worms are then located in the liver. At 8 hr. after injection the worms are gradually making their way back to the mesentery, and by 32 hr. only one-third of them are still left in the liver.

This rapid hepatic shift may conveniently be explained on the ground that the schistosomes lose their muscle tone as soon as the drug reaches them in an effective concentration. They thus lose their grip on the mesenteric blood vessels and are then swept back to the liver by the blood stream within 2 hr. of the drug treatment. The schistosomes start migrating back, however, as soon as the effective concentration of the drug in the blood drops and they begin regaining their muscular activity. The process of re-migration back to the mesentery undoubtedly takes much more time than the actual hepatic shift since the worms have now to travel against the direction of the blood flow.

The hepatic shift thus occurs very soon after the drug is given and is much quicker than had been previously reported by earlier workers. This rapid effect, together with the slow return of the worms to the mesentery, may have an important bearing on the optimum time interval between doses of antimonials and possibly of other drugs administered in the course of treatment of human schistosomiasis.

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RADIOBIOLOGY

Whole-Body Oxygen Consumption during Irradiation for the Survival of Rats after Exposure to X-rays

THE study of consumption of oxygen by animals during irradiation enables one to follow the influence of the intensity of metabolic processes in the organism from the start of the action of ionizing radiation and, consequently, contributes to elucidating the frequent changes in the metabolic processes of the organism evoked by radiation. In previous papers[1,2] we demonstrated the raised consumption of oxygen in rats, rabbits and chickens during their exposure to X-rays. In the present experiment I examined the relation between whole-body oxygen consumption during irradiation (cm.^{3} 100 gm. min.$^{-1}$) and the survival of animals irradiated with 600 r. and 700 r. of X-rays. (The X-ray controlling factors were 180 kV., 15 m.amp., filter 1 mm. aluminium., 0·5 mm. copper, focal distance, 50 cm., dose-rate 50 r./min.)

180 male Wistar rats were used, having an average weight of 190 gm. The rats were divided into two groups, the first consisting of 80 rats irradiated with 600 r. of X-rays, and the second 100 rats irradiated with 700 r. X-rays.

Each animal was placed in a 'Umaplex' chamber and its oxygen consumption measured. The amount of air passing through the chamber depended on the weight of the animal. Measurements were taken and irradiation commenced from the second minute after placing the rat in the chamber. The technique of measurement has been described elsewhere[3].

Fig. 1 shows the oxygen consumption during exposure of rats irradiated with 600 r. of X-rays (equivalent to $LD_{50/30}$) and its relation to their survival. The correlation of oxygen consumption during exposure and the survival of irradiated animals reveals a statistically significant relationship ($r = 0 \cdot 72$, $n = 80$, $P < 0 \cdot 01$), according to which rats with a higher absolute oxygen consumption survive 30 days post irradiation, while animals the oxygen consumption of which during exposure to X-rays is rather low usually perish within the first 10 days after irradiation.

After a dose of 700 r. (equivalent to $LD_{100/12}$) the survival-time of the animals shows a positive correlation with their oxygen consumption during irradiation (Fig. 2). According to this, animals with a lower oxygen consumption perished much sooner. In this part of my experiment the correlation is statistically significant when adding up the absolute consumption of oxygen prior to and during irradiation ($r = 0 \cdot 66$, $n = 100$, $P < 0 \cdot 01$).

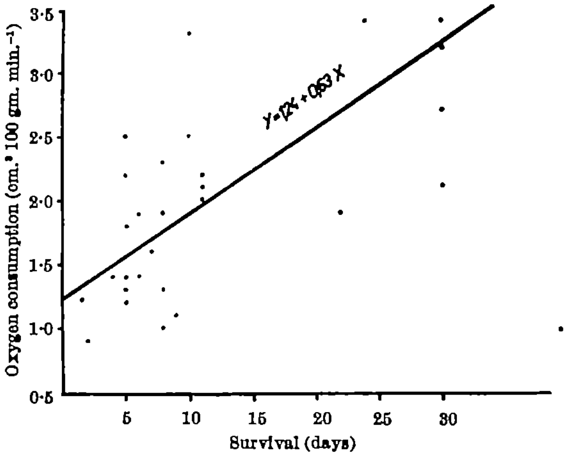

Fig. 1. Relation of oxygen consumption to survival after exposure to 600 r.

The results of these experiments follow my previous work concerning oxygen consumption of animals during irradiation[3,4], in which I demonstrated that raised oxygen consumption of rats during irradiation is dependent on the peripheral nerve tracts passing through the spinal cord being intact[5].

It is obvious that a rise in the oxygen consumption during exposure to X-rays is an indication of the reactivity of the irradiated animal. Since the oxygen consumption of rats immediately after being placed in the chamber is not effected in the same way for all animals[6], the absolute values for oxygen consumption obtained are, under given conditions, determined to a considerable extent by the way in which the individual animals react to the X-rays.

Consequently, it would seem that the relation between oxygen consumption by rats during exposure to X-rays and their survival is dependent above all on the state of reactivity of the animals during irradiation.

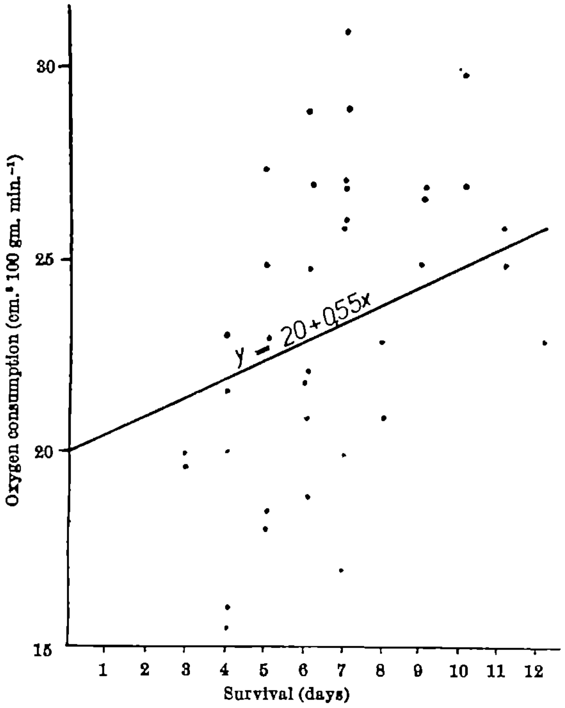

Fig. 2. Oxygen consumption during exposure to 700 r. and its relation to survival

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Radiation Protection with Sulphur and Some Sulphur-containing Compounds

It is known that many biological systems can be protected in part against the effect of ionizing radiation by the presence of very small amounts of additives. It is often difficult to assess the mechanism of this protection due to the complex nature of the system. The effect of radiation on simple organic polymers can also be reduced by various additives. It is believed that this offers many analogies with protection in the more complex biological systems.

The present investigations involve the use of both non-aqueous and aqueous polymer systems; the former being by polydimethyl siloxane fluids and the latter dilute aqueous solutions of polyvinylpyrrolidone. We examined the effect of a wide variety of additives on the behaviour in these systems when exposed to γ-radiation.

The purpose of this communication is to report the high efficiency shown by colloidal sulphur suspensions in very low concentrations (in the range of 5–50 μgm./c.c.) in inhibiting the cross-linking of the investigated systems. This was readily shown by an increase (by a factor of 2 or 3) in the dose to bring about gelation. For the aqueous solutions, for example, this dose was increased from 2 to 6 megarads. This degree of protection is even more remarkable when it is remembered that the sulphur was present in the form of relatively large colloidal particles and not as molecules dispersed at random. A high efficiency in protection by sulphur in aqueous protein solutions was previously reported[1,2].

A similar effect of protection was shown by many sulphur-containing compounds which were used in the dilute aqueous polyvinylpyrrolidone solutions. It is not yet established whether the protection by these compounds is offered by an initial release of sulphur or by an alternative route.

The ability of sulphur to protect macromolecules against cross-linking under irradiation was found to be too large to agree with previously proposed mechanisms involving radical scavenging. This mechanism is, however, present with certain other additives. Further details of this work will be published shortly.

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Experimental Oxidation of Hæmoglobin Effects of Irradiation in Rats

The sensitivity of human intracellular hæmoglobin to oxidation by nitrite ions is raised during growth, pregnancy in malignant disease and in some other conditions[1]. It has also been shown to be increased in similar circumstances in the rat[2,3] and in this animal the changes appear to be related to riboflavin metabolism[2–4]. Both riboflavin deficiency[5] and X-rays interfere with growth and are teratogenic. The effect of radiation on the stability of hæmoglobin against oxidation by nitrites has now been studied in the rat. A dose of 500 r. was chosen ($< \mathrm{MLD}_{50}$) and was administered at approximately 20 r./min.

The age of the rat at irradiation had a definite effect on the reaction of the animal to this dose (cf. ref. 6). The growth-rate of suckling rats was only slightly affected, weaned but immature rats suffered a temporary pause of about one week in their growth, while mature rats lost weight for the week following irradiation.

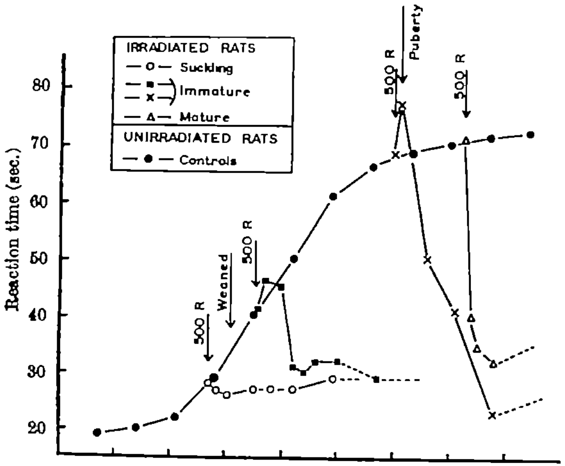

Fig. 1. Change in the reaction time (the time taken to convert the hæmoglobin of a standard saline red cell suspension to methæmoglobin using 0·1 per cent sodium nitrite (*in vitro*) with age and the effects of 500 r. cobalt radiation at various ages. (The *S.D.* of any point is approximately ± 5 sec.)

While the ultimate effect on the hæmoglobin oxidation sensitivity—marked increase in the hæmoglobin oxidation sensitivity for at least three months—is identical whatever the age at irradiation, the initial changes did vary with age (Fig. 1). Suckling rats merely retain the increased sensitivity of the rapidly growing animal into adult life. Mature rats, from puberty to old age, respond to irradiation with an immediate increase in their hæmoglobin oxidation sensitivity to levels found normally in the young or pregnant. Weaned, but still immature, rats, however, exhibit the ultimate increased sensitivity of their hæmoglobin only after an initial period of 3–4 days decreased sensitivity.

The increased hæmoglobin oxidation sensitivity of starving, young or pregnant rats can be altered towards the normal by 2 mgm. of riboflavin given subcutaneously[3,4]. That riboflavin or flavin mononucleotide is without effect in the increased sensitivity following radiation but is effective when given in conjunction with adenosine triphosphate (Table 1) supports the suggestion[7] that some at least of the effects of radiation are due to interference with oxidative phosphorylation.

Table 1. The Effect, in Irradiated Adult Rats, of the Injection of Various Substances on the Time (Sec.) taken to oxidize their Intracellular Hæmoglobin to Methæmoglobin by a 0·1 per cent Solution of Sodium Nitrite *in vitro*. (*S.D.* given, samples of five animals.)

Substance injected	Reaction-time at intervals after injection			
	Before	1 hr. after	2 hr. after	4 hr. after
2 mgm. Riboflavin	35±3	36±6	36±2	37±5
2 mgm. Flavin mononucleotide	39±7	—	37±2	36±3
25 mgm. Flavin mononucleotide	35±4	35±5	35±5	35±5
2 mgm. Riboflavin 2 mgm. Adenosine triphosphate	32±4	31±6	38±3	31±5
2 mgm. Riboflavin 5 mgm. Adenosine triphosphate	29±2	30±2	47±5	34±3

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Dose Effect Curve of Somatic Mutations in *Ephestia kühniella* Z. for Low Dose-range (0–200 r.).

Ephestia kühniella Z., the meal moth, proved to be a good object for investigation of quantitative relations between somatic mutations and ionizing radiations[1,2]. These former investigations were concerned with relative high doses (100–1,000 r.). It seemed interesting to extend the experiments to the lower dose-range, since there is a complete lack of knowledge of this part of the dose effect curves of somatic mutations in animals.

On each of the hind-wings of *Ephestia* there are about 6,250 scales of uniform shape and colour. After irradiation at an early stage of development clusters of scales of different appearance are found, which have been described as somatic mutations[3]. After irradiation of 10–16 hr. old pupæ these mutations only affect single scales, so that the mutation-rate can be measured directly from the proportion of mutated scales to the total number of scales. This calculation is valid only on the assumption that the irradiation has no lethal effect on the mother-cells of the scales, no matter how high the dose. In order to ascertain this fact the number of scales on 6 wings of individuals irradiated with 10 resp. 200 r. was determined and the mean values proved to be not significantly different (6,270/wing and 6,233/wing, $P = 0{\cdot}8$).

Groups of at least 40 pupæ were exposed to one of the different doses (10, 20, 40, 80, 200 r., and control), and both wings of 30 randomly chosen individuals were examined for mutations. This experiment was repeated twice, so that for each dose 3 × 30 × 2 × 6,250 = 1,125,000 scales were classified. For the irradiations an *RT* 100 X-ray apparatus (C.H.F., Hamburg) was used, running at 100 kV., with a 1·7 aluminium filter and a dose-rate of 133 r./min.

Table 1. *ES* 1 Mutation Rates for Controls and for Different X-ray Dosages

Dose (r.)	Exp. I	Exp. II	Exp. III	Total
0	9	4	6	19
10	7	8	7	22
20	15	14	10	39
40	17	12	15	44
80	31	25	19	75
200	69	66	67	202
Total	148	129	124	401

Several mutant types are found in *Ephestia* scales, but here only the results of the most frequent type (*ES* 1) are reported. Table 1 shows the number of mutations obtained in the three experiments. The differences between experiments all lie within the range of chance deviations. Fig. 1 presents the sum total of mutations for each dose with the confidence limits for a $P = 0{\cdot}05$, calculated according to the method proposed by Stevens[4]. It is possible to fit a linear regression line to these data (line *a*). A χ^2 test based on the differences between the observed values and the values expected from a linear regression results in a $P = 0{\cdot}5$–$0{\cdot}3$, which shows that the experimental data are consistent with the hypothesis of an increase of mutation frequencies proportional to the dosage of 0–200 r., with no indication of a threshold.

However, earlier experiments with higher doses (100–1,000 r.) resulted in a dose-effect curve, which was decidedly non-linear but was proportional to the square of the dosage ($P = 0{\cdot}5$–$0{\cdot}3$) (Fig. 2). It is therefore to be expected that in the lower dose-range the curve follows the same rule. A χ^2 test for a straight regression line after a quadratic transformation of the abscissa, however, results in a $P = 0{\cdot}05$–$0{\cdot}02$. The main contribution to the high χ^2 value comes from the control- and 10 r.-points. A χ^2 test, which omits these two values, so that only the doses 20, 40, 80, and 200 r. are included in the calculation, yields a $P = 0{\cdot}9$–$0{\cdot}8$, which signifies a very close fit to the expectation (Fig. 1, line *b*). A linear regression for the same dose-range has a *P* of only 0·3–0·2. The same trend shows up not only in the sum total of the data but also in each experiment separately. These results therefore justify the conclusion that in the range of 20–1,000 r. the increase in mutation frequency is proportional to the square of the dose, whereas for 0 and 10 r. this relation no longer holds good. Both values are significantly smaller than expected. If very low doses have any effect at all, then it must be much smaller than would be expected from the results of irradiations with higher doses.

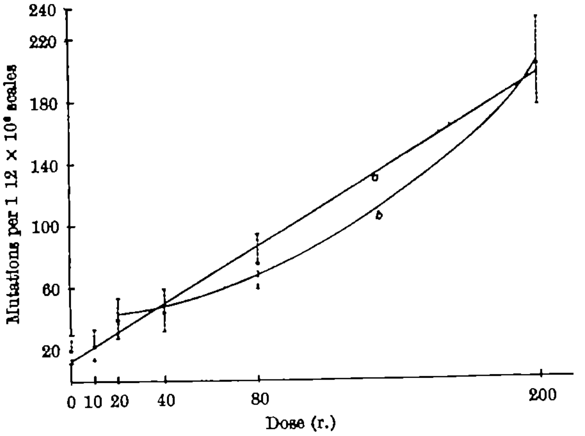

Fig. 1. *ES* 1 mutation-frequencies at X-ray doses of 0–200 r., with 5 per cent fiducial limits. *a*, Regression line proportional to dose; *b*, regression line proportional to dose²

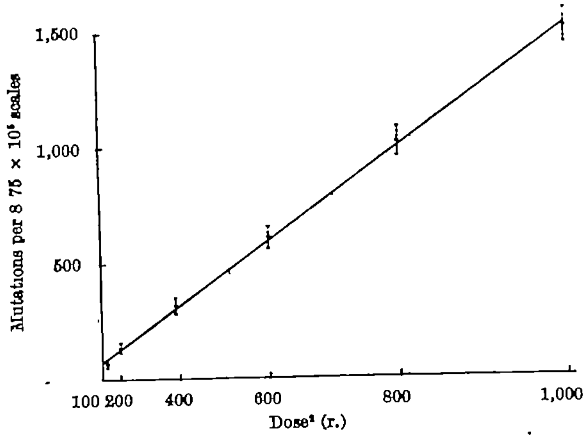

Fig. 2. *ES* 1 mutation-frequencies at X-ray doses of 100–1,000 r., with 5 per cent fiducial limits and a calculated regression line proportional to dose²

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BIOLOGY

Dry-Mass Distribution of Bone Marrow Free Cell Population in the Mouse as determined by Interference Microscopy

The hæmopoietic function of the bone marrow is of primary importance in the defence and normal maintenance of the body. Rapid formation of the cellular dry mass in the precursor cells of the bone marrow is essential to produce more blood cells to replenish the diminishing reserve pools in times of stress or emergency. The determination of a distribution curve for bone marrow cellular dry mass in the normal marrow cell population is essential, for this curve can serve as a base-line for the investigation of changes in dry mass distribution of bone marrow after irradiation, drug treatment, toxicity, trauma or disease, or as an assessment of the extent of damage and/or of recovery following insult to the body.

The femoral bone marrow of healthy LAF_1 black male mice from the Jackson Memorial Laboratories. Bar Harbor, approximately 3 months of age and 22–25 gm. body-weight, were used as a source of material for this work. Live cell slides from the marrow were obtained by killing the animals by a quick blow on the head. The two femurs were immediately severed from the body and the two ends of the femur were cut off with a sharp knife. Only a short middle section was retained for its marrow. The marrow was washed out from the section with a drop or two of phosphate buffered (*p*H 7) isotonic saline by inserting a No. 25 needle on a 2-ml. syringe

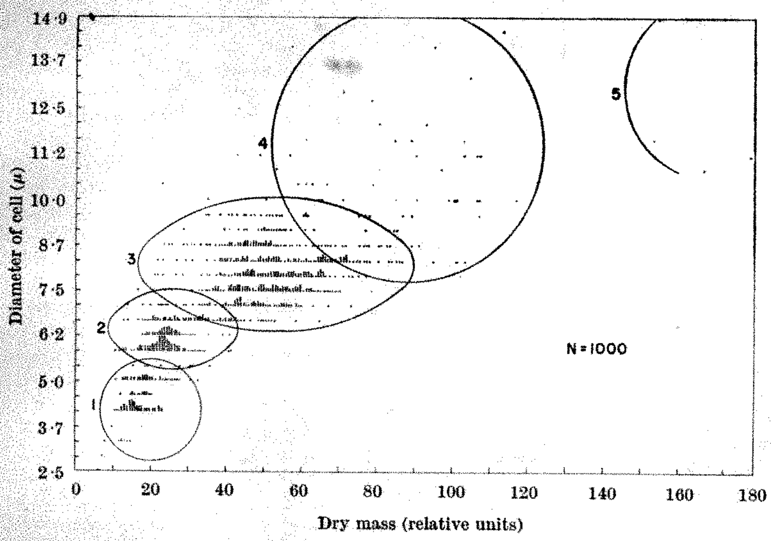

Fig. 1. Size and dry-mass dispersion of the *LAF*$_1$ mouse femoral bone marrow cells. The cells can be divided into five groups (circled areas) according to their diameter and dry mass

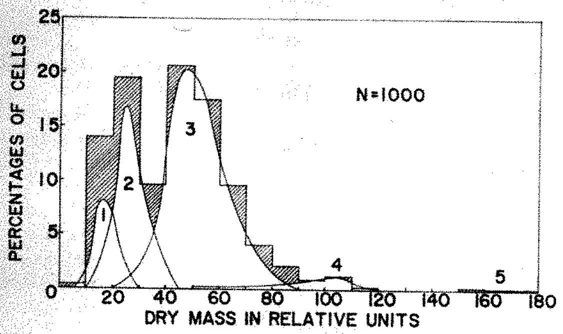

Fig. 2. Dry-mass distribution of the *LAF*$_1$ mouse femoral bone marrow cells. The cells were divided into five dry-mass groups according to the data obtained from Fig. 1. A curve for each group was approximated and fitted into the histogram to show the approximate dry-mass distribution of each group

into the bone marrow cavity. The saline with the washed-out marrow was carefully deposited on a clean slide. The cells were dispersed evenly in the saline by shaking the slide a few times in a rotary motion. A cover-glass was then placed over the cell suspension and gently pressed down to flatten the cells slightly without injuring them. The excessive fluid around the cover-glass was blotted off and the cover-glass was sealed on the slide with paraffin. The slide was immediately put under the interference microscope for dry mass determination. Twenty-five single cells chosen at random were measured from each femur, making a total of 50 cells from each animal. In all, a total of 1,000 cells were measured from 20 animals. The megakaryocytes were excluded from these measurements. They were mostly crushed when the cover-glass was pressed down to flatten the other cells, an essential for accurate measurement. The dry mass of the cells was calculated according to the method published previously[1].

An A—O interference microscope with a × 100 water immersion shearing objective and condenser, and an attached half-shade eyepiece were used for the determination of dry mass. The mercury green line ($\lambda = 0{\cdot}546\mu$) isolated from a mercury lamp served as the light source.

Table 1

Group No.	Diameter (μ)	Dry mass in relative units
1	2·5– 5	10– 30
2	5·4– 7·1	10– 45
3	6·6–10	20– 90
4	8·3–14·5	50–120
5	11·2–	150–

A scatter diagram (Fig. 1) was made to show the dispersion pattern of the cells in relation to their dry mass and average diameter as determined by the mean diameter of the long and short axes of the cell. A histogram (Fig. 2) for the dry-mass distribution was also made from the results obtained. The diameter of the cells ranged approximately from 2·5 to 14·5μ and the dry-mass values ranged from less than 10 relative units to 180 relative units. A study of the scatter diagram revealed that the cells could be divided into five different groups according to their size (diameter) and dry weight (dry mass). Table 1 shows the approximate range in size and dry mass values of each group.

Group 1 cells were easily identified as erythrocytes, while cells in the other groups were not differentiable under the interference microscope. Group 5 cells were possibly small megakaryocytes which escaped our screening for their exclusion.

With the above information as a base, individual dry mass distribution curves for each group were approximated and fitted into the histogram (Fig. 2). From the histogram we found that the modes for the dry-mass values were: 10–20 units for group 1, 20–30 units for group 2, 40–50 units for group 3 and 100–110 units for group 4. There were too few cells in group 5 to give valid mode data. These curves as well as the histogram and the scattergram have proved valuable in our preliminary investigations of the changes of dry mass values of mouse bone marrow after X-irradiation. From previous results of changes in cellular mass after drug or serum treatment of Ehrlich mouse ascites cells[1-3], we believe our present studies may be applicable as a means for assessing the toxic side-effect of drugs.

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Reciprocal Transplantation of Blastocysts between Rats and Mice

IN an earlier communication[1] it was shown that a mouse blastocyst, transplanted beneath the kidney capsule of a mouse, not necessarily of the same genotype, gave rise to an embryo surrounded by a highly invasive trophoblast. The trophoblast destroyed and infiltrated the host organ.

Table 1. RESULTS OF TRANSPLANTING RAT AND MOUSE BLASTOCYSTS TO THE KIDNEY

	No. of experiments	No. of trophoblasts only	No. of trophoblasts and embryos	Total No. of implants × 100/ No. of experiments (per cent)
Rat→mouse	30	4	0	13
Mouse→rat	43	13	2	28
Mouse→mouse	123	69	27	78
Rat→rat	54	10	0	18

Successful implantation of ova following xenospecific transfer has never been recorded except between goat and sheep[2,3]. This communication deals with the results of transplanting 4½-day-old rat blastocysts beneath the kidney capsule of mice, and 3½-day-old mouse blastocysts to a similar position in rats. Only 1 blastocyst was transplanted to any 1 host organ.

Table 1 shows that the reciprocal transfers were not equally successful. More than a quarter of the mouse blastocysts transferred to the rat 'implanted', and gave rise to a flourishing trophoblast. It invaded and phagocytosed the rat kidney, and exhibited all the characteristics of a mouse trophoblast developing on a homospecific host organ (Fig. 1). In 2 cases the inner cell mass of the blastocyst had differentiated into small and partly necrotic embryos (Fig. 2).

In the rat→mouse transfers the percentage success was much lower. Furthermore, the resultant trophoblast was stunted and poorly developed. No proliferation of the inner cell mass occurred. A comparable difference in the viability of ova following transfers between rat and mouse has been observed by Briones and Beatty[4]. In their experiments, 8-celled mouse ova transferred to the rat uterus underwent one further cleavage division; but in reciprocal transfers rat ova failed to undergo any further development.

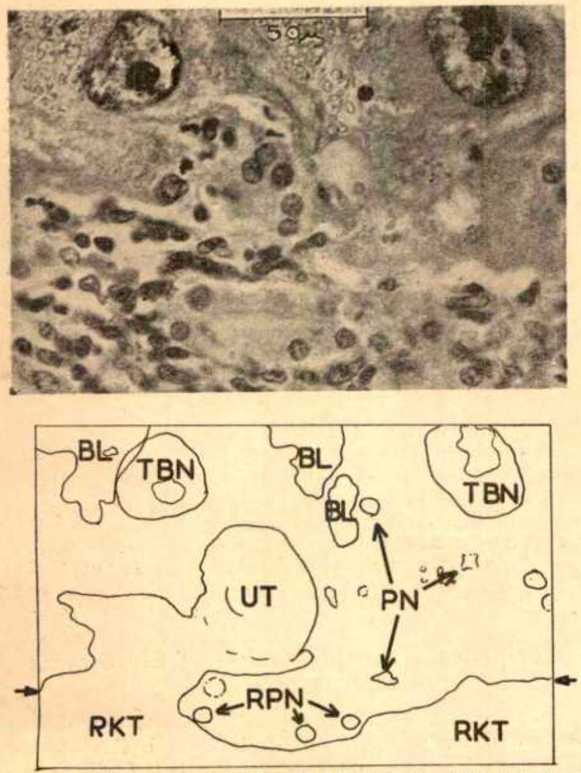

Fig. 1. Mouse trophoblast invading rat kidney. *TBN*, Nucleus of trophoblast cell; *BL*, lacuna of extravasated host blood; *PN*, phagocytosed nuclei; *RPN*, recently phagocytosed nuclei; *UT*, uriniferous tubule; *RKT*, rat kidney tissue. Marginal arrows indicate the limit of the trophoblast

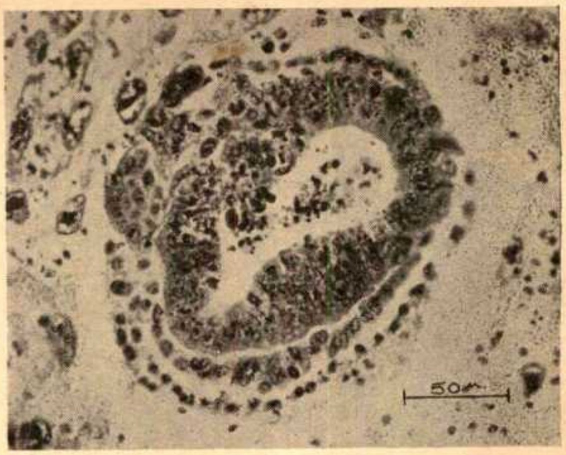

Fig. 2. Section of a mouse embryo developing on the kidney of a male rat. The embryo, and embryonic membranes, are of fairly normal configuration. Note that several cells of the embryonic ectoderm are undergoing mitosis. Autopsy six and a half days after the transplantation of one blastocyst

The homospecific transfer of blastocysts to the kidney in both species further demonstrates the superior ability of the mouse blastocyst to develop in an alien environment (Table 1). Mouse blastocysts are more than four times more successful in 'implanting' on the mouse kidney than rat blastocysts on rat kidney. This proportion of successful rat to rat transplants is very similar to that obtained by Nicholas[5]. My results differ considerably, however, in that he found no invasion of the kidney cortex, no differentiation of the trophoblast, and excessive proliferation of embryonic tissues. I am unable to confirm these findings (Table 1).

The remarkable tolerance of the host to the heterospecific mouse trophoblast may be attributed to two factors. First, the antigenic nature of the trophoblast appears to be weak, although this weakness cannot be ascribed solely to its embryonic character. The transplantation of a 6½-day-old embryo, the associated trophoblast of which has been removed, evokes, in a similar period of time, a strong reaction as manifest by the massive accumulation of leucocytes around the graft. The response to the trophoblast is very much weaker. Secondly, it has been demonstrated recently[6] that the human trophoblast absorbs during pregnancy anti-trophoblastic antibodies. The mouse trophoblast may have a similar function, and any antibodies that are produced would be removed from circulation.

The reason for the failure of the rat trophoblast to proliferate on the mouse kidney is, at present, not clear.

I thank Drs. M. Fischberg and A. W. Blackler for advice. This work was carried out during the tenure of a Ford Foundation research fellowship.

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Brood-production after Hypophysectomy in the Viviparous Teleost *Mollienesia latipinna* Le Sueur

Among the predominantly oviparous teleost fishes the habit of viviparity is widespread, and appears to have arisen repeatedly and independently in various families. Details of viviparity are best known for the cyprinodont family Poeciliidae, which includes several popular aquarium fishes such as the guppy, platyfish and swordtail, but even for this group there is very little known about the physiological specializations involved in gestation, which occurs within the ovarian follicle, and in the birth of the young fish[1]. Evidence so far has suggested the involvement of pituitary hormones in these processes[2], but the essential step of hypophysectomy of pregnant females has not been performed, possibly because of the surgical problems inherent in the small size of these fishes. Recently I have developed a technique for hypophysectomy in the poeciliid *Mollienesia latipinna*, which permits removal of the pituitary gland with good survival in fish down to about 35 mm. length. The operation will be described in detail in a later paper, and this communication is concerned only with the salient effects of hypophysectomy on brood production in the female.

A black domesticated variety of *M. latipinna* ('black mollie') was used, and the fish were kept in stock tanks and individually in two-litre jars under conditions of constant temperature (27 ± 1° C.) and illumination (16 hr. a day). Normal fish were kept in dechlorinated Liverpool tap-water, but it was soon found that, like *Fundulus heteroclitus*[3], hypophysectomized mollies cannot live in freshwater. *M. latipinna* is strongly euryhaline[4], and operated fish were accordingly kept in 33 per cent sea water (for up to three months), individuals being acclimatized to this medium for at least one week before the operation.

Under the above conditions, normal females isolated with males in jars repeatedly give birth to broods of between one and 40 young fish at intervals of about 30 days. After a brood has been born, the ovary contains only partly developed oöcytes, some of which then grow rapidly to maturity in five days. When ripe the yolk-filled eggs are not ovulated, but are retained in their follicles where they are fertilized by sperm introduced into the oviduct by the male in copulation. Each embryo then develops on top of its egg within the follicle; when development is completed the follicles rupture (all within an hour or two of each other) and the motile young are freed into the ovarian lumen. Muscular contractions of the ovary and oviduct then expel them to the exterior.

Removal of the pituitary gland from a pregnant female does not interfere with the current brood. On the contrary, the embryos complete their development, are born in the normal manner, and are as lively and survive as well as young fish born to normal females. This conclusion is based on observations of 12 operated females in which hypophysectomy was shown to be complete by total cessation of growth and by the development of acute distress symptoms within 24 hr. after transfer to freshwater. (Sham-operated fish, in which the pituitary gland was exposed but left *in situ*, grew vigorously and lived indefinitely in freshwater.) In addition, the absence of pituitary remnants was confirmed in eight of these hypophysectomized females by examination of serial sections of the middle part of the head. The interval between hypophysectomy and birth of a brood varied from 1 hr. to 26 days. Six other pregnant females which were hypophysectomized either died or were killed before their broods were born, but in every case the ovary at autopsy contained living or newly-dead embryos. In none was there any signs of resorption or death of the embryos as a result of the withdrawal of maternal pituitary hormones.

These experiments have also confirmed that in this viviparous fish, as in oviparous teleosts[5], pituitary function is necessary for the second-phase (vitellogenic) growth of the oöcyte. Early second-phase oöcytes present in the pregnant ovary at the time of hypophysectomy were resorbed at the same time as the embryos were developing although, as in normal females, the occasional fully-grown egg that had escaped fertilization (or in which embryonic development had been arrested) was retained in its follicle and released along with the living young at parturition. Following parturition in hypophysectomized fish the younger oöcytes failed to embark on the normal rapid yolk-accumulation and growth, the ovary thereafter remaining in an immature state with only small pre-vitellogenic oöcytes. In sham-operated females, in contrast, such oöcytes grew after parturition at the same rate as in normal fish.

It appears that in *Mollienesia*, and presumably in the other live-bearing poeciliids, adenohypophysial hormones are directly concerned in oöcyte development but are not involved in maintaining gestation, nor in the ovulation-like release of the young from the follicles. Nevertheless, some kind of humoral coordination of follicular rupture seems to be indicated by the retention of non-fertile eggs and their ovulation at the same time as the motile young fry are expelled from their follicles, and by four cases in which the 'brood', born to normal females at the predicted time, contained only one motile fish together with eggs, partly-developed embryos and dead fry.

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Interaction of 6-Methylaminopurine and Adenine in Division of Soybean Cells

Cell division in many cultured plant tissues is known to be promoted by kinetin (6-furfurylamino purine) and related compounds[1]. Among these related compounds is a series of 6-*n*-alkylaminopurines. Tests at the University of Wisconsin with tobacco callus tissue revealed no activity for 6-methylaminopurine (MAP), a slight activity for the ethyl compound, and increasing activity through the series *n*-propyl-, *n*-butyl-, *n*-pentyl-, and *n*-hexylaminopurine, with the hexyl and pentyl compounds giving much the same results; activity then decreased with increasing size of the alkyl group[2]. A similar alignment was obtained for these compounds by Kuraishi[3] using a radish leaf disk test dependent on cell enlargement rather than division. Kuraishi, however, did observe a slight activity for the 6-methylaminopurine. Recently, I have obtained almost identical results for the series in another cell division test[4] which utilizes tissues derived from soybean (*Glycine max*, var. Acme) cotyledons.

I have been particularly interested in MAP since others have reported that it occurs in some soluble ribonucleic acids[5] and since there is considerable evidence that kinetin-type compounds (which may be regarded as derivatives of MAP) may influence growth through effects on ribonucleic acids and perhaps afterwards on protein synthesis. MAP, when added alone to the test medium[4], not only failed to stimulate division of the soybean cells at all concentrations used, but also actually inhibited the growth expected in controls when it was used in fairly high concentrations (exps. *A* and *B*, Table 1). This inhibition was most noticeable when the control pieces showed considerable growth as a result of carry-over of kinetin from the previous medium. That MAP can prevent kinetin-induced growth is evident from exp. *B* of Table 1.

Since MAP is an adenine derivative, I suspected that the methyl compound was inhibiting adenine-requiring processes in the cells. That this probably is so is apparent from the complete reversal of the inhibition by adenine added to the test medium even though adenine itself, being rather erratic in its effects, gave either no promotion or an inhibition (Table 1). The riboside adenosine was as effective as adenine in the reversal and by itself showed little inhibition or a slight promotion. Compounds which were ineffective include hypoxanthine, xanthine, guanosine, caffeine, theobromine, theophylline, cytosine, orotic acid, thymine and uracil.

Table 1. EFFECTS OF MAP, ADENINE AND KINETIN ON CELL GROWTH IN SOYBEAN TISSUES

Concentration of additive (mgm./l.)			Fresh weight per piece (mgm.)	Dry weight per piece (mgm.)
MAP	Adenine	Kinetin		
Exp. *A*, 34 days growth				
—	—	—	214	18
0·14	—	—	213	15
1·49	—	—	199	17
7·45	—	—	111	10
14·9	—	—	63	6
22·3	—	—	56	4
—	41	—	116	11
0·14	41	—	118	11
1·49	41	—	193	16
7·45	41	—	518	48
14·9	41	—	548	50
22·3	41	—	789	66
—	—	0·5	1,943	150
—	41	0·5	1,721	142
Exp. *B*, 27 days growth				
—	—	—	54	4
22·3	—	—	37	3
22·3	50	—	841	42
—	—	0·5	973	72
22·3	—	0·5	101	10

Not only did adenine reverse the MAP inhibition, but also the combination of the two caused continuous cell division as though kinetin had been added. The fresh and dry weights resulting from this combination are presented in Table 1; microscopic examination showed that the increased weights were correlated with cell division. Adenosine again was as effective as adenine.

I interpret the results to mean that added MAP has at least two types of action in soybean cells. The compound acts as an inhibitor of certain adenine-requiring processes but also acts as a rather inefficient kinetin-type compound. When a concentration of MAP high enough to cause cell division (in the manner of kinetin) is used, the inhibitory effect is so pronounced that no division takes place. If, however, the inhibition is reversed by adenine, the kinetin-like action becomes quite apparent in terms of cell division. The correlation between activity and the length of the chain in the 6-alkylaminopurines strongly suggests that the alkyl group brings about either a particular distribution or orientation of the compound within the cell and that this is necessary for activity. Thus far, all active kinetin analogues have a fat-soluble group in the 6 position[2]. If such is the only function of the alkyl group, then an assignment of a natural role to MAP is difficult in view of the very high concentrations of the compound required for promotion of cell division. If, however, the alkyl group should be found to have an additional function such as to block the amino group of adenine, we could visualize a critical methylation of adenine already properly located within the cell.

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Effect of Temperature on *in vitro* and *in vivo* Reactions of Diethyl Sulphate

RECENT work[1-3] has established that diethyl sulphate has characteristics which are of special interest to geneticists in many fields of research. These special characteristics include the induction of a high frequency of mutations accompanied by a high survival of the treated population and a low frequency of visible chromosome aberrations[1]. Similar properties have been found for ethyl methane sulphonate, a related alkylating compound[4-6].

The unique properties exhibited by diethyl sulphate can best be understood when they are related to the reaction kinetics of this agent. However, accurate and complete data on the hydrolysis of homogeneous aqueous diethyl sulphate solutions over the temperature range 0–40° C. have not been reported.

The chemical properties of diethyl sulphate have been reviewed by Suter[7]. Hydrolysis occurs in accordance with the equation:

$$\text{step 1: } C_2H_5OSO_2OC_2H_5 + H_2O \longrightarrow C_2H_5OH + C_2H_5OSO_2O^- + H^+$$

$$\text{step 2: } C_2H_5OSO_2O^- + H_2O \longrightarrow C_2H_5OH + SO_4^{=} + H^+$$

To obtain evidence on the quantity and nature of mutagenic substances in diethyl sulphate solutions, it was important to establish whether or not an appreciable amount of ethyl hydrogen sulphate hydrolysed in test periods at 0–40° C. Evans and Albertson[8] showed that the first ethyl group was removed much faster than the second. In our experiments, the rate of hydrolysis of ethyl sulphuric acid (step 2) in the temperature-range 0–40° C. was found to be insignificant. Diethyl sulphate solutions maintained at 30° C. for four weeks before adding barium chloride as a test for sulphate yielded no precipitate. Evidence for a quantitative yield of one mole of hydrogen ion per mole of diethyl sulphate, obtained by titration of hydrolysed solutions with sodium hydroxide to a phenolphthalein end-point, confirmed this result.

Additional experiments on the rate of formation of ethyl sulphuric acid established that the step 1 hydrolysis of diethyl sulphate in acid aqueous solutions over the pH range 1·0–7·0 follows first-order kinetics. Furthermore, the rate of formation of ethyl sulphuric acid was not affected by the accumulation or addition of the hydrolysis products. Similar results were obtained earlier by Kremann[9] in experiments with heterogeneous diethyl sulphate systems. However, he reported a temperature coefficient (Q_{10}) of approximately 4·0 over the temperature range 24–44° C. for the hydrolysis of diethyl sulphate, hence he concluded that the Q_{10} was not influenced by temperature.

In contrast, we found that the Q_{10}, expressed as a ratio of rate constants, was not constant but changed from 4·5 for the 0–10° C. temperature-range to 3·1 for the 30–40° C. temperature-range (Table 1). These values were established from investigations of homogeneous aqueous diethyl sulphate solutions conducted over a wider range of temperatures than used by Kremann. The activation energy values calculated from the rate constants obtained for the *in vitro* reaction were found to range from 23·2 kgm./cal./mol. for 0–10° C. to 21·4 kgm./cal./mol. for 30–40° C. Thus we have established that the *in vitro* hydrolysis Q_{10} is dependent on temperature. This fact must be considered when attempting to evaluate data from biological experiments conducted over a range of temperatures.

Table 1. INFLUENCE OF TEMPERATURE ON THE *in vitro* HYDROLYSIS OF DIETHYL SULPHATE AND ON THE *in vivo* GROWTH REDUCTION INDUCED IN BARLEY SEEDS BY NON-BUFFERED AQUEOUS DIETHYL SULPHATE SOLUTIONS

	In vitro system			*In vivo* system	
Temp. °C.	First order rate constant (min.)	Half-life (hr.)	Hydrolysis Q_{10}	Treatment time* (hr.)	Biological effect Q_{10}
0	$1·95 \pm 0·04 \times 10^{-4}$	59·2		39·0	
5	$4·18 \pm 0·01 \times 10^{-4}$	27·6	4·5		2·69
10	$8·80 \pm 0·08 \times 10^{-4}$	13·1	4·2	14·45	
15	$1·77 \pm 0·02 \times 10^{-3}$	6·53	3·9		2·69
20	$3·45 \pm 0·02 \times 10^{-3}$	3·34	3·6	5·37	
25	$6·43 \pm 0·01 \times 10^{-3}$	1·80	3·3		2·69
30	$1·15 \pm 0·03 \times 10^{-2}$	1·00	3·2	2·0	
35	$2·08 \pm 0·05 \times 10^{-2}$	0·555	3·1		
40	$3·57 \pm 0·04 \times 10^{-2}$	0·323			

* Time of exposure to 0·0076 M diethyl sulphate solutions (changed after 29 per cent hydrolysis) required to obtain the same seedling growth response.

Once this more complete information on the kinetics of the *in vitro* system was available, the use of a relatively constant concentration of diethyl sulphate in treatment solutions was possible. Experiments were then initiated to establish the relationship of the *in vitro* concentration of active mutagen available for reactions with cellular components to the biological response obtained from these reactions in the *in vivo* system. In these experiments, seeds of the hull-less barley variety, Himalaya *C. I.* 620, with 13 per cent moisture, were treated by immersing them in freshly prepared aqueous non-buffered 0·0076 M diethyl sulphate solutions. The solutions were changed at intervals corresponding to the time of 29 per cent hydrolysis in order to maintain the concentration of active agent. Non-buffered solutions were used in order to avoid the complication of extraneous factors in these initial experiments. It had previously been established that barley seeds did not show damage when exposed for even longer periods of time to greater concentrations of the hydrolysis products (pH as low as 1·3) than were used in these experiments. Thus the pH (approx. 2·7) of 0·0076 M diethyl sulphate solutions changed after 25 per cent hydrolysis was not damaging to seeds treated for the period described.

To determine the effect of temperature on the induction of damage to barley seeds, lots of 50 seeds were treated at 0°, 10°, 20°, and 30° C. for varying periods which were estimated first on the basis of the *in vitro* Q_{10}. The seeds were washed for 1 hr. in distilled water at 30° C. after their exposure to diethyl sulphate and then were grown on wet blotters in Petri dishes. After six days at 23° C. under continuous illumination, the height of seedlings was measured and compared with controls.

The treatment times required to retard the growth of seedlings to approximately 63 per cent of the controls was found to vary from 2 hr. at 30° C. to 39 ± 0·4 hr. at 0° C.

The results obtained, confirmed by subsequent experiments, established that the *in vivo* Q_{10} is nearly constant, with values between 2·67 and 2·70 (av. 2·69) for different treatments (Table 1). It follows that the exposure period required to produce a given level of injury to barley seeds by treatment with diethyl sulphate may be calculated according to the formula:

$$t_2 = t_1 \times 2·69 \left(\frac{T_1 - T_2}{10}\right)$$

where t_1 is time of treatment at temperature T_1 and t_2 is time of treatment at temperature T_2.

The nature of the rate-controlling factor mainly responsible for the Q_{10} of 2·69 is under active investigation.

A more extensive comparison of physical and biological data will be published later. Preliminary results of experiments on the mutation response of diethyl sulphate-treated barley seeds, which show the same injury response, indicate that both the frequency and spectrum of mutations recovered may be influenced by the temperature of the treatment solution. Similar work on the kinetics of *in vitro* and *in vivo* reactions of ethyl methane sulphonate is now in progress.

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Fluorophenylalanine as a Systemic Fungicide

In previous publications[1,2] some amino-acids, not naturally occurring, were shown to be chemotherapeutically active against certain fungal diseases of plants. Since no fungitoxic action of the compounds could be demonstrated *in vitro* or in the sap of treated plants, the increase in resistance of the plants might be explained as the result of changes in their metabolism caused by interference of the foreign substances.

We have found that both DL-*p*- and DL-*m*-fluorophenylalanine can protect cucumber seedlings against *Cladosporium cucumerinum* and *Colletotrichum lagenarium* in concentrations of 0·0050 and 0·0025 *M* applied to the roots before inoculation. The roots sometimes show some loss of turgescence, while higher concentrations also affect the green parts. DL-*o*-fluorophenylalanine appears to be more phytotoxic and to have a smaller protective effect.

Fluorophenylalanine, being an amino-acid not found in plants, might be expected to have an indirect effect like the amino-acids mentioned before, for example, D-serine. It is known, however, that fluorophenylalanine inhibits growth of some micro-organisms[3] and its effect on spore germination and mycelium growth was investigated *in vitro*. Using the paper disk bioassay with *Cladosporium cucumerinum* as a test fungus an inhibition zone of 30 mm. was found for a solution of 250 p.p.m. *p*-fluorophenylalanine or *m*-fluorophenylalanine, while *o*-fluorophenylalanine in the same concentration and under the same conditions produced only a zone of scanty growth. A similar difference in toxicity could be demonstrated for *Glomerella cingulata* and *Aspergillus niger*. The latter did not germinate on plates of a glucose-mineral salts agar containing more than 5 p.p.m. *p*- or *m*-fluorophenylalanine, but 50 p.p.m. *o*-fluorophenylalanine did not inhibit germination.

From these results it seems likely that the influence of the fluorophenylalanines on the development of fungal diseases is due to their fungitoxicity and not to some indirect effect. This was examined further by expressing sap from the hypocotyls of cucumber seedlings—the part of the plants which is most affected by *Cladosporium cucumerinum* and *Colletotrichum lagenarium*—and testing its fungitoxicity by means of the paper disk bioassay. Sap of plants treated via the roots with *p*- or *m*-fluorophenylalanine produced distinct inhibition zones about 45–60 mm. in diameter, but sap of plants treated with *o*-fluorophenylalanine had no effect or only produced a zone of decreased growth. This is in good agreement with the results of the experiments on the activity of the different compounds *in vitro* and in plants, and may indicate that the latter is due to the former.

According to Mitchell and Niemann[3], fluorophenylalanine is an antimetabolite of phenylalanine. This was confirmed in our experiments *in vitro*. The growth inhibition caused by all three fluorophenylalanines could be completely antagonized by adding L-phenylalanine in a concentration twice that of the fluorocompound.

However, treatment of plants with a solution containing fluorophenylalanine (*p*-, *m*- or *o*-) together with phenylalanine in the same or a higher concentration had a similar effect on the disease as a solution of the first substance alone, although addition of phenylalanine seemed to decrease the injury to the plants, especially to the roots. Sap of plants treated with both amino-acids was not fungitoxic in the paper disk bioassay, but when this sap was chromatographed according to Dekhuijzen's method[4] inhibition zones were found with the same R_F as fluorophenylalanine. Apparently fluorophenylalanine is present; but its fungitoxicity is antagonized by some compound of the sap, presumably phenylalanine. This seems to contradict the conclusion that the increase in the resistance of the plants is due to fungitoxic properties of the sap after treatment. The inconsistency can be explained if we assume that there is a difference in the distribution of the two substances among the various tissues of the intact plant. This means that at the spot where fluorophenylalanine exerts its influence on the fungus, the concentration of phenylalanine is too low to reverse this effect. In expressed sap, of course, the contents of all cells are pooled.

To test this hypothesis we looked for a more homogeneous tissue for treatment with fluorophenylalanine and phenylalanine and inoculation with fungus. Disks of potato tuber, var. Bintje, 8 mm. in diameter and 0·5 mm. thick, proved to be suitable for this purpose. The disks were floated in aerated solutions for 24 hr., rinsed with water, dried with filter paper, placed on moist filter paper in Petri dishes and inoculated with a suspension of zoospores of *Phytophthora infestans*. After 5–6 days at 20° C. all control disks were covered with mycelium, whereas only a few of the disks treated with fluorophenylalanine showed some growth. Here the best effect was obtained with *o*-fluorophenylalanine 0·0050 or 0·0025 *M*; *m*-fluorophenylalanine had less and *p*-fluorophenylalanine had scarcely any effect. The protective effect disappeared almost entirely when phenylalanine was added to the solutions.

The fact that both the fungitoxic and the protective effect of the fluorocompounds can be reversed by phenylalanine renders it probable that the first is responsible for the second. The possibility that a change in the metabolism of the host cells is also antagonized by phenylalanine cannot be ruled out, however. It should be mentioned that Samborski and Forsyth[5] found inhibition of rust development on wheat leaves floating on solutions of *p*-fluorophenylalanine, which effect was reversed by phenylalanine.

A difference in sensitivity of the various fungi is probably responsible for the fact that the *o*-isomer was most active in the potato experiments. Because of the difficulties involved in growing *Phytophthora infestans* on simple synthetic media data about its sensitivity are lacking, but mycelium growth of two other Phycomycetes, *Phytophthora cactorum* and *Pythium debaryanum*, was more inhibited by *o*- than by *m*- or *p*-fluorophenylalanine.

The results mentioned here indicate that fluorophenylalanine acts in a different way from the other amino-acids and their derivatives investigated before. In the latter case the effect seems to be indirect, while the fluorocompounds probably behave as real systemic fungicides.

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Preparations of Cell-Walls of Some Fungi

LITTLE is known of the qualitative and quantitative composition that may occur in the cell wall of fungi. Cochrane[1] has claimed that the most serious defect in our knowledge of cell-wall composition in the filamentous fungi is the fact that no 'pure' wall material is yet available. The major sources of error are the inclusion of extraneous substances in the preparation, and the loss of wall material during purification. Several workers have described results of the cell-wall composition based on X-ray diffraction patterns. Results from other methods (isolation of derivatives from a hydrolysate, microchemical tests, etc.) are usually in agreement with those of X-ray analysis, but are in most cases unsatisfactory.

This communication describes a method for the isolation of cell-walls of various moulds. Organisms were grown in Czapek medium shaking at 25° for 48 hr. avoiding sporulation. The resulting mycelium was then collected in a centrifuge and washed three times with water. After resuspension in water the mycelium was partially disintegrated by means of concentric ground-glass cones, washed five times and treated with ethyl ether for 1 hr. A serious difficulty occurred when attempting to disintegrate mycelium before treatment with ethyl ether. The resulting mycelium was washed with water and cell-walls were prepared by the method of Salton and Horne[2], by using the Mickle disintegrator. 10 ml. of the partially broken mycelium suspension were added to each cup of the machine and shaken with 10 ml. of grade 12 Ballotini glass beads for 4 hr. at room temperature. Breakage was followed by microscopic examination of lactophenol blue stained material.

After breakage, intact mycelium and debris were removed by centrifugation at 500*g* and the supernatant was transferred into a narrow glass tube (3 × 100 mm.) and the contents spun down at 1,000*g*. The sedimented material, containing unbroken cells, was discarded. The supernatant suspension was then centrifuged at 5,000*g* for 20 min. to sediment the cell-wall fraction. This material was digested first with crystalline trypsin and then with pepsin, as described by Villanueva[3]. The digest was centrifuged at 5,000*g* and washed once with 1 *M* sodium chloride solution, the precipitate being re-suspended in water and thoroughly washed. The purity of the preparation was checked by light and electron microscopic examination.

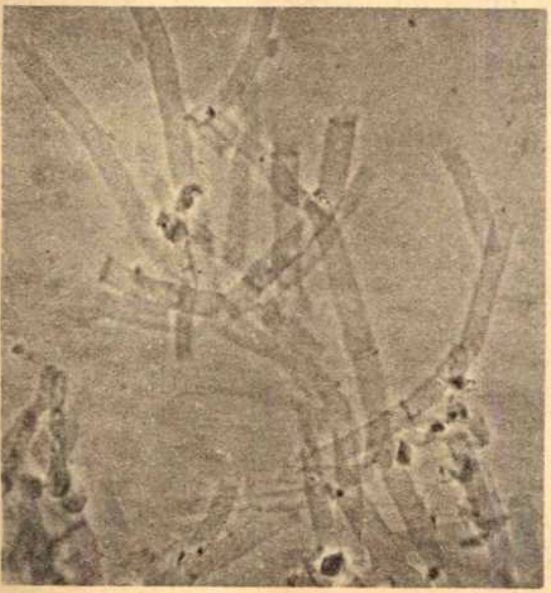

Fig. 1. Purified cell-walls of mycelium of *Aspergillus nidulans*

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Some Fossil Chelonian Fragments from Makapansgat

THE fossil chelonian material loaned to me by Prof. R. Dart, of the University of the Witwatersrand Medical School, for examination consists of 36 fragments from the early Middle Pleistocene grey breccia of Makapansgat Limeworks in the Transvaal. This fossil material was compared with complete and fragmented skeletal material of *Testudo pardalis babcocki*, *Kinyxs belliana belliana* and *Pelusios sinuatus*, all of which occur in the area to-day. The fossil fragments were all from large animals and none was referable to the genera *Chersina*, *Psammopbates*, *Homopus* or *Pelomedusa*, which were also available for comparison.

Twenty-four bone fragments were assigned to *Testudo pardalis*, representing a minimum of eight individuals, but only two fragments are definitely from the same specimen. Most of the material is derived from large animals (exceeding 12 in. in length and 20 lb. in weight) and the majority of the fragments are from the plastron, particularly the thickened gular area. The frequency of occurrence for the various bones is as follows :

Carapace	5th neural	1
	pleurals	4
	pygal	1
Plastron	epiplastra	9
	entroplastra	2
	hyoplastra	3
	hypoplastra	5
	xiphiplastra	3

One bone fragment consists of the gular section of the epiplastra of a moderate sized chelonian which may be *Kinyxs* sp. Unfortunately, there are no other fragments to establish the presence of this genus.

Three plastron fragments are assigned to *Pelusios* sp. They are from a large terrapin(s) comparable in size with *Pelusios sinuatus*.

Six small fragments could not be assigned to any form with confidence. No less than 22 of the 28 fragments identified were from the plastron.

The two terrestrial forms, *Testudo* and *Kinyxs*, are both widely used as food by man to-day, the former being prized because of its large size. The hinged terrapins (*Pelusios*) are aquatic animals, but are often caught on rod and line and are eaten by many Africans. There seems to be little doubt that the chelonian fragments dealt with here are from in-

Table 1. FOSSIL CHELONIAN FRAGMENTS FROM MAKAPANSGAT LIME-WORKS. LIST OF IDENTIFICATIONS.

No.	Species	Bones represented
1	*Testudo pardalis*	epiplastra (same specimen as No. 5)
2	*Testudo pardalis*	pygal
3	*Testudo pardalis*	RHS epiplastron ; part entroplastron and part hypoplastron
4	*Testudo pardalis*	hypoplastron
5	*Testudo pardalis*	LHS epiplastron ; entroplastron and hypoplastron (same specimen as No. 1)
6	*Testudo pardalis*	LHS 6th pleural
7	*Testudo pardalis*	epiplastra
8	*Testudo pardalis*	epiplastra
9	*Pelusios* sp.	RHS xiphiplastron
10	*Testudo pardalis*	LHS epiplastron
11	*Testudo pardalis*	LHS hypoplastron
12	*Testudo pardalis*	RHS hypoplastron
13	*Testudo pardalis*	pleural
14	*Testudo pardalis*	epiplastra
15	*Testudo pardalis*	(?) hypoplastron
16	*Testudo pardalis*	pleural
17	*Testudo pardalis*	epiplastron
18	(?) *Kinyxs* sp.	gular portion of epiplastra
19	*Testudo pardalis*	5th neural
20	*Testudo pardalis*	xiphiplastron
21	*Testudo* (?) *pardalis*	xiphiplastron
22	*Testudo* (?) *pardalis*	epiplastron
23	*Testudo pardalis*	pleural
24	*Testudo pardalis*	LHS part hypoplastron and xiphiplastron
25	*Testudo pardalis*	LHS xiphiplastron
26	*Pelusios* sp.	plastron
27	*Pelusios* sp.	plastron
28	*Testudo pardalis*	hypoplastron
29–34	Unidentified chelonian fragments.	
35–36	Unidentified non-chelonian fragments.	

dividuals eaten by *Australopithecus*. There are no marks of carnivore teeth on the fragments, and the fracturing could have been caused by smashing the reptiles against rocks. Stone age man would have simply chopped through the bridge between plastron and carapace. The only carnivore which habitually preys on large tortoises is the hyæna, which devours them in the open, being unable to carry a large *Testudo pardalis* in its jaws. I have examined many live tortoises bearing deep marks of carnivore teeth, indicating that the shell is not easily broken open.

I thank Prof. Raymond A. Dart for the loan of this material from the Bernard Price Institute and D. K. Blake for assistance in preparing the necessary comparative material and comparing the fossil bone fragments with modern bones.

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MICROBIOLOGY

Morphology of Bacteria treated with Colicin

COLICINS, like bacteriophages, are strain-specific as to their effect. Fredericq[1] proved that specific receptors in the cell wall are necessary for their fixation on the cell. It is interesting that, in my experience, the specificity of colicins is considerably lower than that of *coli*-phages.

Immediately after the fixation of the colicin *ML* on sensitive bacteria, the increase of respiration intensity of the culture, synthesis of both nucleic acids and, in accordance with these, also the growth of the culture are inhibited. After 20 min. the respiration begins to slow up and at the same time the optical density of the culture slightly decreases[2]. I noticed that the cells exposed to colicin quickly lose their reproduction ability and even when washed with broth and re-inoculated on highly nutrient agar they do not form colonies.

In my morphological studies I observed sensitive cells of the strain B_1 on the surface of agar blocks saturated with colicin 18 (ref. 3). The preparations framed with paraffin were observed and photographed in Zeiss phase contrast microscope, using the 1,350-fold direct magnification. From the cells, exposed to the colicin on agar plates for 6 and 48 hr., electron-microscopical preparations were made as well. These cells were, after being washed, gathered on 'Formvar' membranes, shadowed with wolfram-oxide and ob-

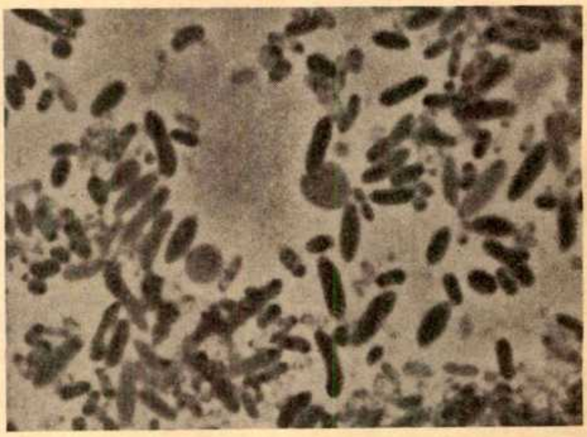
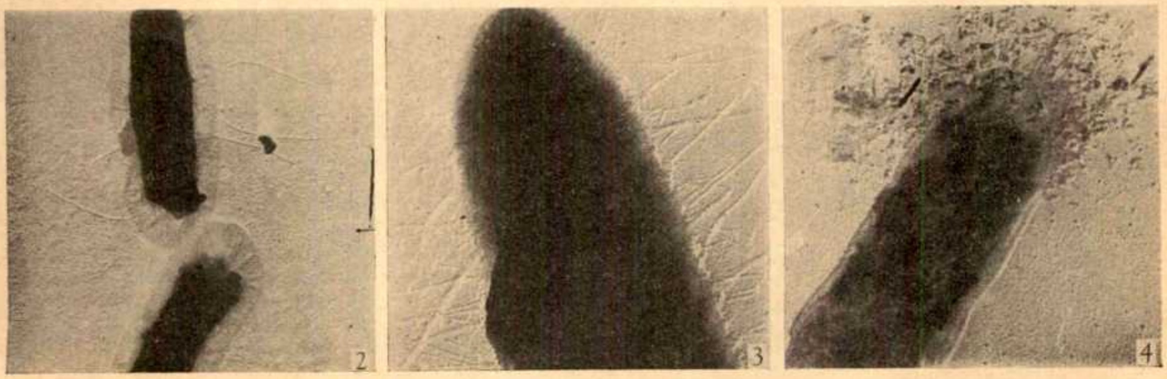


Fig. 1. Sensitive cells of the strain B_1, 26 hr. after contact with colicin. (Phase contrast. × 2,390)



Figs. 2, 3, 4. Cells of the strain B_1. Electron microscope. × *c*. 10,660. 2, Normal cells (control); 3, swollen cell 6 hr. after contact with colicin; 4, lysing cell 6 hr. after contact with colicin served and photographed in a 'Tesla *BS*' 242 electron microscope using the 10,000-fold magnification.

The results in the phase contrast microscope show clearly that the growth and reproduction of sensitive cells stop immediately after the contact with colicin; even the cells which were treated with colicin just in the state of division do not continue to divide. Otherwise no immediate changes were noticed. After about 20 min., however, their volume begins to increase noticeably, they do become more or less round and at the same time their refraction sinks; they swell (Fig. 1). On electron micrographs of these cells it is scarcely possible to distinguish the characteristic edge of the cell wall, typical for such micrographs of normal bacteria (Figs. 2 and 3). The cells continue to swell slowly and the proportion of swelling cells increases until the cell walls burst and protoplasm flows out of them (Fig. 4). At last empty cell walls remain in the preparations.

Controls, that is, non-sensitive cells of *E. coli*, show under the same conditions no morphological changes and multiply quite normally.

With respect to the immediate inhibition of growth and division of the cells by colicin it is possible to assume that colicin kills them by disturbing some elementary physiological reaction; the swelling of the rods is one such result.

I performed similar experiments with spheroplasts of sensitive and resistant cells, using penicillin. Colicin acts on spheroplasts of sensitive cells similarly as on the cells themselves. The order of morphological changes is similar to that of the cells; it just proceeds more quickly. For 8 hr. the majority of swollen spheroplasts suddenly burst and disappear. The higher rapidity of swelling may be caused by the fact that the spheroplast wall is less firm than the cell wall because it lacks the rigid layer[4].

The spheroplasts of resistant cells are not affected by colicin at all: they multiply and regenerate into rods.

These results indicate that the weakened wall of penicillin spheroplasts still contains specific receptors for colicin 18.

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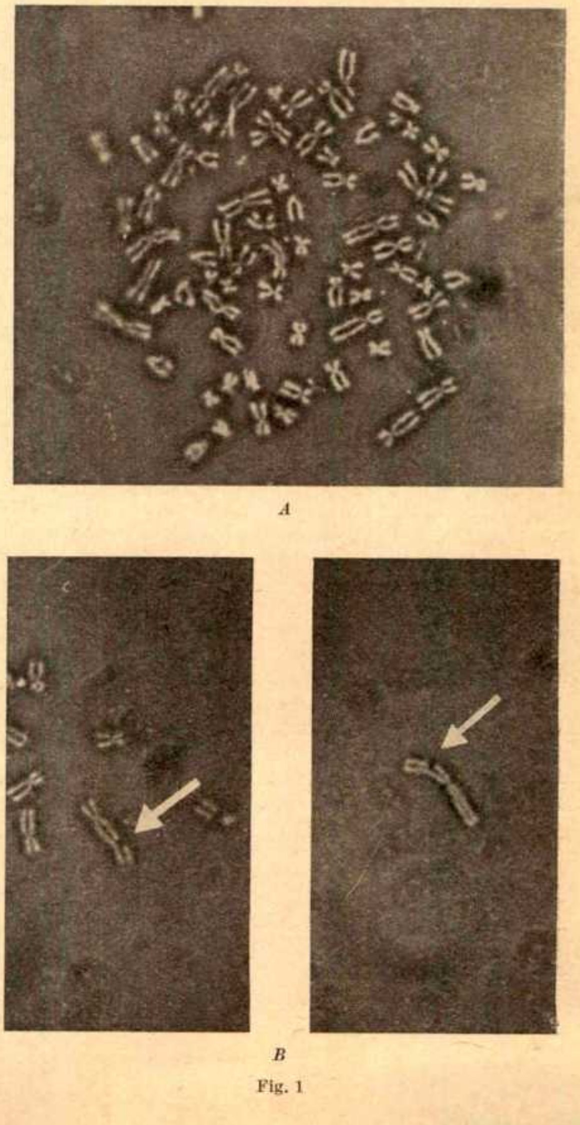
CYTOLOGY

Ozone-induced Chromosome Breakage in Human Cell Cultures

OZONE-INDUCED effects in biological systems may be particularly significant in radiation biology. The decomposition of ozone in water produces the same active radicals (OH and HO_2) which are generally considered the biologically active products of irradiation of protoplasm. Phillips[1] has reported on the effects of active radicals chemically produced by the catalytic decomposition of hydrogen peroxide with ferric ions; this system, however, has a low radical yield, as was evidenced by the extremely low frequency of chromosome aberrations produced over a wide range of reactant concentrations.

The ozone system produces a much higher concentration of these active radicals and in the presence of positive pressures will establish an equilibrium reaction with the hydroxyl ions in solution continuously to produce active radicals so long as the ozone is present[2]. A previous report[3] indicated that exposure of *Vicia faba* to ozone resulted in a high frequency of chromosome aberrations in root meristems. The investigations reported here are an extension of this work to demonstrate the activity of ozone against human cells in culture.

The *KB* human cell line[4] used was grown in Eagle's media[5] and incubated at 35° C. Before exposure to ozone, cells were washed once in a protein-free saline solution to remove extraneous reducing materials. Ozone is very reactive, and small amounts of reducing materials provide considerable protection. After rinsing with saline the bottles were rotated so that the cells were at the top of the flask and any saline would drain to the bottom. The ozone–oxygen mixture was allowed to flow into the bottle continuously in a closed system at a rate of about 100 ml./min. Ozone was produced with a laboratory ozonator (Wellsbach Co., model *T* 22) from cylinder oxygen and the concen-



A

B

Fig. 1

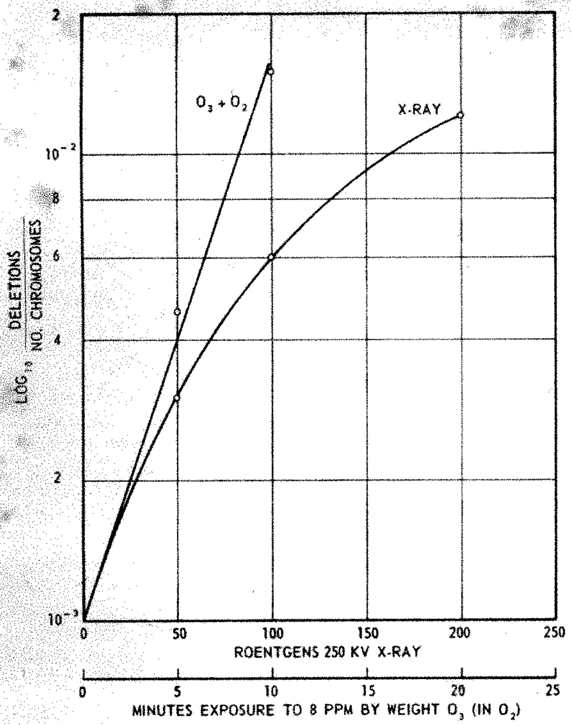


Fig. 2

tration calculated by scrubbing replicate samples of known volume through 2 per cent potassium iodide (*p*H 9), acidifying the solution and then titrating with 0·1 *N* sodium thiosulphate[6]. After exposure to ozone the culture bottles were rinsed with saline and then replaced with a medium containing 2 mgm./l. colchicine. Cultures were incubated for 24 hr., hypotonically treated for 20 min. (20 per cent strength medium), agitated to dislodge the metaphase cells[7] and then fixed by the addition of propionic acid to a final concentration of 25 per cent. With this technique all the metaphase chromosomes analysed were dosed with ozone since their last metaphase—the duplication time of these cultures being approximately 33 hr. The fixed cell suspension was concentrated by centrifugation, air-dried on slides, stained with propionic orcein and made permanent with the dry-ice technique. A number of different types of chromosome damage was investigated and chromatid breaks were selected as the criteria of choice. This type of aberration is a random event requiring a single hit and may be assumed to be independent of other chromosomes in the cell for its expression. The variable chromosome number characteristic of adapted cell lines makes the analysis of chromosome type of aberrations unlikely; but with chromatid breaks the culture may be treated as a population of chromosomes. The type of effect reported is shown in Fig. 1. *A* shows a typical metaphase figure and *B* demonstrates chromatid deletions in two different chromosomes.

Table 1

Experimental group	No. of chromosomes	Deletions	$\frac{\text{Deletions}}{\text{No. chromosomes}}$
50 r.	3322	10	0·003 ± 0·001*
100 r.	1854	11	0·006 ± 0·002
200 r.	1780	21	0·012 ± 0·003
8 p.p.m.† O_3 in O_2, 5 min.	4158	20	0·005 ± 0·001
8 p.p.m. O_3 in O_2, 10 min.	1283	23	0·018 ± 0·004
O_2, 10 min. (control)	3410	0	0·000

* S.E. $= \sqrt{\frac{A}{N}}$, where $A = \frac{\text{deletions}}{\text{chromosome}}$ and N = No. of chromosomes.

† Parts per million by weight.

Table 1 and Fig. 2 present the results of exposing cultures of *KB* cells to 8 parts per million by weight of ozone in oxygen for 5 and 10 min. Higher concentrations of ozone or longer exposure times with this concentration resulted in the cells dislodging from the glass surface, rendering the preparation unfit for analysis. For comparison, cultures were exposed to 50, 100, and 200 r. of 250-kV. X-rays and treated in the same manner as the ozone-exposed cultures.

Chromatid deletions were produced as an exponential function with exposure to ozone. This would be in agreement with the concept that chemically produced active radicals would be dependent for expression on penetration to the chromosomes. This would therefore be a function of concentration and would obey the law of mass action. Such an exponential response has also been interpreted as evidence of a direct action type of effect[8]. The production of chromatid breaks with X-ray follows a linear relationship or 1-hit kinetics as has been demonstrated in many other systems and is usually interpreted as a function of the random distribution of active radicals produced by the radiolysis of water throughout the protoplasm of the cell. This investigation has demonstrated that exposure to ozone is capable of producing chromatid breakages in human cell cultures which are apparently identical to those produced by X-rays. The difference in time-concentration response between ozone exposure and X-rays may be explained in terms of penetration.

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Isolation of Synaptic Vesicles from Nerve Endings of the Rat Brain

SINCE the discovery of 'synaptic vesicles' as a specific component of synapses[1,2] it was suggested that they could be associated with the storage and possibly with the synthesis of transmitter substances. This concept was supported by degeneration experiments[3] and specially by the stimulation of some cholinergic terminals[4], which suggested that within the ending a balance exists between the formation and release of synaptic vesicles. However, the isolation of such a sub-cellular component remained as the most direct approach to the elucidation of the physiological significance and chemical nature of these vesicles.

Recently[5], we demonstrated that the so-called mitochondrial fraction of the brain contained, besides free mitochondria and myelin, a considerable number of isolated nerve endings, and it was postulated

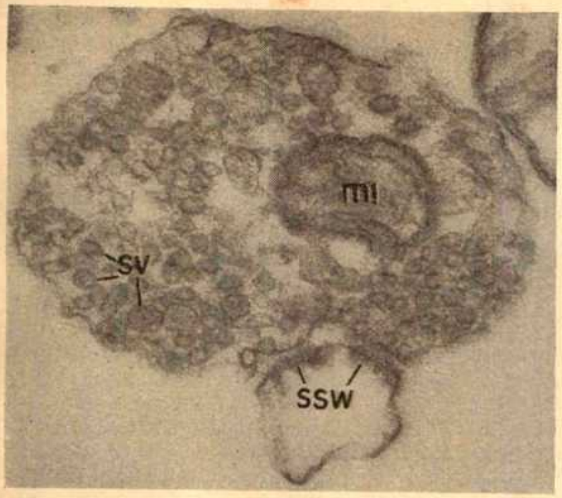

Fig. 1. Nerve ending isolated from the rat brain in fraction *M*. *Mi*, Mitochondria; *SV*, synaptic vesicles; *SSW*, subsynaptic web. Pellet fixed in osmium tetroxide, embedded in methacrylate and sectioned. (× *c*. 83,000)

that this fraction could provide a good starting point for the isolation of 'pure' synaptic vesicles. Independently, Gray and Whittaker[6] published similar observations of nerve endings in the primary mitochondrial fraction of the brain and were able to separate them from myelin and free mitochondria.

More recently, a technique was developed in our laboratory by which the mitochondrial fraction (M) could be fractionated further into five fractions which in order of increased density were: A, myelin; B, fragments of endings and membranes; C, cholinergic nerve endings; D, non-cholinergic nerve endings; and E, free mitochondria[7].

The two sub-fractions C and D of nerve endings provided the starting point for the isolation of synaptic vesicles; but these endings were found to shrink in the hypertonic sucrose, and it was observed that treatment with isomolar sucrose, when prolonged, interfered with the liberation of the synaptic vesicles. On the contrary, the freshly prepared endings of the primary mitochondrial fraction M (ref. 7) showed an excellent preservation and different osmotic behaviour. As shown in Fig. 1, such an isolated ending has a limiting membrane within which there are one or more mitochondria and numerous synaptic vesicles. In addition to the pre-synaptic membrane, a portion of the post-synaptic membrane and the sub-synaptic web are attached[8]. The complex formed by the two synaptic membranes is held together by a system of fine intersynaptic filaments[8].

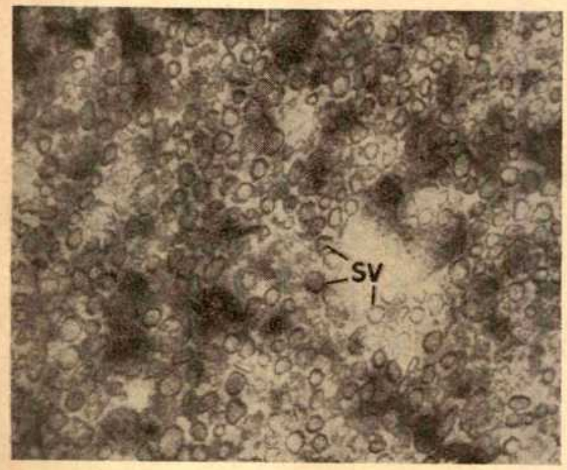

Fig. 2. Synaptic vesicles present in subfraction M_2 after osmotic disruption of nerve endings of fraction M. Pellet fixed in osmium tetroxide, re-suspended in phosphotungstic acid at *p*H 6·8 (negative staining, ref. 9) and observed under the electron microscope. Photographic inversion of the electron micrograph to make easier comparison with Fig. 1. (× *c*. 97,400)

If such isolated nerve-endings are submitted to an osmotic shock in distilled water, the outer membrane of the ending is ruptured, and the synaptic vesicles are liberated. By centrifugation of the osmotically shocked mitochondrial fraction (M), three sub-fractions can be obtained (Table 1) of which M_2 is composed mainly of synaptic vesicles (Fig. 2) and some curved membranes. Fraction M_1 contains myelin fragments, swollen mitochondria and disrupted nerve-endings in which the components of the membrane complex, but not the synaptic vesicles, can be recognized. Table 1 shows the relative distribution of protein and succinodehydrogenase in the three sub-fractions of M. The fact that practically all the succinodehydrogenase is in M_1 demonstrates that the osmotic shock has not so disrupted the mitochondria as to liberate this enzyme.

Table 1. PERCENTAGE DISTRIBUTION OF SUCCINODEHYDROGENASE AND PROTEIN IN THE SUBFRACTIONS OF *M* AFTER OSMOTIC SHOCK IN DISTILLED WATER

M_1 sediment centrifuged at 11,500g for 20 min., M_2 sediment centrifuged at 100,000g for 30 min., M_3 supernatant of M_2

	M_1	M_2	M_3
Succinodehydrogenase	97·2	2·8	—
Protein	71·2	12·4	16·4

Morphologically, the vesicles are similar to those found within the isolated nerve-endings and in sections of the brain cortex. They have a mean diameter of about 500 Å. and comprise a definite membrane surrounding a cavity. This is demonstrated by negative staining with phosphotungstic acid which penetrates into the osmotically shocked vesicles (Fig. 2).

At high magnifications small openings or pores through which the phosphotungstic acid may have entered the vesicles can be observed. Such discontinuities could be due to the drastic osmotic treatment. Work is being carried out to liberate the vesicles by milder treatment. In such preparations investigations are under way to determine the distribution of acetylcholine, and other transmitters and the enzymes involved in their metabolism.

This work was supported by grant *B*-1549 of the U.S. National Institutes of Health and the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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Adrenal Cytology of South Indian Anurans

During the course of investigations on structural variation among the endocrine glands of anurans the pituitary and the adrenal have shown some interesting features with regard to their cell types. The cytology of the pituitary gland, with special reference to the green-frog *Rana hexadactyla*, has been reported previously[1].

In the local species of frogs and toads the adrenal shows the usual interspersed nature of the cortical and medullary components, without any zonation in the cortex. In the cortical cords some of the cells appear blue with Mallory's triple stain and the same cells appear deep magenta in periodic acid – Schiff reaction. These cells are scattered and are occasionally seen in groups of two to three cells. The skipper-frog (*Rana cyanophlyctis*) adrenal shows few periodic acid – Schiff-positive cells while that of the bull frog (*Rana tigrina*) discloses plenty. In the green frog Mallory preparations fail to reveal any blue cells but show light brown ones which are periodic acid – Schiff-positive; the cell outline is either round or oval, but always with the nucleus situated eccentrically. These features correspond with the Stilling cells or eosinophilic cells of the other frogs[2]. These cells contain a mucoprotein which is diastase resistant. They also exhibit a faint metachromasia with toluidine blue or methyl violet. Burgos[3] considers them to be eosinophilic cells which increase in number after hypophysectomy and decrease for pituitary injections. It is found that the periodic acid – Schiff-positive cells which stain blue with Mallory's triple stain are not present in the terrestrial forms *Bufo melanostictus*, *Uperodon systoma* and *Kaloula* sp. In one or two preparations some of the cortical cells of the toads show few minute spherical granules which are periodic acid – Schiff-positive but no blue or magenta red cell occurs. The Stilling cells do not show definite seasonal variation. As yet no one has ascribed the functional role of the Stilling cells. Since the Stilling cells are found only in the aquatic forms and absent in the terrestrial forms, perhaps they may have some importance in the habitat. Hence it has been planned to study the adrenal gland changes, tadpoles and metamorphosis of aquatic and terrestrial forms, which may give some clue to the functional role of Stilling cells.

The other type of cells present in the cortex are mostly cuboidal or elongated and contain lipid droplets of varying size. These cells exhibit cyclical variation in different seasons of the year. It is noticed that there is a regular sequence of storage and elaboration of the lipoid material in all the frogs and toads examined in the present study. These changes appear to be correlated with the changes in the pituitary and the gonad. Active secretion of the lipoidal cells appears to be associated with cytoplasmic vacuolation which reaches a maximum during the pre-spawning months. It has been demonstrated that corticosterone and hydrocortisone are present in the blood[4] and aldosterone in the adrenals[5] of anurans. The former two hormones can bring about ovulation in lower vertebrates[6]. The supporting role of cortisone in anuran ovulation has already been reported[7,8]. Hence, it seems probable that the lipoid droplets of the adrenal cortex of the anurans store potential precursor materials which may be converted into substances of metabolic importance, in response to specific stimulus possibly from the pituitary gland[9]. The adrenal gland of anurans does not show the presence of ascorbic acid, the role of which is debatable.

The ratio between the cortical and medullary components of the anuran adrenal varies in different species. The two components can be distinguished by the so-called chromaffin reaction or by the light-green method of Ira-Ray and Sinha[10]. The cortical tissue constitutes the greater part of the adrenal in all the forms except the green frog in which the medulla appears to predominate.

Two important inferences are made from the seasonal studies of the anuran adrenal glands. First, the Stilling cells are present in all the aquatic forms and are absent in the terrestrial forms; the Stilling cells do not show any seasonal variation. Secondly, the lipoidal cells of the cortex contribute some factor which aids in the growth and maturation of gametes. A detailed report of the changes in the pituitary, gonad and thyroid in relation to the adrenal changes during different seasons of the year will be published elsewhere.

I thank the Council of Scientific and Industrial Research, New Delhi, for a fellowship.

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GENETICS

Slow and Fast Transferrin Variants in the Same Pedigree

By means of starch-gel electrophoresis Smithies[1,2] demonstrated the occurrence of distinct fast and slow variants of transferrin, the iron-binding β-globulin fraction of human serum. Several different electrophoretically distinct transferrin components have been identified. They are termed in order of electrophoretic mobility towards the anode B_0, B_{0-1}, B_1, B_2, C, D_0, D_4, D_1, D_2 and D_3. The discovery of the type D_4 by Harris, Penington, Robson and Scriver[3] after all the other D types is the reason for the use of the designation D_4 for a transferrin migrating between D_0 and D_1. Parker and Bearn[4] designated as B_{0-1} the new variant which they recently found with a mobility intermediate between B_0 and B_1.

Family studies[2,5–7] have indicated that the different transferrins were genetically controlled by a number of autosomal genes. Individuals with two transferrin components, say, B_2C, are heterozygous for the genes controlling the B_2 and C variants, while individuals with one transferrin component only are homozygous for the corresponding gene. The phenotype B_2C occurs with a frequency of around 1 per cent in Europeans. In African populations and Australian aborigines the type CD_1 occurs

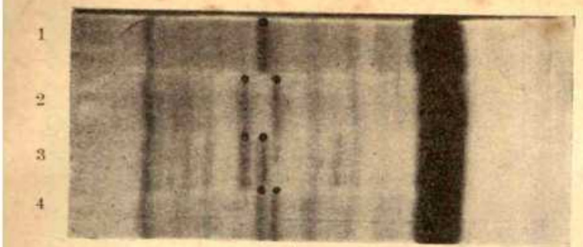

Fig. 1. Photographs of starch gels stained with amido-black 10 *B*. 1, Father $Tf\ C$; 2, mother $Tf\ B_2D_1$; 3, daughter $Tf\ CD_1$; and 4, daughter $Tf\ B_2C$. Transferrin components are indicated by black dots

in a rather high frequency[8]. The remaining phenotypes appear to be rather rare.

So far 10 transferrin components and 12 different phenotypes have been found[4]. Homozygous individuals for aberrant transferrin have been found for the B_1 and D_1 variants. The first frequency studies seemed to indicate that fast variants occurred only in European populations and slow variants only in Africans and Australian Aborigines. Beckman and Holmgren[9] have observed about 2 per cent of the type CD_1 in Swedish Lapps, however, and in a survey of the Swedish population Beckman, Holmgren and Mårtensson[10] found both slow and fast variants. Out of these 25 heterozygous types 18 were B_2C, 4 B_1C and 3 CD_1.

The families of some transferrin heterozygotes found in a sample from Uppsala were examined. Starch-gel electrophoresis of the sera was carried out using a discontinuous buffer system (cf. Beckman and Holmgren[9]). One of these families showed a rather remarkable result.

The proband had the transferrin type CD_1. It was found that a sister of the proband was B_2D (Fig. 1). The father had the normal phenotype C and the mother showed a very rare phenotype, namely, B_2D_1. This female should be the offspring of a mating $B_2C \times CD_1$.

To my knowledge this is the first case where slow and fast transferrin variants have been found segregating in the same pedigree, an observation which greatly strengthens the presumption that the transferrin variants are controlled by alleles at a single locus.

This work was supported by a grant from the Swedish Natural Science Research Council.

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X-ray Sensitivity of Spermatogonia and Spermatozoa in *Drosophila melanogaster*

In a study of X-ray induced crossing-over in young spermatogonia of *Drosophila melanogaster*, Sävhagen (unpublished) has irradiated 17-hr.-old larvæ. These were heterozygous for a series of recessive marker genes in the third chromosome. After eclosion the males were tested during six successive mating periods of three days each. The frequency of males with cross-overs among their progeny decreased for every period. Synchronously the frequency of cross-over per male with cross-overs increased. In all these mating periods the frequency of cross-overs of the total offspring was the same.

The present investigation has been performed in order to clarify if, after irradiation of young spermatogonia, other mutations behave in a manner similar to that shown by the results of Sävhagen. By working with recessive lethal mutations in an autosome the advantage is gained that it can be confirmed with certainty when clusters appear.

The stocks used were one of wild-type (Canton *S*) and one with $al^2\ Cy\ lt^3\ L^4\ sp^2/Pm$. In order to make the second chromosomes isogenic and for lethal testing the CyL/Pm technique was applied. From the wild-type stock one chromosome, free from lethals, was isolated which, when homozygous, provided the flies with a good viability. This chromosome was used in all experiments and was made isogenic at regular intervals in order to avoid spontaneous lethal mutations as much as possible. In every experiment a dose of 900 r. was used. Spermatogonia were irradiated in larvæ, and after eclosion the males were mated in two periods for lethal tests. As material for comparison of lethal frequencies of spermatogonia, spermatozoa in adult males have been irradiated and the frequency of lethals has been stated. In Table 1 it can be seen that the rate of chromosomes with lethals is about three times higher after irradiation of spermatozoa than after the same treatment of spermatogonia. It is true that these stages have been irradiated in different phases of development of the animal. However, Khishin[1] has shown that the sensitivity of the germ cells seems to be independent of their biological environment, and, for that reason, the foregoing comparison should be justified.

Table 1

Stages of germ cells and individual at irradiation	Spermatozoa adults (3–4 day old)	Spermatogonia larvæ (17 hr. old)	
Mating period and type of mating	0–4 hr. after irradiation mass mated	0–3 days after eclosion individually mated	15–18 days after eclosion individually mated
Total No. of tested chromosomes	5·589	7·076	6,021
Total No. of chromosomes with lethals	291	130	94
Chromosomes with lethals/tested chromosome ($\times 10^{-4}$)	521	184	156
Independently induced mutations/tested chromosome ($\times 10^{-4}$)	—	168	111
Chromosomes with lethals/independently induced mutation	—	1·09	1·40
No. of tested chromosomes/male	—	11·4	11·6

Muller[2] has obtained quite a diverting result after irradiation of adult males (3,000 r.). The frequency of lethals in the second chromosome is, according to his work, almost fourteen times greater in spermatozoa than in spermatogonia. Muller's material for irradiated spermatogonia is rather small, and he has not shown if the number of the reported lethals was independently induced or if all chromosomes with lethals are included, thus counting clusters as separate lethals.

In Table 1 it may be seen that a higher rate of independently induced lethals and a lower rate of clusters was found in the mating period 0–3 days than in the mating period 15–18 days after eclosion of irradiated larvæ, while there is no significant difference in the rates of chromosomes with lethals. The results agree then very well with those of Sävhagen on induced crossing-over.

As an explanation of her results, Sävhagen has proposed that primary spermatogonia at division are not always the source of one primary and one secondary spermatogonial cell, as suggested by Tihen[3], but may divide into two primary or into two secondary spermatogonia. Those which have divided into two primary spermatogonia may later divide in a similar manner into two primary or two secondary cells or they may follow the stem cell type of division into one primary and one secondary cell. These later divisions should occur at random and then it would be very possible that some of the mutant spermatogonia would disappear from the testes and some which remained could be the source of large clusters in later mating periods.

Another explanation of the results of Sävhagen and of this work could be that there is some kind of selection mechanism in the spermatogonial tissue. If so this should be reflected in the selective values of the heterozygotes of lethals which have been found following different occasions of irradiation and mating. In order to try to throw light on this problem persistency tests of different lethals which have been found in this experiment are now being carried out.

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BUILDING RESEARCH

Bond of Galvanized Steel Reinforcement in Concrete

GALVANIZING is at present being advocated as a means of combating corrosion of reinforcing steel in concrete, and galvanized high-tensile wire is already being used extensively in stressed concrete structures. A survey of existing literature on this subject yielded little useful information, except that various investigators have reported widely divergent results, often in direct conflict.

Thus, Robinson[1] showed that the average bond stress of ½-in. galvanized steel bars in concrete, at failure, was 54 per cent of that of rusty bars. For ⅜ in. rods the figure is reduced to 41 per cent.

D. A. Lewis and J. A. P. Laurie (private communication, 1958), on the other hand, reported the bond-strength of ⅜ in. galvanized reinforcement and that of black or pickled steel, after 9 months' exposure, to be very similar.

Recent experience, during the preparation of prestressed concrete beams, has been equally confusing. Using a galvanized high tensile wire *A* (coating weight 1·00 oz./sq. ft.) as prestressing reinforcement, very little bonding was obtained, the concrete immediately surrounding the wire being spongy. Extensive cracking occurred when external stress was released. Wire *B*, however, with only 0·5 oz. of galvanizing per sq. ft., did not show this tendency. Fig. 1 illustrates this.

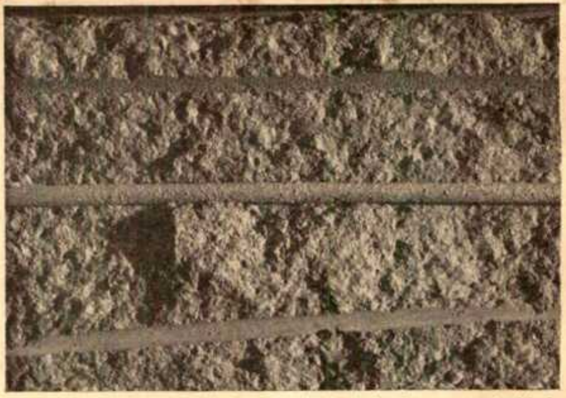

Fig. 1. Concrete in which galvanized steel wires are embedded. Top, Spongy concrete surrounding wire *A*; middle, wire *A* in position; bottom, normal concrete surrounding wire *B*

Initial laboratory experiments indicated that, when pure zinc is immersed in Portland cement extract of *p*H 12·8, no hydrogen is liberated. As soon as a piece of clean steel is brought in contact with the pure zinc under these conditions, however, hydrogen is liberated cathodically on the steel.

From this it was deduced that iron, of relatively low hydrogen overpotential, exposed to wet fresh concrete, either as zinc–iron alloys or at imperfections in the zinc coating of a galvanized rod, would lead to hydrogen evolution which would impair the bond. Tests were carried out to substantiate this theory.

Microscopic inspection of cross-sections of the wires *A* and *B* showed clearly that, while the outer layer of the galvanized coating on wire *B*, although thin, consists essentially of pure zinc, the coating on wire *A* is comprised almost entirely of zinc–iron alloy.

Micro-hardness test results, together with micro-section photographs, showed that the coating on the galvanized bars with a specified coating of 2·0 oz./sq. ft. used by Lewis and Laurie in their experiments had a substantial outer layer consisting mainly of pure zinc. When embedded in concrete no loss of bond due to hydrogen liberation resulted, confirming their original results. When similar bars, which had previously been anneeled at 400° C. for 2 hr. to allow alloy-formation to extend to the coating surface, were placed in concrete, hydrogen was evolved with resultant disruption of the bond.

When the galvanizing on unannealed rods was removed by filing to expose steel at several points along their length and the rods embedded in concrete, hydrogen was evolved on the exposed steel surfaces. The surrounding concrete became porous with little bond to the exposed steel; the bond to intact galvanized surfaces remained unaffected.

Present indications are that, although the amount of gas liberated is sufficient to affect the bond, the galvanic cell between the galvanized surfaces and iron is rapidly polarized and hydrogen evolution ceases within 1 hr.

Thus, the adverse consequences of using galvanized reinforcement where alloys appear at the coating surface, having ungalvanized cut-ends, a damaged coating, or where it is used with ungalvanized steel binding wire, are clearly evident. Specifying a minimum thickness of coating does not necessarily obviate the problem since, as has been shown, even a coating of 1·00 oz./sq. ft. may have zinc-iron alloys extending to the surface.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 28

University College, Department of Biochemistry (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. R. K. Morton (University of Adelaide). "The Flavohæmoprotein Cytochrome b_2—a New Type of Dehydrogenase".*

University of London (at the Wright–Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Prof. E. S. Lennox (New York): "Antibody Synthesis Analysed at the Cellular Level".* (Last of a series of four Almroth Wright Lectures.)

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. F. Caldin: "The Logic of Discovery in Chemistry".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Owen Lattimore: "The Geography of Ghengis Khan" (The Dickson Asia Lecture).

Monday, May 28—Saturday, June 2

Fourth International Instruments, Electronics and Automation Exhibition (at Olympia, London).*

Tuesday, May 29

University of London (in the Physics Lecture Theatre No. 2, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Prof. Paul Malliavin (University of Caen): "On Some Problems of Harmonic Analysis".*

Tuesday, May 29—Wednesday, May 30

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. on Tuesday and 10 a.m. on Wednesday—Informal Discussion on "Machine Tools and Automatic Control—Technical and Economic Appraisal".

Wednesday, May 30

British Nuclear Energy Society (at 1–7 Great George Street, London, S.W.1), at 5.30 p.m.—Sir Roger Makins: "Nuclear Power—a Year of Promise".

Thursday, May 31

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. Michie and Mr. M. F. A. Woodruff: "Induction of Specific Immunological Tolerance of Homografts in Adult Mice by Sublethal Irradiation and Injection of Donor Type Spleen Cells in High Dosage"; Mr. J. F. A. P. Miller: "Effect of Neonatal Thymectomy on the Immunological Responsiveness of the Mouse".

Society of Chemical Industry, Plastics and Polymer Group (at the Film Theatre, Shell Mex House, Strand, London, W.C.2), at 6 p.m.—Annual General Meeting.

Polarographic Society (in the Optics Lecture Theatre of the New Physics Building, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Papers on "Polarographic Analysis in Organic Systems".

Thursday, May 31—Thursday, June 7

Institution of Electrical Engineers, Electronics and Communications Section (in association with the Television Society, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—International Television Conference.

Friday, June 1

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. G. Bennett: "The Biology of the Astronaut".

Monday, June 4

University of London (at the London Hospital Medical College, Turner Street, London, E.1), at 5.15 p.m.—Prof. F. Halberg (University of Minnesota Medical School). "External and Internal Timing in Organisms".*

Monday, June 4—Thursday, June 7

British Electrical Power Convention (at The Dome, Brighton)—Fourteenth Annual Meeting. Theme—"The British Electrical Industry in the World Scene".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Sociology—The Registrar, The University, Leicester (June 2.)

Assistant Lecturer, an Experimental Officer (preferably graduate in physics or electrical engineering, or qualified in electronics) to take charge of the electronic equipment, and a Research Demonstrator (honours graduate in physics) in the Department of Physics—The Registrar, The University, Leicester (June 2).

Assistant Lecturer and a Tutorial Assistant in Sociology—The Registrar, The University, Leicester (June 2).

Lecturer in the Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (June 4).

Assistant Lecturer (preferably with qualifications in social psychology) in Psychology—The Registrar, The University, Sheffield (June 5).

Lecturer (preferably with African field experience and interested in the geography of soils and the conservation of resources) in the Physical Geography of Intertropical Lands and in Survey and Cartography—The Secretary, The University, Edinburgh (June 5).

Senior Tutor (with a degree and preferably teaching experience) in the School of Mathematics, University of New South Wales—Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (June 8).

Experimental Officer or Senior Experimental Officer in the Department of Mechanics of Fluids, for duties which will include the design and construction of electronic and optical apparatus for studying turbulence, aerodynamic noise and flows at very high speeds, including their interaction with magnetic fields—The Registrar, The University, Manchester 13 (June 9).

Lecturers (2) in the Department of Mathematics—The Secretary, The University, Edinburgh (June 9).

Scientific Officer (male, with a good honours degree), to engage in research on sugar beet breeding, with special reference to the exploitation of male sterility and polyploidy—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (June 9).

Lecturers and Senior Lecturers in Philosophy at Monash University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (June 12).

Demonstrator (with, or about to obtain a good honours degree) in Physics—The Registrar, The University, Keele, Staffs (June 15).

Lecturer/Assistant Lecturer (preferably with experience in the field of magnetic resonance or microwave techniques) in the Department of Physics—The Registrar, The University, Keele, Staffs (June 15).

Lecturer in Geography at Fourah Bay College, University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 15).

Lecturer (with an honours degree in botany and postgraduate experience in taxonomic work) in the Department of Botany, University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 19).

Lecturer or Assistant Lecturer in Physics at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 28).

Research Fellow in the Surface Physics Group, to join a team investigating mechanism of photoelectric emission—The Secretary and Registrar, The University, Southampton (June 30).

Chair of Physics in the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 7).

Assistant Lecturer in Agricultural Economics and Farm Management; and an Assistant Lecturer in Animal Husbandry—Clerk to the Governors, Essex Institute of Agriculture, Writtle, Chelmsford, Essex.

Lecturer or Demonstrator in Genetics, with special reference to cytology—Dr. C. B. Goodhart, Museum of Zoology, The University, Downing Street, Cambridge.

Master to teach Biology in VI form to A level; also General Science and/or Mathematics in Middle School—The Headmaster, King's School, Rochester, Kent.

Research Assistant in Organic Chemistry—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

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FUTURE OF PRIMARY AND HIGHER EDUCATION

THE report* of the independent committee under the chairmanship of Sir Charles Morris which, at the invitation of the National Union of Teachers, studied the expenditure needed for future educational development was published some two months before the Government announced its proposals for university development in the next quinquennium. As its title, *Investment for National Survival*, indicates, the report is, however, entirely pertinent to the discussion on Government policy inasmuch as, although primarily concerned with the supply of teachers to implement current proposals for educational reform, its main emphasis is on the cost of the necessary measures, which the Committee frankly recognizes will be expensive in manpower and womanpower as well as in finance. Nevertheless, its conclusions are framed in the belief that our national survival depends wholly on the manner in which we use our human resources. "If the country is to use its reserves of ability to the full it must create an educational system which provides adequate opportunities at all levels, which is staffed throughout by well-qualified teachers, and which offers both a broad, liberal education, and that degree of specialist training which will enable the individual to pursue his chosen career and contribute to the needs of industry, commerce or the professions." Furthermore, the Morris Committee recognizes that any proposals to this end must be executed quickly.

Now it is the contention of the Government in commending its proposals to Parliament that Britain's economy needs an increase—at least 35 per cent—in the supply of trained men and women coming from the universities but that the country's economy is such that it cannot afford to set aside just now the financial resources which those best qualified to judge believe are necessary to achieve the Government's target. In particular, the Government has postponed authorizing the salary changes that are regarded as essential to ensure an adequate supply of university teachers of the high quality required. The Government, in making its decisions, was fully aware that the whole pattern of higher education was already under review and did not attempt to anticipate the findings of the Robbins Committee. Neither, however, did it seek to ensure that those findings could be implemented without prejudice.

This report from the Morris Committee does not attempt to evaluate the different proposals for educational reform at present before the public but rather to consider the cost in money and teachers of implementing them and such other demands on education as will be exerted on the economy, by changes in population and by the demand for more education which is now making itself felt, and which while receiving less public attention have considerably increased the magnitude of the task. A valuable feature of the report is, in fact, the background paper in which J. Vaizey and S. Pratt estimate quantitatively the probable pattern of education now and up to 1970 and 1980, the demand for teachers, the supply of teachers and buildings and the probable costs. By 1970, they suggest, it is probable that more than three-fifths of our children will be taking courses of at least five years duration in their secondary schools and we may need 95,000 additional teachers to implement existing policies as well as about 10,0000 new teachers over the decade in higher education. To achieve 175,000 places in the universities and 40,000 elsewhere in the colleges of technology by 1970 would require 22,000 teachers, at about 1,700 a year to allow for wastage.

In all, they suggest we may need to find on average more than 30,000 teachers a year during the nineteen-sixties at an annual increment of £10–£20 million in the bill for education. Total capital investment in primary and secondary schools is put at £550 million with some £600 million for higher education by 1980, compared with the present rate of about £150 million a year. Unless the national income rises by at least 6 per cent a year the share of the national income to education is bound to rise, and, in the absence of new means of financial support, educational development must slow down or public expenditure rise. If, however, 4 per cent of the gross national product continues to be spent on education Mr. Vaizey and Mr. Pratt estimate it should be possible to double expenditure on primary education, with maximum classes of 20–25, to provide higher education for 600,000 and to double expenditure on secondary education, with education up to the age of 17–18 in classes of 20–25 and substantial margins for adult education.

The vital point they make is that it is extremely likely that a higher rate of educational expenditure now will help the national income to grow faster, and the faster the nation spends in the next decade, the easier it will ultimately be to provide a really lavish system. If higher education is expanded to yield an average gross flow of 30,000 teachers a year, all the reforms proposed can be achieved by 1970, but to sustain this rate of expansion the nation has both to expand higher education rapidly and to find the finance for an adequate educational system. This probably means an increase of £40–50 millions a year for at least twenty years and this is admittedly a heavy burden for the national exchequer and the local rates.

The Morris Committee believes that this investment should be made and the real issue with the Government is not so much on the magnitude of the investment as on its timing. Under the present plans it expects at least £1,300 million to be spent on public education in 1970, and changes in salaries and prices could well bring this to £1,500 million by that date—a figure which was anticipated by Sir David Eccles in the House of Commons fully two years ago. There is no minimizing the magnitude of this effort in terms either of manpower or expenditure, but in the full context of the economic situation the report maintains that it is essential for economic growth and the investment should be made now: to delay will not merely impair the nation's economic strength but will probably considerably increase the cost of achieving the objectives to which the Government is already committed if, indeed, it does not prejudice their attainment.

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* *Investment for National Survival.* (The Report of an Independent Committee under the chairmanship of Sir Charles Morris on the Expenditure Needed for Future Educational Development.) Pp. vi+42. (London: National Union of Teachers, 1962.)

The economic argument on which the Government seeks to justify the proposals announced on March 14 appears, in fact, to be one of the most telling indictments of the policy of delay. It is a matter of timing as well as of values, and this is fully supported by the analysis which Mr. John Vaizey has recently published under the title *The Economics of Education* *. Reviewing primarily what has already been published on the economic aspects of education and the returns from education whether regarded as consumption of investment, he approaches the question what should be spend on education. Emphasizing that such expenditure should be studied in the context of the whole structure of public finance, he directs attention to factors affecting the expansion of education, such as the supply of teachers, over which Government spokesmen have passed too easily in the present discussion, and to the way in which that expansion has to be related to prior decisions about the rate of growth of the economy and about its general characteristics.

Fundamentally, Mr. Vaizey believes that decisions as to the role of education in the economy and the rate of expansion of education must rest not simply on a balanced view of the whole system of education but also on a reasoned discussion of its social purposes. We must be clear as to the ends to be served before sound decisions can be taken as to the means to be used. This is fundamental whether we are considering the development of secondary education or the expansion of the universities, but having willed the ends, we must also will the means.

This is the gravamen of the present charge against the Government over the expansion of the universities, but it is at least encouraging to note that in much of the evidence submitted to the Robbins Committee the purpose of particular sectors of education is fully considered. The memorandum submitted by the Manchester Local Association of University Teachers, for example, considers first the purpose and functions of universities, and it is from this that the Association proceeds to urge the vital necessity of institutions in which teaching and research proceed side by side. The Committee of Principals of Colleges of Advanced Technology likewise discusses the nature and specialized functions of higher educational institutions in general before outlining its views on the full development of colleges of advanced technology; and, if this is not done in the memorandum submitted by a group of Fabians *, this is because during discussions it was decided that it would be helpful if the Fabians, with their traditional interest in administration, concentrated their evidence on the structure of higher education rather than on broader issues of content.

In so far as the Government's decision to set aside the recommendations of the University Grants Committee have led to the expression of fresh doubts as to the adequacy of the existing administrative arrangements this Fabian pamphlet, *The Structure of Higher Education*, is of special interest at the present time. The effectiveness of the University Grants Committee has already been questioned in respect of university research, and in the recent debate in Parliament it was equally questioned in respect of research, when Mr. R. P. Hornby suggested that responsibility for the University Grants Committee should be transferred to the Minister for Science or the Minister of Education. There appears to be a considerable volume of opinion in favour of removing the responsibility from the Treasury; but opinion is divided as to whether it is preferable to give the Minister of Education responsibility in this way for all State educational services, including the colleges of technology as well as the universities or whether, on the contrary, the advantage lies in giving this responsibility to the Minister for Science, particularly in view of the research aspect and his responsibilities for the Research Councils.

Another possibility which has been suggested is the creation of a separate Ministry of Higher Education responsible for universities, colleges of technology and all centres of advanced training. Despite the solid advantage of the colleges of technology and universities being handled by one Minister, possibly assisted by a Technological Grants Committee, balancing the University Grants Committee and advised by the Advisory Council on Scientific Policy there are objections or difficulties to any of these proposals, though scarcely such as could not be overcome without serious duplication of effort, given real determination and goodwill. The Fabians lean to the second of the three proposals advocating the creation of a Higher Education Authority under a single department responsible for the whole field of higher education.

The proposed Authority would lay down lines of higher educational policy for Britain as a whole, and it would also co-ordinate the activities of the regional higher education authorities (of which about ten are visualized) which would be responsible for interpreting national policy in the light of local circumstances. The national authority, which would be appointed by the Minister of Education, would itself appoint the Regional Authorities and their chairman, and, since higher education is conceived as essentially a national rather than a local problem, would apportion financial grants from the Exchequer among the regional authorities. Nevertheless, the proposal is designed and intended to ensure the maximum interest and support from the local authorities.

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* *The Economics of Education.* By John Vaizey. Pp. 165. (London: Faber and Faber, Ltd., 1962.) 21*s*. net.

* Fabian Tract No. 334: *The Structure of Higher Education.* (Socialism in the Sixties.) Pp. 20. (Fabian Society, 1961.) 2*s*. 6*d*.

The Fabian Group visualizes the real practical division in higher education lies between universities, colleges of advanced technology and teachers' training colleges on one hand and all other institutions on the other, and so far from wishing to discard the University Grants Committee it contemplates similar bodies for colleges of technology and for teachers' training colleges. None of the proposals, however, seems to contemplate the present situation in which the Government, having asked its responsible advisory body to formulate proposals to achieve a specific objective, has refused to will the means. No system can function effectively unless used with sense and insight, and whatever proposals may in the upshot come from the Robbins Committee, its report should at least bring a forcible reminder to the Government that if and when the nation's economic position is such that specific limits are necessarily set on the resources which can be allocated to particular ends, the appropriate institution should be advised before and not after it has formulated its plans. Failure to modify an objective when necessary at an early stage can result in serious waste as well as frustration, and it is difficult to clear the Government at this junction of the charge of causing waste as well as endangering the objectives it had set.

Meanwhile, it cannot be emphasized too strongly that even to maintain existing standards and serve the generally accepted ideas of universities as institutions in which teaching and research proceed side by side will involve the universities in expenditure which will go far to absorb the increased funds which the Government proposes to place at their disposal. Apart from the question of salary increments, there is the creation of more senior posts, such as readerships and professorships, and, at the other end, the provision of technicians and craftsmen and other ancillaries, lack of which is at present a serious handicap to research. The organization as well as the finance of research also present problems which will call for increased financial provision before we can wisely contemplate any expansion in student numbers with existing resources, and while it is possible that considerable economies should be obtainable in the cost of university education by modification in teaching methods and in buildings without necessarily lowering standards, it is scarcely reasonable to expect to achieve these with staffs that are already overstrained if not frustrated. Nor does it seem prudent to attempt more than exploratory measures when the whole structure of higher education and its balance is the subject of authoritative inquiry.

On almost every count therefore the Government policy appears to be ill-judged, and likely to prejudice rather than assist Britain's economic development. The-Robbins Committee can scarcely be expected to expedite the completion of its report by more than a month or two, and in the meantime the soundest course would be to ensure that the way is clear for whatever action may be recommended. Even from the strictly economic point of view, a first desideration in policy should be the removal of causes of waste and friction and, above all, of the frustration which could deny us the trained manpower and womanpower essential when the lines of expansion have been decided.

CANADIAN UNIVERSITY PROBLEMS

Canadian Universities Today

Symposium presented to the Royal Society of Canada in 1960. Edited by George Stanley and Guy Sylvestre. ("Studia Varia" Series, Vol. 6.) Pp. x + 97. (Toronto: University of Toronto Press, 1961. Published for the Royal Society of Canada.) 4 dollars.

THIS short book consists of papers contributed to the annual symposium of the Royal Society of Canada by eleven of the Society's Fellows. Four of the essays deal with the philosophy of higher education: the threat of over-professionalization; the search for an integrating theme in an age of abundant and narrow specialities; the preservation of the university as a centre of social criticism at a time when much of education is described as "adjustment to society". The remaining seven papers deal with severely practical problems facing Canadian universities: the population boom; the financial difficulty; the scientific lag in French–Canadian universities (three papers touch on this); and the need to join technology with pure science in a university setting.

Perhaps the most incisive chapter in the book is that on "The University and its Finances". The Vice-Principal of Queen's University informs us that "twenty-five years ago about two-fifths of a cent out of every dollar of gross national production . . . was devoted to higher education. To-day it is still only two-fifths of a cent, in fact slightly less, although the average material standard of living has more than doubled. In the same period student enrolment . . . has gone up three times, a rate of increase substantially greater than the growth in total population". By 1970 it is estimated that university enrolment will be double its present level; furthermore, costs per student are bound to rise substantially. Unless new money becomes available soon "a critical situation could be reached in a very few years. . . . Something would have to give way—it could be a considerable limitation on the number of students that may be admitted or it could be a serious deterioration in quality". Government, incidentally, provides 55 per cent of operating revenues at present; business and private benefactions account for only 4 per cent of income.

The editors assume that the conflict between science and humanism is "more apparent than real, more smoke than fire". The scientists among the contributors seem less certain. Dr. Steacie, president of the National Research Council, observes that "by refusing to take any interest in man's environment, there is no question that the humanists have surrendered their right to regard themselves as liberally educated, and have left a gap which must be filled". By comparison with the humanities, liberally supported by the Canada Council, "the sciences seem to be in something of the position of the labourer in the vineyard who toiled all day for one penny".

Due to their heavily classical tradition, the universities of French Canada (Laval, Montreal and Sherbrooke) have had great difficulty in adjusting to modern scientific culture, and have, so far, been unable to play their full part in scientific research. For the most part, we are assured, French and English-speaking Canadians get on well together in the national academic community. But there are indications in at least one paper of the familiar (and understandable) French-Canadian sense of injustice, which, in recent months, has led to a revival of Quebec 'nationalism' or 'separatism'.

Clearly, Canadian universities have their problems, some familiar enough to English academics, but some peculiar to themselves. BRIAN HEENEY

GEOGRAPHY AND ITS RECENT PAST

A Hundred Years of Geography

By T. W. Freeman. (The "Hundred Years" Series.) Pp. 335. (London: Gerald Duckworth and Co., Ltd., 1961.) 30*s*. net.

"THE history of geography is long and honourable," said Mr. J. N. L. Baker, in his presidential address to Section E (Geography) of the British Association in 1955, "No geographer need apologize for it or be ashamed of it". But the actual writing of the history of the subject during the past hundred years has awaited an author of Mr. Freeman's courage, breadth of thought, and fluency of writing. The story of the progress of any subject over the course of a century is difficult to relate—as other books in the "Hundred Years Series", of which this volume is the latest to appear, clearly show: but perhaps the achievement of geographers is especially hard to describe in a small compass if only because of their range of interest and of the many-sided development of geography, particularly during recent years.

Geographers will have abundant reasons to be grateful to Mr. Freeman, who is reader in economic geography in the University of Manchester and who has obviously greatly enjoyed his task of assessment, compression and selection. They will not always agree with his observations or accept all the innuendos: they may not invariably appreciate all the asides. But they will be glad to receive his guidance and can scarcely fail to profit from it, as they are led through the different branches of the subject as each has unfolded and developed over a century. Fortunately, the author is essentially catholic in his sympathies. On one hand he has a deep respect for our forbears, the nineteenth-century pioneers, who laid splendid foundations for the subject because of their concern for what Jean Gottmann has called "the broad and permanent problems of the world's partitioning": on the other hand he sees the future of geography lying increasingly in "closer study of a more restricted field, but by far more people than were available a generation ago".

Mr. Freeman looks first with understanding and appreciation on the subject as it existed in the mid-nineteenth century and then in the period immediately before the First World War. He evaluates the varied contributions that have come through exploration and through education, by the efforts of the numerous geographical societies which were founded and flourished during the nineteenth century, and from the developments in the academic world that owed so much, in their early days, to the encouragement of these societies. An attempt is made to consider these developments over a wide range of the subject, including physical, economic, social and political geography as well as cartography, while one chapter is devoted to what the author describes as "that most controversial of geographical topics, 'the regional approach'."

Quite often the reader may be tempted to query statements or interpretations: occasionally he may be surprised at omissions in the valuable and far-reaching bibliographical entries supplied in the notes and references that are—unfortunately and no doubt at the behest of the publisher—placed altogether at the end of the book. There is, for example, no specific reference to the boldest, if not perhaps the most carefully organized, of the attacks on the regional concept in geography. While there are useful biographical notes of about ninety geographers now deceased (some of them fortunate perhaps to find themselves in so select a company), some of the leading figures in British geography to-day qualify for no reference whatsoever. Consideration of the environmental determinism of Ratzel, Miss Semple, Ellsworth Huntington and others seems nowhere to be linked to the rebirth of some of these philosophical disputes during recent years. There might also be fuller references to some of geography's most significant growing-points to-day: the remarkable progress of historical geography, for example, or the growing concern of geographers for tropical studies, or developments in biogeography. Perhaps, though, there is point in Mr. Freeman's whole-hearted endorsement of H. R. Mill's contention that geography is "old even in the expression of ideas that we are apt to consider most modern": indeed he says as much when suggesting that "many ideas are produced, ignored, and revived fifty years or so later with good results".

Now, thanks to Mr. Freeman, geographers can look back over a hundred years of progress and change and thus appreciate better the status and prospects of their subject to-day. We can be grateful for the author's simple and forthright re-statement of some vital truths that must be recognized if the steady, balanced development of geography is to continue. It is good that the experts in particular fields should be reminded that "systematic and regional geography need not be thought of as rivals but rather as complementary, for each can fructify the other". Others can profitably be told that "too often regional study has been discredited by its superficiality and its readiness to generalize on inadequate premises; the macrogeographical picture must rest on the micro-geographical basis". Even the terse reminder that "neither statistics nor distribution maps in themselves are more than useful tools" has real relevance to-day. Geographers will therefore react to this book in different ways: some will be provoked, others may be tempted to question, but all should be encouraged. Readers who are not geographers will be given insight into both the history and the methods of a subject that is widely studied to-day but is still insufficiently understood or appreciated in many quarters.

In 1895, Mr. Freeman reminds us, the sixth International Geographical Congress was held in London and was hailed as "an opportunity, such as has never occurred before in this country . . . of demonstrating the importance of geography as a science of high precision and vast extent, rich in results of theoretical interest, and of practical value, which affords an unequalled discipline in education when rightly applied". Two-thirds of a century later geographers may have lost some of this evangelical fervour and would probably express their point of view a little more cautiously. But when the Congress comes again to Britain in 1964 geographers intend to seize their opportunity once more, and in their advance planning they will be fortified by the valuable reminder of the history of the recent past with which they are provided in this volume. R. W. STEEL

PHOTOGRAPHIC INTERPRETATION OF GEOLOGY

Photogeology

By Victor C. Miller, assisted by Calvin F. Miller. (International Series in the Earth Sciences.) Pp. vii+248. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1961.) 104*s.* 6*d.*

THIS book is an elementary introduction to the principles of photographic interpretation of geology. It is a lavish production and contains eighty halftone reproductions of stereopairs or single photographs which illustrate 'text-book' examples of different geological structures or geomorphological features, all from the United States or Canada. Each example is supported by a brief analysis of the features which can be interpreted from the photography and there are outline maps of the localities which can be used for practical exercises. The reproduction of the photography is excellent, and the plates can be examined through an ordinary lens stereoscope of small magnification without serious loss of quality. The book is specially bound with a ring back to allow the pages to lie flat for stereoscopic examination.

Although this book is worthy of serious study for the sake of these examples, the remaining text is of uneven quality. The chapters relating to the qualitative interpretation of geological features are elementary but sound. The author rightly emphasizes the importance of geomorphological analysis as the basis of any photo-geological interpretation.

The preliminary chapters which describe the elementary geometry of the aerial photograph, the principles of stereoscopy and the methods of measurement and plotting are the least satisfactory part of the book. It is reasonable to assume that a book of this sort should instruct the geologist how to transfer the results of his interpretation to a base map by simple graphical methods. Moreover, the ability of the geologist to use a parallax bar, or even a third-order photogrammetric plotter, greatly increases the scope of the work which can be done by allowing him to use quantitative techniques also. These subjects are referred to in the early chapters; but many of the essential elements are treated superficially and some are not mentioned at all. For example, the perspective geometry of a tilted photograph is only described for the representation of a plane surface; relief displacement is only considered for the truly vertical aerial photograph. The combined effects of tilt and relief displacement are not described although these effects occur in practically every aerial photograph which the geologist will examine. The method of plotting principal point base-lines which is described depends on the assumption that the image of each principal point can be transferred from one photograph to the next by inspection. There is no mention of the methods which have to be used in the common case of these points lying in areas of comparatively featureless terrain. The section describing stereoscopic parallax is most unsatisfactory. The parallax equation, which is introduced without proof, is incorrect and there is no explanation of the kinds of errors which will be experienced in determining height differences from simple parallax measurements on tilted photographs. The radial line assumption is glossed over in no more than a sentence and the use of anharmonic ratios as an alternative method of transferring detail from the photograph to the base map is not mentioned. A variety of second order and third order photogrammetric instruments are splendidly illustrated, but the reader is left in some doubt about how these instruments can be used or their relative merits for compilation of photogeological maps.

D. H. MALING

BIOLOGY OF PARASITES

Parasitology

The Biology of Animal Parasites. By Prof. Elmer R. Noble and Dr. Glenn A. Noble. Pp. 767. (London: Henry Kimpton, 1961.) 82*s* 6*d.*

THIS book does not, so its authors say in their preface, attempt to duplicate the contents of a text-book of clinical parasitology. It attempts, in fact, the difficult task of summarizing our knowledge of the biology, rather than other aspects, of parasites of all kinds. One of the aims of its authors is the admirable one of offering parasitology to the undergraduate as a major field of study, and the undergraduate thought of is the one who has already completed at least one year of introductory biology or zoology and yet is surprisingly called by the authors "advanced". The research worker, however, is also catered for. He is warned that he will not find in the book exhaustive consideration of his own field of work, but he will, the authors hope, find the various fields of general animal parasitology summarized and brought up to date.

This combined aim of trying to cater, in one volume, for the needs of the young undergraduate and the research worker is bound to be difficult; some will doubtless feel that it is impossible. So much will depend on what the young student has already been taught, and on how it has been taught and on whether the undergraduate can advance beyond the facts he has already. There is so much detail in the text of the book, and also in its illustrations, that some students may be intimidated by the complexity so rightly recorded here. On the other hand, there will, one hopes, be other students who will welcome this complexity and will find in it a challenge which will lead them on to serious, independent work and later to discovery.

For senior workers the book contains much that will be valuable. Its first eight chapters deal with the biology of parasitic members of the main taxonomic groups of animals, including an all too brief chapter on the parasitic mesozoans, coelenterates and other groups which standard text-books, dealing only with species of économic importance, often leave out. In Chapter 9 the physiology and biochemistry of the parasite are discussed. In Chapters 10–14 we learn about their ecology, and in Chapter 15 about their evolution. At the end of each chapter there is a useful brief bibliography. Well printed and handy in size, the book gives us many illustrations, most of which are good, while some are unique, and others could have been improved. Most of them are borrowed from other books or from research papers.

But the main value of the book is the fact that it brings together our knowledge, not so much about the systematics, structure and great economic importance of the parasite, but about its biology and about the basis of the parasitic mode of life. It was high time that such a book was prepared. If it contains here and there some statements which can be criticized, it should be remembered, not only that it is easy to criticize and very difficult to write a book of this kind, but also that nobody yet has written a book that does not, in some respect, arouse controversy. It is good, in fact, that it should be so, for the senior reader can, from his own experience, correct what he does not accept; and the student can refer to him whatever may puzzle him.

So far as I am concerned, I am glad to have this book. It breaks new ground and illustrates the gratifying fact that parasitology, which for so long has been one of the 'Cinderellas' of biology, is nowadays getting the attention it has always deserved. The parasite, as will be realized by the reader of the early pages of this book, may be, perhaps, indefinable, but it does take us, the more deeply we study it, far beyond biology and straight to the very roots of our general philosophies. G. Lapage

CUP FUNGI

British Cup Fungi and Their Allies

An Introduction to the *Ascomycetes*. By R. W. G. Dennis. Pp. xxiv+280+40 plates. (London: The Ray Society, 1960. Sold by Bernard Quaritch, Ltd., 11 Grafton Street, London, W.1.) 80*s*.

No more generous publication has been offered the student of British ascomycetes. By a handy, well-printed and copiously illustrated volume this flora of myriad fungi from *Eurotium* to *Morchella* has been reopened. "Most of the genera said to occur in Britain are illustrated." About 1,000 species in 450 genera are described. For some 180 species taxonomic points are explained in twenty figures of line-drawings. Forty colour plates depict fructifications and spores of nearly 500 species. Most, if not all, descriptions and illustrations in the tradition of Kew mycology are original; they reflect the patience and skill which has been devoted over many years to this artistic product.

The keys to the families and genera are concise and appear to be satisfactory; but in the case of large genera, for which numerous species are described, the absence of specific keys is annoying. The major key to the orders is strange. It is not, as stated, a key to the larger ascomycetes but one to all the ascomycetes in minutest detail, such as will baffle the beginner. The name 'cup-fungi' has been retained throughout the book, and pyrenomycetes are mixed up with discomycetes inasmuch as these two standard groups are, without explanation, completely passed over. Plectascales are associated with Tuberales, and *Nectria* becomes a cup-fungus. A great opportunity has been missed both to help the beginner and to explain the new alternative. The book is stern, but it will make its way overseas because most genera are cosmopolitan and a comparable introduction is scarcely available. Lexicographers will find hidden in the text new combinations.

In all this advantage there is a vexatious omission. Has the author, so expert in this group, turned his back on science? "Taxonomy . . . not a science but an art" (p. xvii). Many of these fungi have been objects of research. To have left this knowledge out merely pales the introduction but, names having been changed and the clue to research residing in the twisted thread of synonymy, to have left this out is ungracious. Where is *Ophiobolus graminis*, and is it rightly named? Where is *Peziza aurantia*, the standard cup-fungus of modern botanical teaching? Without explanation it becomes *Aleuria aurantia* (not ascertainable from the key on p. 1). *Aleuria* is "one of a residual assemblage of genera defined largely by negative characters". *Peziza* becoming *Aleuria*, Aleurieae become Pezizeae, and vice versa, as one might swop *Amanita* and *Lepiota*. The student is referred to a monograph where this *Peziza* is called *Aleuria* (cum *Galactinia*), and the author toys in small print with the popular name "Orange peel Peziza". Scientists try to respect taxonomy. When, artfully or artlessly, it gets in this mess, minds begin to work on a new and scientific taxonomy. The stirrings are abroad, and this book, with its great appeal, will promote them. E. J. H. Corner

HYDRA

The Biology of Hydra and of some other Coelenterates

1961. Edited by Howard M. Lenhoff and W. Farnsworth Loomis. Pp. xv+457. (Coral Gables, Florida: University of Miami Press, 1961.) 4.50 dollars.

This book consists of twenty-three papers delivered by North American workers at a symposium in Miami during March 1961, with thirteen exclusively on *Hydra*, and six more general in nature. Topics covered include: electron microscopy, nematocysts and their toxins, nutrition, growth and differentiation, regeneration, colony formation, and sexual differentiation.

Here is an unusually good example of a single animal treated by widely varying techniques, and it will prove stimulating to alert readers who, if prepared to think for themselves, will achieve some of the "interdisciplinary synthesis" aimed at by the editors. This is helped greatly by the inclusion of the discussions which followed the papers and the additional one on the nervous system of *Hydra*.

Its main value, however, even if rather ephemeral, is for research workers, and for them the aggregation of such diverse material, much of it previously unpublished, under a single cover must be applauded. Most chapters are followed by an almost invariably up-to-date bibliography. Unfortunately, comment on the systematics of *Hydra* is omitted yet frequent discrepancy apparently within species is described.

This is a well-produced volume. Its tables, diagrams and plates are variable but generally adequate and sometimes good; many of the electron micrographs are excellent though occasionally spoilt by the absence, or only obscure insertion, of a magnification indicator.

It is more than two centuries since Trembley's *Memoire* appeared and yet the problems of nematocyst discharge, nerve net and sexual differentiation in the coelenterates still remain unsolved. Mary Campion

Audubon and his Journals

Edited by Maria R. Audubon. With zoological and other notes by Elliott Coues. Vol. 1: Pp. xiv + 532. Vol. 2: Pp. viii + 554. (New York: Dover Publications, Inc.; London: Constable and Co., Ltd., 1960.) 2 dollars each volume.

THESE two 'Dover books' (in stiff paper covers but suitable for rebinding in cloth) represent an unabridged and unaltered republication of the work originally issued in 1897. It consists of a biography (some 70 pp.) of John James Laforest Audubon by his granddaughter, supplemented by certain of Audubon's own journals and a number of short 'episodes' from his publications. The whole provides a picture of the personality and career of a man whose name has become in the United States—in the titles of national and local societies—synonymous with the conservation of wildlife, notably birds. Audubon was born in Louisiana, probably about 1780, the son of a French admiral and his Spanish Creole wife. He was brought up mainly in France but returned to North America as a young man; there he settled, married, suffered many vicissitudes, and became famous as a naturalist and artist, dying in 1851.

Of special interest from the Old World point of view are the European journals, 1826–29, covering the period when Audubon worked in London and elsewhere on the publication of parts of his illustrated folio *The Birds of America*, now a rarity. He naturally met many contemporary figures and, while spending part of the time in Edinburgh, he had William MacGillivray as a collaborator on the scientific side. The Labrador journal, 1833, and the Missouri River journals, 1843, give a good idea of travel, conditions, and wildlife in those parts more than a century ago. The 'episodes' deal with many different matters; in so far as they are dated, they refer to the period 1808–33 and help to fill gaps where journals have not survived.

LANDSBOROUGH THOMSON

The Mathematical Theory of Linear Systems

By Prof. B. M. Brown. (Automation and Control Engineering Series.) Pp. xv + 267. (London: Chapman and Hall, Ltd., 1961.) 50s. net.

THE mathematics underlying the many analytic methods now used in modern control engineering is of necessity omitted from most of the text-books on the subject at present available, and has also not been obtainable hitherto in one book. This gap is filled by the present volume, which is the second of a series entitled *Automation and Control Engineering*.

The work presents clearly yet comprehensively the basic mathematics of linear systems, and in particular of feedback control systems, developed in a logical manner from a starting point no more complex than the basic concepts of differentiation and integration.

The first five chapters are devoted to introduction and to the solution of linear equations by operational, Laplace, and Fourier methods. In particular, the author stresses the value of the bilateral Laplace transform, and compares the applicability of each method in various situations. The mathematics of conventional linear systems is completed by chapters on step and impulse functions, system analysis, feedback and stability. Two chapters are then devoted to statistical methods and the optimization problem, and the final four chapters to finite difference methods and their application to sampled data systems.

The text includes many worked examples of the methods described, and also references, and concludes with twelve substantial appendixes of topics too specialized to be included in the main development. The clear and logical treatment followed throughout is particularly attractive, and renders this book suitable as a teaching text.

E. M. DEELEY

The Veterinary Annual, 1961

Edited by W. A. Pool. Pp. xxiv + 406 + 34 plates. (Bristol: John Wright and Sons, Ltd., 1961.) 42s.

WITH this, its third edition, the *Veterinary Annual* is now firmly established, and has achieved its own distinguished place in veterinary literature. A noteworthy feature is its wide appeal; designed primarily to help practitioners to keep in touch with recent work it is to-day quite widely used by research workers, advisory officers and teachers.

Following what has become its established pattern this year's volume contains, in addition to contributed short reviews on many subjects, a number of special articles. There are eight of these dealing with veterinary medicine in West Germany; in Switzerland; Army Veterinary Services; the ecological approach to veterinary medicine in the United States; parasitological complications of intensive sheep grazing; hides, skins and leather; the physiology of the udder; animal trypanosomiases in Africa; and the unusual but topical subject of retirement from the Colonial Service. These have all been written by prominent workers with close contact with the various subjects and they add greatly to the interest of the book.

A feature is the provision of a list of selected references to each topic, quite a number of them from foreign journals. The value of the lists would be improved if each item from a foreign publication gave information on (*a*) the language in which the original is written, and (*b*) any abstract in English which may be available.

Concise History of Logic

By Heinrich Scholz. Pp. xv + 140. (New York: Philosophical Library, 1961.) 3.75 dollars.

THIS slender manual is a translation from the author's *Abriss de Geschichte der Logik* (second, unchanged edition), which appeared in 1959. Valuable as it is in many ways, it is a pity that steps were not taken to bring the sources more nearly up to date. Bochénski's *Formale Logik* had appeared in German in 1956, and became available in English last year. These events do not detract from the importance of the late Prof. Scholz's masterly sketch so far as it goes, but with these recent developments in view, one can scarcely expect a wide circle of readers in Britain.

The amount of material incorporated is astonishing, and this alone, with the copious notes and references, will assure a ready welcome. A good feature is the general treatment of 'types' of logic (rather than by any other classification), for this brings out the basic distinction between classical formal logic and modern formal logic. Thus, the greatness of Aristotle is seen in correct proportion, not as an obsession, and the unique position of Leibniz is well displayed.

Unluckily, however, the very personal style of the German original has made the translator's task exceptionally difficult, and some of the passages are far from idiomatic English. There are also too many tiresome colloquialisms.

The author's erudition is impressive: this alone commends the book to students who are willing to grapple with some decidedly difficult reading.

F. I. G. RAWLINS

RADIOCHEMICAL RESEARCH LABORATORIES AND THE LAW

By DR. HUGH D. EVANS

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THE legal requirements which have to be fulfilled by persons or organizations using radioactive materials are not clearly understood by the vast majority of workers in this field. There is, in fact, very little Statute Law, though this state will not continue for much longer; what is so often forgotten is that there is considerable scope for Common Law actions in Great Britain.

The discharge of noxious wastes, which can include radioactive material, has long been controlled by legislation such as the Salmon and Freshwater Fisheries Act, 1923, the Public Health Act, 1936, the Public Health (Drainage of Trade Premises) Act, 1937, the Water Act, 1945, and the Rivers (Prevention of Pollution) Act, 1951; but much more specific control will, in the near future, be exercised by the Radioactive Substances Act, 1960.

The handling of radioactive materials in factories is partly covered by the Factories (Luminising) Special Regulations, 1947, and, since February 1, 1962, by the Ionising Radiations (Sealed Sources) Regulations, 1961. The terms of reference of the latter will be extended by a new Act, in the relatively near future, to include unsealed radioactive materials[1].

Apart from these Factories Acts, the only other relevant legislation affecting laboratories outside the United Kingdom Atomic Energy Authority is the Nuclear Installations (Licensing and Insurance) Act, 1959, which is concerned primarily with criticality aspects and the provision of adequate insurance cover in the event of a nuclear accident.

Let us now look at the situation from the point of view of research laboratories. In the first place, since they are exempt from the provisions of any of the Factories Acts, the Factories (Luminising) Special Regulations, 1947, the Ionising Radiations (Sealed Sources) Regulations, 1961, and the companion Act covering unsealed radioactive materials[1] do not apply. This exemption applies to research laboratories in universities, Government establishments, research associations and even in factories; but in the case of the last-named, they must be used exclusively for research and not, for example, combined with a process control laboratory.

Clearly, the Nuclear Installations (Licensing and Insurance) Act, 1959, applies to any organization operating a reactor or processing nuclear fuel, and a few research laboratories, both in industry and the universities, will therefore be affected.

The Radioactive Substances Act, 1960, will control the discharge of radioactive waste from all types of establishments, including research laboratories. It may not be evident at first sight, however, that its powers are much wider, since it requires users of radioactive materials to register with the Ministry of Housing and Local Government; if practice in an organization is found to be unsatisfactory, it will be possible to stop work by withdrawal of the licence.

Apart from this, it would appear that research laboratories have considerable scope for malpractice in the use of radioactive materials, and it is to be regretted that in many, due largely to ignorance of the necessary precautions, work is carried out under conditions which, if not an immediate danger to health, are potentially hazardous. What is so often forgotten is that such practices open the door to justifiable or spurious claims under Common Law. Many injuries or diseases (for example, leukæmia and cataract) which occur in a normal population, have increased incidence in persons exposed to radiation and it is impossible to differentiate between those which are or are not radiation-induced. If working conditions have not been of the highest standard, a legal claim has a fairly high chance of success; but if the employer can show that the precautions taken were adequate (often a debatable point), that all reasonable means of measuring personal dose were provided and that the employee did not at any time receive doses of radiation in excess of those recommended by the International Commission on Radiological Protection[2], then it is unlikely that the Court will uphold the claim. It must be remembered that there is, as yet, very little Case Law on this subject, so test actions are probable and these may well be taken to higher courts. Even for the winner of such actions, costs are likely to be high and a settlement out of court may be preferred. In a recent case (March 1962) it was alleged that a man had contracted leukæmia and cataract as a result of working at Windscale; the United Kingdom Atomic Energy Authority settled out of court for £4,500, without admitting liability.

Clearly, a reputable research organization must institute the safety measures appropriate to its work, not only for the well-being of its staff, but also to protect itself against Common Law actions. This is often accomplished by means of a Code of Practice, which does not have the force of law, outside the establishment, but can be made obligatory within.

One of the earliest Codes in the field of radiation and radioactivity was issued by the Ministry of Health in 1957 [3], principally for the benefit of hospitals, who were at that time probably the major users of radioactive materials outside the United Kingdom Atomic Energy Authority; this Code is at present under revision. Other Codes have been issued by Imperial Chemical Industries, Ltd.[4], the International Atomic Energy Agency[5] and the International Labour Office[6].

The universities have also produced similar documents. The first advisory booklet was published by the University of Cambridge in 1957 [7] and this was followed, also in 1957, by the Code of Practice of the Imperial College of Science and Technology, London[8]. The latter was the first university Code to lay down specific internal regulations and has been widely used in universities and in industry, as a basis for their own regulations; a fourth edition is at present in preparation.

In 1958 the Committee of Vice-Chancellors and Principals of the Universities discussed the whole problem, especially with regard to the possible repercussions of forthcoming legislation on university research. As a result, a sub-committee was formed, with the task of preparing a Code of Practice designed for university conditions; this was published in February 1961[9] and provides a basis for introducing a uniformly high standard of radiological protection in the universities.

Besides these Codes, there is yet another in preparation by the Ministry of Labour, which will be the companion of the Ionising Radiations (Sealed Sources) Regulations, 1961, and the Act which will derive from the Unsealed Radioactive Substances Regulations[1]. This will cover research laboratories in Government establishments, hospitals, research organizations and the universities, but unlike the two Factories Acts, it will not have the force of law. Other Codes in Great Britain will almost certainly be revised to make them compatible with it, although many will continue to be more restrictive.

Clearly, no research organization can afford to be complacent about precautions where radioactive materials are concerned. Though not rigidly bound by Statute Law, the implications of Common Law actions are far too serious to be neglected. There is, however, as has been shown in this article, a wide range of information available, and it is to be hoped that greater advantage will be taken of it.

[1] *Unsealed Radioactive Substances Regulations.* (*Preliminary Draft of Regulations.*) (H.M.S.O., London, 1961.)

[2] *Recommendations of the International Commission on Radiological Protection* (Pergamon Press, London). (*a*) *Report of Main Commission* (1959); (*b*) *Report of Committee I* (*Permissible Dose for External Radiation*) (in preparation), (*c*) *Report of Committee II* (*Permissible Dose for Internal Radiation*) (1959); (*d*) *Report of Committee III* (*Protection Against X-Rays up to Energies of 3 MeV, and Beta- and Gamma-Rays from Sealed Sources*) (1960); (*e*) *Report of Committee IV* (*Protection Against X-Rays above Energies of 3 MeV. and Heavy Particles Including Neutrons and Protons*) (in preparation); (*f*) *Report of Committee V* (*Handling of Radioactive Isotopes and the Disposal of Radioactive Waste*) (in preparation).

[3] *Code of Practice for the Protection of Persons exposed to Ionising Radiations* (H.M.S.O., London, 1957).

[4] *Ionising Radiation Hazards: A Guide to the Safe Use of Ionising Radiations. I.C.I. Engineering Codes and Regulations* (*Safety Series*), Group *D*, 1, 5 (Imperial Chemical Industries, Ltd., London, 1960).

[5] *Safe Handling of Radioisotopes* (Intern. Atomic Energy Agency, Vienna, 1958).

[6] *Manual of Protection against Radiations in Industry, Part II; Model Code of Safety Regulations for Ionising Radiations* (International Labour Office, Geneva, 1958).

[7] *Precautions to be Observed in the Use of Radioactive Materials in the Laboratories of the University* (General Board of the Faculties of the University, Cambridge, 1957).

[8] *Code of Practice against Radiation Hazards* (Imperial College of Science and Technology, London, 1957); (second ed., 1958, third ed., 1960).

[9] *Code of Practice for the Protection of Persons Exposed to Ionising Radiations in University Laboratories* (Assoc. Univ. British Commonwealth, London, 1961)

SAFETY IN LABORATORIES

By PROF. R. A. MORTON, F.R.S., and DR. J. GLOVER

University of Liverpool

As universities and similar institutions grow larger, and the work done in laboratories and workshops becomes more complicated, there is greater need to maintain and strengthen safety precautions. Some years ago the University of Liverpool appointed a retired fire officer to inspect and maintain portable fire-fighting appliances throughout the university and to see that they were placed in the best positions. Later, many hand-operated fire-alarm bells were hung in carefully chosen places, and additional ways of quitting buildings in an emergency were agreed on and sign-posted. Members of staff, technicians and senior students were instructed as to their duties in case of fire.

The next step was to appoint a member of the staff of the Department of Physics to act as 'radiation officer', responsible for the safety of persons using radiation equipment and radioactive isotopes in university laboratories.

Important as the fire and radiation hazards undoubtedly are, there are many other risks. Safe working requires foresight, organization and discipline, all of which can be sensed by visitors to the more 'safety conscious' industrial premises. The many types of building which make up a university present very different hazards; thus, in a chemistry laboratory cuts from glass, splashes of chemicals in the eye and organic solvent fires are ever-present dangers, while mechanical, electrical and radiation hazards are more frequent in physics and engineering laboratories.

At Liverpool, the Fabric Committee, after conducting investigations, and making visits, recommended the appointment of a full-time safety officer with industrial experience. This was agreed to and the post has been filled. It supplements the provision already made through having a fire officer and a member of staff responsible for protection against harmful radiations.

During the Lent Term this year courses of lectures and demonstrations have been given on safety in laboratories with co-operation from the Department of Extra-Mural Studies. The main course was held on Wednesday afternoons during ordinary working hours and was attended by representatives of the teaching staff, research students, senior technicians, tradesmen and porters. The audience numbered about a hundred. The course was planned and organized for the University by Dr. L. J. Burrage (laboratory administrator, Research Department, Imperial Chemical Industries, Widnes), who has specialized in this field and was able to call on leading experts to deal with particular topics.

The course was introduced by H.M. chief inspector of factories (Mr. T. W. McCullough), who displayed statistics on the nature and causes of industrial accidents. Of the annual total number (190,000) of accidents recorded (involving three days or more lost time) some 26 per cent occurred in handling goods and another 16 per cent involved power machinery. Untidiness, accounting for 8 per cent, emphasized the importance of 'good housekeeping'. Mr. McCullough also stressed the need for care in designing machinery and suitable guards to ensure safe working.

With the latter in mind, Dr. Burrage gave his first lecture on "Safety Equipment and its Design", and traced the development of a range of safety measures appropriate for the chemical laboratory. In his second lecture he discussed in more detail various types of guards for laboratory equipment and machinery. Protective clothing, eye shields and goggles were exhibited and illustrated by lantern slides. Many of the devices described were the outcome of years of experience in dealing with laboratory and workshop hazards. Particular stress was laid on protection at the actual source of danger so as to inculcate in staff and students habitual attention to safety in conducting chemical and physical experiments. Advice was given on the proper screening of apparatus which in use was attended by risk. Much attention had been paid to safe ways of carrying dangerous chemicals from one place to another. It was important that each item of safety equipment should be correctly sited and given regular inspection and maintenance.

The third session was devoted to 'housekeeping'. Dr. Burrage laid stress on the fact that true tidiness helped to generate the right attitude towards safety among staff, technicians and students. This led to the conclusion that many accidents can be prevented by adopting some simple but important measures: (1) the benches and floors should be cleared of flasks and apparatus not actually in use; this would reduce breakages as well as risks; (2) reagent bottles should be properly labelled and kept in allotted places with labels to the front; (3) large bottles, winchesters or dangerous chemicals should not be placed on shelves above eye-level; (4) all laboratory workers should be taught to assemble apparatus correctly and neatly and to avoid trailing electricity leads or unnecessary lengths of rubber or plastic tubing.

Valuable suggestions were made on where to place and how to move gas cylinders and fire-fighting equipment. The disposal of waste products always merits strict adherence to a well-considered plan.

Dr. Burrage gave a fourth lecture on the "Correct Handling and Use of Glass". He made many points on care and attention in handling and cleaning glassware and glass tubing so as to reduce the number of accidents. Of the total injuries reported in the chemical laboratories of Imperial Chemical Industries some 30 per cent involve glass cuts. Vacuum and pressure lines ought to be correctly fitted and erected and protective screens should be in use. The lecture was well illustrated with lantern slides.

On the fifth and sixth occasions the audience was offered the choice between lectures on the hazards met in science or engineering laboratories.

Good and bad ways of handling quite ordinary, as well as dangerous, chemicals were described in the science lectures. The proper use, care and maintenance of fume-cupboards was discussed fully. The dangers of explosion which are associated with ammoniacal silvering solutions, or isopropyl ether left to stand, or through contact of strong oxidizing agents with organic matter, were pointed out and the appropriate precautions were detailed. When mercury is spilt it can run out of sight and be forgotten. In a poorly ventilated space it can give rise to toxic amounts of mercury vapour. Misuse of a mercury-diffusion pump without a liquid nitrogen trap is also hazardous. Awareness of these risks is the main thing; they can then easily be eliminated by responsible persons. Strict precautions to be taken in dealing with specially dangerous chemicals, for example, fluorine, chlorine and bromine, strong acids and gases such as hydrocyanic acid and acetylene were also discussed.

The engineering lectures were given by Mr. R. K. Mawson (deputy senior engineering inspector of factories, Ministry of Labour), who discussed the "Guarding and Safe Operation of Various Types of Mechanical Equipment", and by Mr. S. J. Emerson (senior electrical inspector of factories, Ministry of Labour), who dealt with "Electrical Risks and their Elimination". In the seventh session "Personal Protective Equipment" was discussed by Dr. Burrage more fully and a number of specimen articles were on show and their use was demonstrated.

In giving the eighth lecture, Mr. T. E. Hall (principal, Liverpool College of Building) suggested that a more positive attitude should be taken to safety in laboratories and similar buildings by training people in 'safe working' rather than in 'accident prevention'. Simple features of the design of buildings were often important factors in safety, for example, the way in which a door opens into a room and even the exact position of the handle. The treatment given to floors should minimize the risk of slipping; and uneven flights of stairs should be eliminated. A pleasant room with a good layout of equipment lessens accidents by reducing fatigue.

Finally, Mr. Hall emphasized that in addition to correct siting and maintenance of equipment it was important to give full instruction, particularly to the younger workers, on how to use machines and instruments. In the remaining sessions, Dr. Burrage discussed fire-fighting and first-aid equipment. All this he thought should be kept off the floor in panels, preferably near the door to make it strike the eye and be accessible for use, or inspection. Again, fire-alarms should be prominent and audible in every part of the building when people are working. The respective merits of the different types of fire-fighting equipment were explained and advice was given regarding the use of extinguishers and the number of pieces required in various types of premises. The most frequent laboratory fire hazards and the steps which must be taken to avoid them were then described. Dr. Burrage pointed out that everybody who works in the laboratory or other building should be instructed in the use of the equipment, so that the most appropriate piece is chosen in an emergency.

This also applies to first aid, where training of staff is invaluable. In general no one should be allowed to work in a laboratory alone, particularly at night; a second person can usually render first aid and can summon help quickly in the event of a serious accident. All workers should make themselves familiar with the nearest first-aid supplies and especially with the location of wash solutions specially provided for accidental splashes on the skin or eyes. They can be assisted in this by sign-posting in green, following the convention that this colour is associated with safety. Dr. Burrage also recommended that when showers are provided they should give a vigorous spray of water from the side rather than from overhead. He then discussed the treatment of some chemical burns and the action to be taken when workers have been accidentally exposed to doses of toxic substances.

The lecture course was concluded with an excellent film on "Safety in the Laboratory", shown by courtesy of Imperial Chemical Industries (General Chemicals), Ltd. The film brought out very successfully many of the points covered in the previous lectures and emphasized them in a forceful and gripping way.

Dr. Burrage and the other lecturers are to be congratulated on giving a most useful course, which was very well attended and broke new ground in the University. By striving for safety in its own premises a university community may incidentally be providing a desirable educational service to its students, who will in due course bear responsibility elsewhere.

ELECTRICAL MACHINES OF THE FUTURE

Substance of a Friday Evening Discourse at the Royal Institution

By Dr. E. R. LAITHWAITE
Electrical Engineering Laboratories, Manchester

THE subject 'electrical machines' may cover a great variety of devices from turbo-alternators and induction motors to computing machines and particle accelerators. Here, however, I am mainly concerned with solid objects which move, in short, with what were included in the heading 'electrical machines' perhaps fifty years ago. Such machines are mainly used for converting electrical power into mechanical power, or vice versa, and the object of the designing engineer is always to produce a machine which gives the maximum return for the minimum outlay of money. For example, he may be concerned with getting the highest efficiency out of his machine, that is to say, wasting the minimum of power in the conversion process. On the other hand, he may not care about this aspect too much, provided he can get the maximum amount of power from a given size of machine. In some cases, he may forgo a great deal of either of these aspects, provided he makes a machine which is guaranteed to operate year in, year out, without attention of any kind.

In design problems the engineer is generally faced with so many alternatives that it becomes almost impossible to write down equations which represent the problem, and it is quite surprising to what extent design is still an art as much as a science. If a bunch of keys be dropped on to a table it seems scarcely possible to calculate the final rest position of each key, yet to do it experimentally is a simple matter and there is one and only one answer, which means that a unique theoretical solution should be possible. Very often, in complex problems, we make use of models, and the electrical engineer often makes a mechanical model of a problem to help him appreciate the implications better. There is a real danger in pursuing such techniques too far, however, for often one can pin such faith in the model as to believe that it represents the original problem in every way, whereas in fact the basic concept of the original problem may not be understandable.

Such may be the case with our commonest form of electric motor—the induction motor. A simple form of induction motor consists of an iron ring carrying coils which are supplied with polyphase alternating current. When switched on, nothing which is detectable chemically occurs in the centre of the ring. The space is still filled with air. But something curious has happened, for if an aluminium or copper ball be placed inside the ring it will be rotated even though it makes no physical contact with the ring at all. The forces are applied across space and are of the same nature as those of gravity. The honest answer to the question of how it works is: "We do not know".

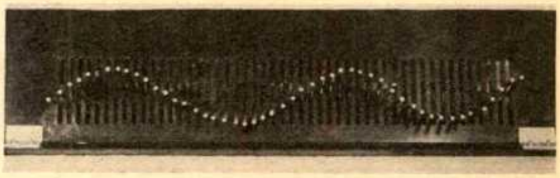

Fig. 1. Mechanical model illustrating travelling waves

What we can do, however, is make a mechanical model which together with other analogues helps us to believe that we understand, in fact so much so that we can exploit the principles of induction very well indeed and make very useful motors.

The first analogue we use is that of the rotating magnetic field. If a pair of horseshoe magnets be mounted on a wheel with their poles facing a copper disk, so that they can be rotated around the face of the disk, they are found to try to drag the disk around with them. We say that the moving magnetic field cuts the conductor and induces a voltage into it, which causes a current to flow. The current in turn produces a field which reacts with the original field to produce the torque. In saying this we have really 'explained' very little; but what we have done is to suggest that whenever a moving magnetic field is produced it is capable of exerting a force on a piece of conductor, no matter how we produce that field. Now, in the case of the ring and the ball we produced our moving magnetic field by means of stationary coils.

To try to understand how this is done we may make a mechanical model such as that shown in Fig. 1. Each of the metal rods is capable of being moved vertically, but not horizontally, since it is confined within a vertical slot. If we arrange to move the rods up and down rhythmically in a certain order we can produce the effect of a travelling wave, like a ripple in water. If, however, we seriously consider just what does move sideways we are forced to the conclusion that it is nothing more than an 'idea' in our heads. The most interesting demonstration, however, consists of placing a ball on the set of rods when the wave is being produced. The ball is moved along horizontally and the 'idea' has become a reality. In the case of the induction motor the rods are represented by the coils on the ring, each of which produces a magnetic field which goes up and down in value; but the coils are so phased with respect to each other as to produce the effect of a travelling magnetic field. It is when a conducting object is placed in this field that the idea of movement is turned to reality by a process which we do not understand.

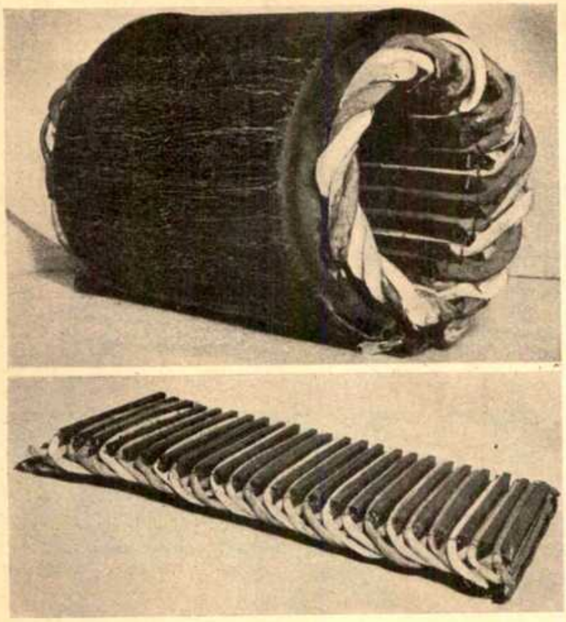

Fig. 2. Top, rubber model of the stator of a conventional induction motor; bottom, model split and opened out flat

Most of our electric motors have rotating fields and produce rotary motion of a shaft. There is no reason, however, why the motion cannot be reproduced in a straight line. Fig. 2 (top) shows a rubber model of the stator of a conventional induction motor. This model can be split along one side and opened out as shown in Fig. 2 (bottom). An actual motor constructed in this form is shown in Fig. 3, and it is capable of accelerating conducting objects in a straight line.

When one considers the number of industrial processes where motion in a straight line is used, the first natural question to ask is: "Why is the linear motor not being used ?" The final answer to this question is that induction motors are only good machines if their surface speed is high enough, and unless the speed exceeds 20 ft./sec. the machine is rarely good enough to be acceptable economically.

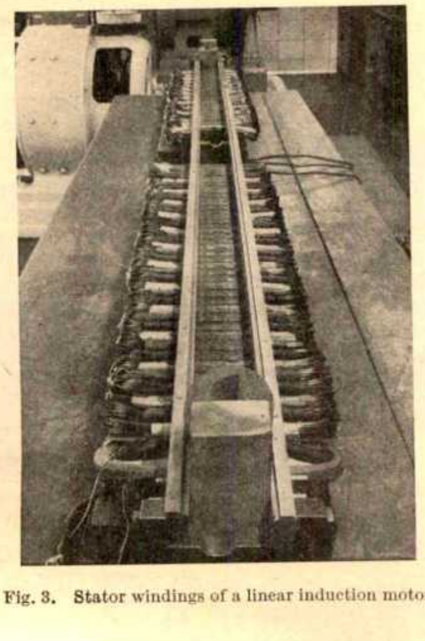

Fig. 3. Stator windings of a linear induction motor

One possible application where a high speed is required is that of propelling a shuttle across a weaving loom. One of the main problems here is to obtain reversal of motion. Normally, it is necessary to interchange two phases of the supply to an induction motor in order to reverse the direction of motion; but such an operation would demand switching of highly inductive circuits perhaps 200 times/min. By suitable design of linear motor, however, all switching can be eliminated, and the motor made to oscillate by itself. The principle of self-oscillation is shown in Fig. 4. Two linear motors are placed back-to-back so as to accelerate the 'rotor' or shuttle towards the centre. At first sight it appears that a rotor will oscillate in the manner of a pendulum under gravity, finally coming to rest in the centre because of friction and other imperfections. This, however, need not be so, since the force on the rotor can be made to vary with speed in the manner shown here. A rotor starting at A is accelerated towards the centre by forces Oa to p arriving at velocity v, say, as it crosses the centre. On entering the reversed field it is effectively moving against the field and therefore at speed $-v$ and is brought to rest at B by forces q to a. Now these forces are less than the accelerating forces and in the absence of friction $x_2 > x_1$. Each succeeding traverse will get bigger until the peak force is exceeded and the rotor approaches the field speed at the centre. Beyond this no further increase can occur.

One of the more recent applications for this device is the movement of a thread guide in a yarn package winder and speeds of 900 traverses/min. have been achieved in laboratory models.

Returning for a moment to the idea of 'unrolling' a conventional induction motor to obtain a linear machine, if we could roll up the linear machine about an axis perpendicular to the original we should make a tubular motor in which the field travelled, as it were, up the 'barrel', for we have made a form of electromagnetic gun. Elementary forms of tubular motor without any iron in the stator are very simple to construct but very wasteful of power. For example, a gun 8 ft. long which can fire a 2-lb. dart at 100 m.p.h. consumed 200 amps/phase at 440 volts line. Well-designed tubular motors with good magnetic circuits have been used for pumping liquid metal in atomic power stations.

Two further applications of linear motors are at present under consideration. In the first, the motor drives directly on to a flexible metal belt. This is the conveyor drive. The obvious advantage is that belt slip is impossible since the force is not imparted to the belt through rollers or other friction-dependent device, but through space over a large area of belt. Unfortunately, most conveyors are too slow to exploit the device to its best advantage.

The second application is concerned with electric traction. The arrangement is virtually the same as that of the conveyor, except that the secondary conductor is fixed and the energized coils are allowed to move. A pair of linear motors is mounted on either side of a conducting rail as shown in Fig. 5. Such a traction unit would be completely devoid of moving parts. The rotor losses of a conventional induction motor, which prevent the machine from having the high starting torque required for traction, are no longer a problem with the linear drive, for the 'rotor' in this context is the conducting rail which, as it is heated, is left behind to cool and fresh cold

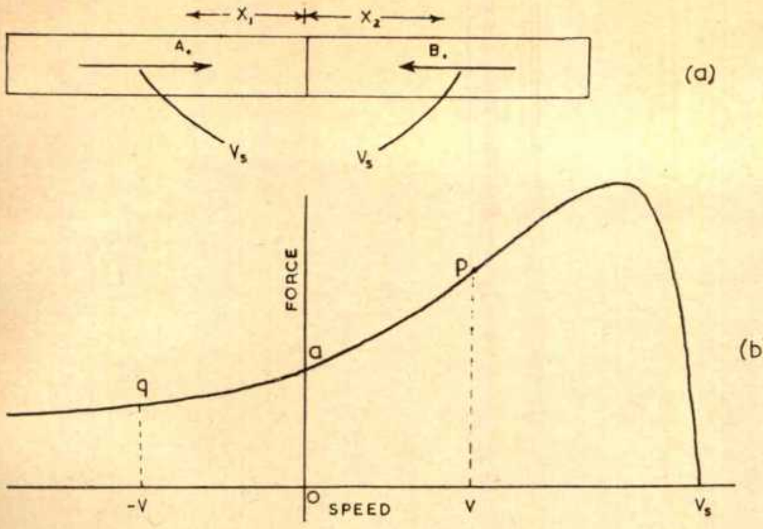

Fig. 4. Principle of the self-oscillating induction motor

rotor is continuously being fed into the motor as the vehicle moves. The first experimental track in the University of Manchester is shown in Fig. 6. The unit develops about 200 lb. of thrust up to a speed of 25 m.p.h. A second model, designed to have 1,000 lb. of thrust up to 33 m.p.h., is at present under construction in collaboration with the British Transport Commission.

In speculating about the future of linear motors it may be convenient to divide them into three classes, the first of which consists of high-powered, high-speed devices. These are likely to be relatively few, but the train may be one of them. In the second class are included all devices of effectively zero output. If the output is zero the efficiency is zero and one is only concerned with doing a job. The package winder is one example. Devices for producing push at negligible speed also fall into this class, and these could be very important. One American firm is producing a tubular actuator which produces a 2-lb. push, continuously, for 80 watts of input in a device weighing 7 lb. and costing at the present time £20.

The third class of linear motor which has a chance is the device for producing energy. Rocket launching is one such example. The object is not to produce power output, although power is needed—not to produce force—although force is needed, but to produce $\frac{1}{2}mv.^2$. Here it must be remembered that it is a fundamental of induction machines that for every joule of output energy produced, at least one joule is lost in heating the rotor and this may well exclude it as a serious proposition for rocket launching.

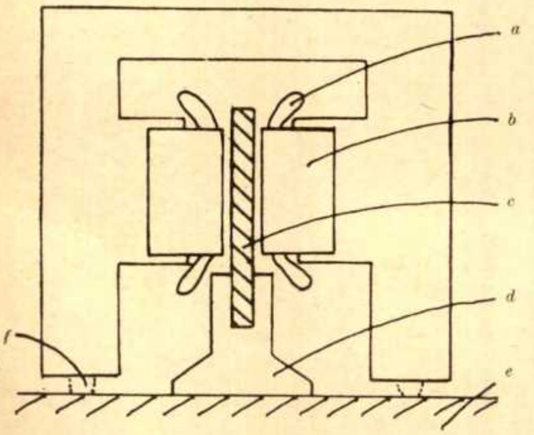

Fig. 5. Cross-section of experimental railway traction unit using linear induction motors
a, Stator coils; *b*, stator core; *c*, conducting rail; *d*, rail clamp; *e*, concrete 'road'; *f*, weight-supporting device (wheels or air-jets)

Of future developments in the broader field of electrical machines than that of the linear induction motor, the search for efficient continuous speed control of a brushless motor continues and may finally succeed.

Electronics and power engineering have now joined forces, and the new semi-conductor devices at power-level are opening up many new possibilities in the field of machines. Some of these are bound to prosper.

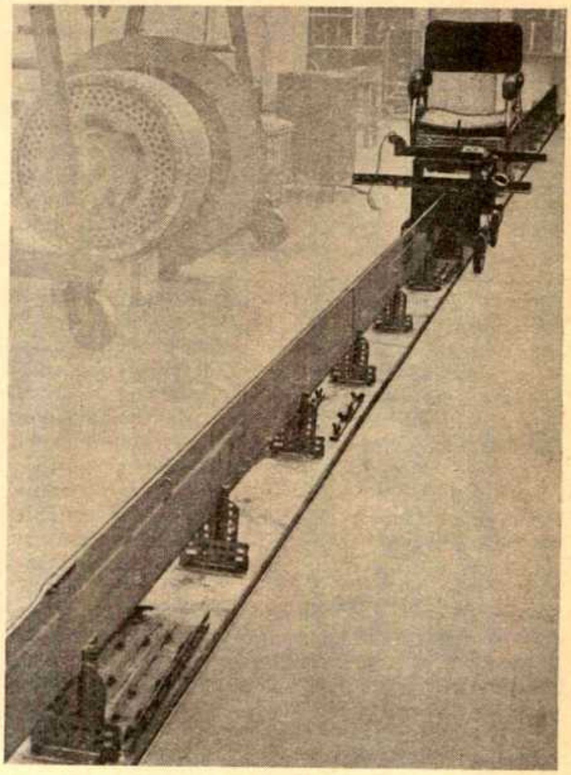

Fig. 6. Experimental railway traction layout in the University of Manchester

In an even broader field, some interesting electrical machines of the electrostatic kind may emerge. Recent work of zoologists on the reactions of living things to alternating electrostatic fields has suggested that control of emotions may not be outside the scope of this kind of machine. There is a real challenge for the electrical engineer, with possibly greater opportunities than in any time in the past, for the stakes are higher and the prizes so much the greater.

FUNDAMENTAL CANCER RESEARCH

THE sixteenth annual symposium on fundamental cancer research was held at the M. D. Anderson Hospital and Tumor Institute, Houston, during March 1–3, and its title ("Conceptual Advances in Immunology and Oncology") is indicative of the renewed interest in the immunological approach to the problem of cancer. It is equally significant that two out of the three days should have been devoted to fundamental immunology: even those who remain profoundly sceptical of the possibility of an all-embracing immunological interpretation of the cancer problem must concede that many of the techniques used in immunology are proving to be of immense value in its study.

The conference began auspiciously with discussions by Sir Macfarlane Burnet and F. Haurowitz on theories of immunity, one taking up the cudgels on behalf of an elective theory implying some kind of genetically built-in cellular responsiveness which is merely triggered off by antigen, and the other championing the instructive theory, which postulates that it is antigen, acting as a template, which impresses specific complementariness on the newly formed γ-globulin of cells. Burnet made it clear that although he could no longer believe in the truth of the clonal selection hypothesis as originally proposed by himself he still felt that the experimental evidence—not least his own, concerned with graft-against-host reactions of cells transplanted to the chorioallantoic membrane of the chick embryo—pointed to an elective theory; it would be extremely difficult to reconcile any other kind of theory with the fact that, during the course of development, the body 'learns' not to react against its own substances. Haurowitz, on the other hand, described experiments which indicate that antigen may persist intracellularly for many months and that antibody may be formed in very small amounts as long as one year after primary stimulation with bovine serum albumin. He felt that re-interpretation of the anamnestic response with these facts in mind was perfectly in line with the idea that antigen acts in an instructive capacity. However, both speakers agreed that at present there could be no final proof either one way or the other, and that in the last analysis a compromise between these two concepts may turn out to be nearest to the truth.

To fill the gap in the programme caused by the regrettable absence of N. K. Jerne, several short papers were read by the 'home team'. Of these, the most relevant to the problem of antibody formation was that of J. J. Trentin, who outlined a technique which, he thought, should at last provide a conclusive test of the clonal selection hypothesis. The technique entailed the re-population of the lymphoid organs of lethally irradiated mice with cells thought to belong to a single 'clone' and the subsequent challenge of these mice with four *Shigella* antigens. In giving his results, Trentin was careful to stress that much work remained to be done before they could be accepted as conclusive; but it appeared that the majority of a small number of mice was able to respond to several of the antigens. However, the crucial assumption—that the cells were, in fact, derived from a single donor strain cell—remained to be proved.

One of the most exciting advances in recent years has been the recognition that certain diseases such as Hashimoto's disease and acquired hæmolytic anæmia are caused by autoimmune responses, and a number of other diseases are now strongly suspected of an autoimmune origin. In discussing theories of autoimmunity, W. Dameshek suggested that experimentally induced 'runt disease' (which is due to the immunological response of genetically foreign lymphoid cells against the tissue antigens of the host) provided a revealing model for the autoimmune disorders. Autoimmunity could be antigen-mediated as in thyroiditis, which is almost certainly made possible by the fact that antigen in the form of thyroglobulin is sequestered during embryonic development and is therefore unable to induce immunological tolerance in the body's antibody-forming cells; or it could be due to abnormal immunologically competent cells owing their existence either to genetic causes or to somatic mutation.

Several papers were devoted to the control mechanisms of antibody synthesis. In describing the detailed cellular changes which occur when antigen is introduced into sensitized animals, F. J. Dixon provides the morphological background against which all immunological reactions must be viewed. He particularly stressed the fact that the nature of the morphological changes depends to a very large extent on whether immunity is mediated through cells or through serum. D. H. Sussdorf critically reviewed past and present experiments designed to reveal antibody activity in cell-free systems and pointed out many of the pitfalls inherent in experiments of this kind. R. W. Dutton described a series of experiments revealing the capacity of sensitized spleen cells to synthesize deoxyribonucleic acid (DNA) *in vitro*: addition of antigen stimulated the incorporation of thymidine labelled with carbon-14 into the DNA of the spleen cell, a phenomenon which was specific for the antigen with which the cell donor had been sensitized. Increase in thymidine incorporation was accompanied by an increase in the number of engaged cells rather than by an increase in the degree of incorporation by the same number of cells.

The properties of interferon (first uncovered by A. Isaacs at the National Institute for Medical Research, London) were comprehensively reviewed by S. E. Grossberg. Interferon was the name given to certain proteins produced by cells after exposure to viruses, and these substances had the power to protect other cells against infection. The resistance conferred by interferon was non-specific for virus, but it showed a curious species-specificity in the sense that interferon extracted, for example, from mouse tissues would not protect chicken cells. The action of interferon was intracellular, for it had been shown not to affect virus adsorption and penetration; nor was it, in all probability, able to influence release of virus. Although its action was still far from clear, Grossberg suggested that it might well be based on the uncoupling of oxidative phosphorylation.

R. S. Schwartz discussed the chemical suppression of immunity, that is, the elimination of the immunological response by prolonged treatment with antimetabolites such as 6-mercaptopurine. Although in a limited clinical study some of the patients suffering from hæmolytic anæmia responded well to chemotherapy, Schwartz went out of his way to stress the fact that, owing to the toxicity of the drug, treatment of this kind could be applied only with the most expert clinical and laboratory supervision. He also raised the interesting if speculative point that treatment of tumour-bearing patients with chemical agents capable of suppressing immunological responses might well deprive the patient of the means of resistance to the tumour.

A whole day was set aside for the discussion of the nature of the antigen–antibody reaction. C. W. Parker explained that for certain kinds of antigen–antibody reactions the spectrofluorometric method, which depends on a decrease in fluorescence when antibodies specifically interact with haptens, has important advantages over other methods. Using this method he had confirmed that R. R. Porter's immune γ-globulin fractions I and II (produced by digestion with papain) titrated as 'univalent molecules', but that fraction III was inactive. J. J. Cebra showed that water-insoluble papain may be used to great effect in the examination of the structure of antigen–antibody complexes, their molecular weights and the valence of the antigens. That the treatment of antibody with pepsin, followed by reduction of a single highly labile disulphide bond, can produce fragments closely resembling Porter's fractions I and II was demonstrated by A. Nisonoff, who went on to describe the formation of 'hybrid' antibodies by the oxidative coupling of univalent sub-units obtained from antibodies of two different specificities. Nisonoff also presented evidence suggesting that hybridization had occurred as a result of random recombination.

Z. Ovary described experiments which showed that although non-aggregated low molecular weight haptens with one determinant group did not provoke passive cutaneous anaphylaxis they could inhibit it, as well as neutralize *in vitro* specific rabbit or guinea pig antibody. When a second determinant group was added *in vitro*, neutralization was again complete but *in vivo* results were variable: with some guinea pig and rabbit sera the hapten now provoked passive cutaneous anaphylaxis, whereas with other guinea pig sera it acted only in the feeblest manner.

The chemical nature of the combining site of antibody molecules was discussed by D. Pressman, who made the point that owing to the heterogeneity of antibodies the study of anti-hapten antibodies was of the greatest value; unlike antibodies directed against proteins or other natural substances, such antibodies were directed against a single known chemical grouping. Pressman also said that there was now reasonable evidence from pepsin and papain experiments indicating that antibodies of even a single molecular weight may be of several different types, and that the fractions discovered by Porter were more complex than had originally been thought. J. R. Marrack suggested that the formation of aggregates, joined by specific bonds between bivalent antigens and multivalent antibodies, was not sufficient to account for precipitation. One of several possible reasons for failure to precipitate was the possibility that, because antigens carried determinants of various kinds, univalent antibody to one of the determinants was present in the system.

Finally, O. J. Plescia explained that although pure DNA had not been shown to be antigenic, it had been possible to prepare it in an immunogenic form from *Brucella abortus*. Several antigens were associated with this DNA; those susceptible to treatment with DNase were almost certainly DNA-carbohydrate complexes which could be dissociated only with the greatest difficulty, and the released DNA had comparatively little antigenicity. The antigenic groups were therefore unlikely to be part of the DNA molecule.

The highlight of the symposium was the presentation of the Bertner Foundation Award to G. D. Snell, director of the Roscoe B. Jackson Memorial Laboratory—an award given for outstanding achievement in the field of cancer research. In his Foundation Lecture on the immunology of tissue transplantation, Snell gave a comprehensive and up-to-date survey of a field which is continuing to change very rapidly and to which he and his colleagues have made such important contributions. He stressed that it had become necessary to distinguish between two kinds of immunological response, the one mediated through conventional serum antibodies and the other through antibodies associated with circulating lymphoid cells. Although both responses frequently occurred side by side, leukotic cells and normal lymphoid cells were susceptible to serum antibodies whereas most solid tumours and normal tissues such as skin were susceptible only to a cellular immunity. Snell also reviewed the phenomenon of immunological enhancement and made it clear that he and his colleague, H. J. Winn, had come to the conclusion that enhancement was brought about by central inhibition of the immunological response by serum antibodies.

Snell's lecture set the scene for a session devoted to tissue transplantation and immunological tolerance. L. Brent described experiments which confirmed that unresponsiveness to skin homografts can be brought about in adult mice by repeated injection of allogeneic hybrid spleen cells. He and G. Gowland had analysed this unresponsiveness and had come to the conclusion that it had almost all the properties of neonatally induced tolerance; although different methods had to be used to induce them, these two phenomena were probably identical at a cellular level. J. J. Trentin and J. Session described experiments using chromosome marker methods which indicated that in mice made tolerant at birth with allogeneic spleen cells the lymphoid tissues were largely repopulated with donor strain cells. They also found that tolerance of skin grafts was more durable when it was induced with hybrid rather than with parental strain cells. That donor cells persist in mice made tolerant with lymphoid cells either at birth or in adult life was demonstrated very clearly by C. Martinez, who used the spleens of tolerant mice to induce tolerance of donor strain antigens in isogeneic new-born mice. The degree of repopulation of the hosts' lymphoid tissue depended largely on the genetic relationship between donor and recipient strains and also on the manner in which tolerance had been brought about in the first instance.

M. W. Chase reviewed the work he and his colleagues had done on the acquisition of immunological tolerance of simple chemical allergens such as picryl chloride; guinea pigs repeatedly fed with such substances were unable to [illegible] to the allergen with a delayed-type contact hypersensitivity or with circulating antibodies. When especially powerful antigens were used abundant serum antibody was formed but inhibition of delayed hypersensitivity remained unaltered. Chase felt that present theories of tolerance would have to be amended so as to take these facts into account. In a most interesting contribution, M. Feldman explained that he and his colleagues had shown that the immunological responsiveness of sub-lethally irradiated rats could largely be restored by the injection of cells from normal animals together with the antigen (sheep red blood cells). This was also true for rats made unresponsive to human serum albumin by treatment with 6-mercaptopurine: immunological responsiveness could be restored not only with spleen cells but with cell-free extracts and DNA-digests as well. Feldman believed that these experiments were incompatible with the clonal theory of tolerance.

The final session was concerned with the question of cancer-specific antigens. The papers by K. Habel and H. O. Sjögren were beautifully complementary in demonstrating that the cells of tumours induced by polyoma virus possessed a new antigen which was foreign to the host. The experimental evidence was partly based on the observation that mice can be made resistant to the tumour by pre-treatment with the virus or with virus-induced tumour cells; but Habel also showed that resistance could be transferred with lymph node cells but not with serum from resistant mice, and that tolerance of the cellular antigen could be induced in new-born mice. In addition, Sjögren confirmed that methylcholanthrene-induced tumours, too, have a new cellular antigen. The antigenic properties of methylcholanthrene-induced tumours were discussed by R. T. Prehn, who stressed that, in contrast to polyoma virus-induced tumours, immunological cross-reactions among various tumours were rare. The fact that antigenicity was sometimes lost after repeated transplantation suggested the possibility that non-antigenic tumour cell variants had been selected by a hostile environment. Prehn suggested that several experimental observations indicated that one of the principal facets of carcinogenic activity might be the suppression of immunological responsiveness by the carcinogens.

B. Björklund described the production in horses of a cancer-specific antiserum following sensitization with sixty human carcinomas. This antiserum was specific for human neoplastic cells because its cytotoxic activity was retained after absorption with normal human cells. Björklund's conclusion that human carcinomas are characterized by specific antigens was not accepted by all immunologists present.

W. Braun *et al.* claimed to have shown that DNA digests from calf thymus or the spleens of very young mice reduced the incidence of spontaneous tumours when injected repeatedly into young mice. Furthermore, pretreated mice rejected transplantable tumours obtained from untreated old mice—a finding which was complicated by the observation that the same animals died more quickly of growing tumours when challenged for a second time two months later. Finally, R. H. Wilson described a clinical study in which 32 patients with terminal malignant disease were injected with their own tumour cells emulsified with hyaluronidase and Freund's adjuvant. A rise in specific serum antibody titre occurred in 20 patients, and Wilson *et al.* concluded that a specific antigen must have been present in the neoplastic cells of these patients.

The symposium was attended by many hundreds of cancer research workers, and it undoubtedly succeeded in giving them an excellent bird's-eye view of the manifold new developments in immunology and cancer research. However, the very size of such a conference seriously limits the value of the discussions, and this is to be regretted.

LESLIE BRENT

OBITUARIES

Mr. Fred Scholefield, M.B.E.

FRED SCHOLEFIELD, who died on March 10 at his home in Hale, Cheshire, at the age of seventy-eight, and who spent almost the whole of his long professional life in the service of tinctorial technology, will best be remembered as the head of the Department of Textile Chemistry in the College of Science and Technology, Manchester, a department which he brought into being. He came late to the service of university education, for, after a meritorious performance as a student in the Departments of Chemistry and of Dyeing and Tinctorial Chemistry at the Yorkshire College (later the University of Leeds), he was for twenty-two years engaged in the industrial practice of textile dyeing and allied chemical treatments, becoming ultimately the chief chemist and works manager of Burgess, Ledward and Co., a large yarn-processing firm near Manchester.

In an old industry in which traditional usages and modern chemical and engineering discovery need to be promptly combined by the more enterprising firms, Scholefield foresaw more strongly than many of his generation the need for a much greater number of carefully and soun[illegible] trained technologists than seemed to be in p[illegible] end of the First World War. In 1926 he gave up his industrial position to join the Department of Applied Chemistry at the College, taking the place left vacant by the death of Edmund Knecht and the retirement of Julius Huebner. Soon after his arrival he was able to have a separate Department established, of which he was to remain the head until his effective retirement from academic work in 1951. During his régime the Department developed greatly in activity and importance, students came from many parts of the world and the record of research and teaching was very substantial taking into account the small numbers of his staff.

Scholefield's main interest in the field of dyeing research was in the oxidative changes brought about in cellulosic and other fibres when they were dyed with certain vat dyes, and activation occurred either by light or through other chemical agencies. Although later workers have extended our knowledge of the fundamental mechanisms on which these phenomena could be based, there is, even now, very little further understanding of the distinctive effects of specific dyes in actual textile products.

Scholefield was a lucid and inspiring teacher, a kindly, sociable and generous man, widely read and widely travelled, with many friends in Britain and abroad. He had unusual powers of analysing a complex situation and giving his opinion in precise and graphic terms. This, coupled with his retentive memory and his liberal outlook, made him particularly effective in committee. During the whole of his professional life, he was an active member of the learned societies appropriate to his interests. He served two terms on the council of the Royal Institute of Chemistry and was chairman of its Manchester and District Section; he was a founder-member and past president of the British Association of Chemists and did a great deal of work to forward its interests with which he was greatly in sympathy. He was on a number of committees of the Textile Institute and the City and Guilds of London Institute, most of them concerned with technical education and professional status. The Textile Institute made him an honorary life member and awarded him the Institute Medal: the City and Guilds made him a special award of its insignia. The Society which made the strongest claims on his attention was the Dyers and Colourists, of which he was a member of council for many years and in the varied activities of which, local and national, he played a very special part. He held the Research Gold Medal of the Dyers' Company, the Society's own Gold Medal and was a honorary life member, and its president in 1950–51. One of his last public activities was as a member of the inter-society committee which organized the celebration of the Perkin Centenary. His former students, part-time and university, were widely scattered throughout the textile processing and allied industries in responsible positions, and during his later years he had many tokens of their gratitude and esteem.

On his retirement from academic life, he became technical adviser in Manchester to the Dyestuffs Office of the Board of Trade, and his wide knowledge of the dye-making and dye-using industries, with his many personal connexions in both, enabled him to give further valuable service to the tinctorial interests of Britain. For it he was made a member of the Order of the British Empire in 1959. In the past few years serious illness curtailed his activities; but on all occasions possible he attended at his office until final retirement in 1961.

He lived to see the Department that he founded increase greatly in size, equipment and numbers with the expansion of the College itself. He leaves behind many pleasant memories in all those who were his friends and associates.

H. A. TURNER

Mr. W. G. Kendrew

THE death on April 4 of W. G. Kendrew will be deeply regretted by climatologists and meteorologists throughout the world. For more than fifty of his seventy-seven years he took a great interest in climatology and his name soon became a household word in climatological circles. At Oxford, after taking a pass degree, he joined, in 1909, the teaching staff of St. Catherine's Society as classical don and eventually also as dean.

But Kendrew's main inspiration sprang from geography, in which in 1910 he gained with distinction the certificate and diploma. Geography at Oxford was then under Prof. A. J. Herbertson, who had transported personally to Britain the ideas prevalent in Germany on world climatic belts and regions. It is probable that Kendrew was impressed by public criticisms of Herbertson's speculative global schemes (1912), such as the desirability of 'avoiding' wherever possible sharp boundaries to thermal zones; of using accumulated and extreme temperatures rather than averages; of taking account of rainfall and of evaporation, particularly from the human body. In any event, Kendrew turned to the more quantitative study of the climates of individual areas, and on these and climatic principles he lectured and tutored, with breaks for War service, from 1912 until 1950, the last ten years as reader in climatology.

Whatever he said or wrote reflected his classical leaning and his geographical enmeshment. It was highly intelligible, carefully and concisely phrased, and geographically rather than meteorologically applied; his use of quotations and his translations from foreign texts were brilliant; his technique of providing, prominently, but not too disruptively, a simple statistical basis to climatic phenomena inspired confidence. Consequently, the general public and the non-specialist student found his expositions attractive and most helpful.

His earlier climatic writings appeared in Herbertson and Howarth's *Survey of the British Empire* (1914). Within eight years these regional accounts had been extended to all the land-masses and *The Climates of the Continents* began its long career. The book aimed at filling a gap caused by the lack in English of any adequate description of the actual climates of the countries of the Earth, considered regionally. It was hailed as 'a pioneer work of its class' and of great value as bringing together in one volume a vast amount of information. A fifth edition, considerably revised and enlarged, appeared in 1961 and to date about 30,000 copies in all have been sold.

In the meantime, Kendrew, although well aware of the existence of several excellent works on the subject, compiled *Climate* (1930), a systematic account of the general principles of climatology treated mainly from a geographical and distributional point of view. A leading British meteorologist welcomed, *inter alia*, the valuable chapter on humidity and temperature in relation to the human body and thought the volume very readable and probably the best and then most up to date of its particular scope and purpose. What was virtually an extensively rewritten third edition appeared as *Climatology* in 1950 and was revised in 1957.

On retiring from Oxford, Kendrew worked for some years in Canada for the Government, in rewriting for general use the detailed reports by specialist investigators of climatic conditions in parts of that country. In 1955 the results, *The Climate of Central Canada* (with B. W. Currie) and *The Climate of British Columbia and the Yukon Territory* (with D. Kerr), evoked warm appreciation for the readily available data, the condensation of a vast mass of statistics, and the undoubted suitability for the general public of the illuminating and invitingly readable narrative.

Kendrew will long be remembered as an outstanding advocate and exponent of climatology, but his practical qualities should not be ignored. From 1935 until 1950 he directed the Radcliffe Meteorological Station at Oxford and he was a life-long connoisseur of weather and skyscapes. Partly in pursuit of climatic experience, he travelled widely, and for him there was always, besides meters and gauges, the human recorder, if possible in the form of Wilfrid George Kendrew.

R. P. BECKINSALE

NEWS and VIEWS

Molecular Biology at Cambridge

H.M. THE QUEEN opened the Medical Research Council's Laboratory of Molecular Biology, Cambridge, on May 28. The Laboratory, which is situated about two miles from the centre of Cambridge, has been built in association with the new accommodation for the University Department of Radiotherapeutics on the site of the new Addenbrooke's Hospital in Hills Road, and these buildings also were opened by the Queen. The Medical Research Council were represented at the ceremony by its chairman, the Right Hon. Lord Shawcross, and secretary, Sir Harold Himsworth.

The Council's Laboratory provides new permanent accommodation for the staff of the former Molecular Biology Research Unit, which was set up in 1947 in the Cavendish Laboratory at Cambridge, and has made outstanding contributions to knowledge about the structure of proteins and nucleic acids and in the field of molecular genetics. In the new Laboratory, the Unit's research workers will be joined by others who have been working in the same field. These will include the team led by Dr. F. Sanger, whose work on the chemical structure of insulin gained him a Nobel Prize in 1958, and who has until now been a member of the Council's staff at the School of Biochemistry, Cambridge, and by another member of its staff, Dr. H. E. Huxley, formerly at University College, London, who is well known for his discovery of the mechanism of muscular contraction. Dr. J. D. Smith, at present at the Biological Division of the Californian Institute of Technology, distinguished for his work on the chemistry of nucleic acids, and Dr. A. Klug and his virus research group from the Physics Department at Birkbeck College, London, will also be joining the Laboratory. The Laboratory will be under the general direction of a small committee, composed of the heads of divisions, under the chairmanship of Dr. M. F. Perutz. There will be three main divisions: (*a*) Structural Studies under Dr. J. C. Kendrew; (*b*) Molecular Genetics under Dr. F. H. C. Crick; (*c*) Protein Chemistry under Dr. F. Sanger.

U.S. National Bureau of Standards :
Dr. J. A. Simpson

DR. JOHN A. SIMPSON has been appointed chief of the Electron Physics Section at the National Bureau of Standards, U.S. Department of Commerce, in succession to Dr. L. L. Marton, new chief of international relations. Born in Toronto, Ontario, in 1923, Dr. Simpson received his secondary education in Stratford, Conn. He attended Lehigh University in 1940 under the New England Regional Scholarship. After serving in the Army Signal Corps during the War, he returned to Lehigh, receiving his B.S. in engineering physics in 1946 and his M.S. in physics in 1948. He then joined the staff of the National Bureau of Standards, where he has conducted research in electron physics with special emphasis on instrumentation. His dissertation for Ph.D. at Lehigh University in 1953 was based on his work on the electron interferometer. He has also published on electron optical schlieren problems and on the characteristic energy-losses of electrons in metals.

Nuclear Tests and the World Federation of Scientific Workers

THE president of the World Federation of Scientific Workers, Prof. C. F. Powell, has issued a statement calling for the stopping of all nuclear tests and for further expert assessment of the possibility of identifying tests. The Federation, which links 200,000 scientists in 32 countries, expresses grave concern at the resumption of nuclear tests in the atmosphere and does not understand how the possibility which still remains of concealing the smallest underground nuclear tests can be rationally used to justify the resumption of atmospheric tests on the largest scale which undoubtedly cannot be concealed. The statement asserts that the resumption of atmospheric tests gravely prejudices the possibility of progress in general disarmament; facilitates the acquisition of nuclear weapons by powers not at present possessing them; constitutes a biological hazard through dangers from fall-out; and is likely to lead to a further deterioration in international relations and an intensification of the cold war.

The Federation welcomes the initiative of the representatives of the neutral powers at the Geneva Conference in suggesting a compromise solution to the differences between the Anglo-American and Soviet positions on nuclear tests. In view of recent advances claimed in the identification of small underground explosions, the Federation calls, as an urgent measure, for the establishment at Geneva of another conference of scientific experts to assess the present position with regard to the detection of nuclear tests, using only national detection systems supplemented by international monitoring stations on the territories of uncommitted powers willing to accommodate them. Pending the presentation of the report of such a conference, the statement urges all nations to refrain from further nuclear tests of any description. The statement has been sent to heads of States already possessing nuclear weapons, to the United Nations, and to the heads of all Delegations to the Geneva Conference on Disarmament.

U.S. Projects on Economic and Social Implications of Science and Technology

Current Projects on Economic and Social Implications of Science and Technology, 1961, issued by the National Science Foundation, is the third in a series of annual surveys of projects relating to some aspect of social-science research on science and technology (NSF-62-4. Pp. vii+116. Washington, D.C.: Government Printing Office, 1962. 40 cents). The surveys, which are based on investigators' reports of their projects, supplement the Foundation's programme of statistical studies. Of the 262 projects listed, only 40 are financed solely by the investigator and more than 200 have full or partial financial support outside the Foundation. The projects are classified under 14 subject headings and there are indexes of authors and of institutions.

Industry in Katanga

A COLLECTION of papers on the industries of Katanga has recently been published in French by the State University of Elisabethville (Publications de l'Université de l'État à Élisabethville. 2: *l'Industrie Katangaise—Réalisations et Perspectives*. Pp. vi+404. Elisabethville: Université de l'État à Élisabethville, 1961). These were read at a conference held last July in connexion with the celebrations of the fiftieth anniversary of the foundation of the City of Elisabethville, and together they provide an authoritative and detailed survey of practically all aspects of the country's economy, including transport, electricity production and transmission, agriculture, mining, the metal and chemical industries, building, and the food industries. In a preface, the Rector of the University (M. J. Frenkiel) points out that, in the older countries, collaboration between universities and industry has had very beneficial effects on both parties, and in particular it has been especially helpful to small and medium-sized firms which cannot afford to provide their own research laboratories. Similar collaboration, he emphasizes, is even more valuable in a young country such as Katanga. A concluding paper on the principal characteristics, structure and prospects of Katangan industry by Prof. René Clemens and M. Grosjean, of the University of Liège, provides thoughtful comment not only on the specific problems of Katanga's economy but also, more generally, on problems of economic progress in under-developed countries. The authors of this paper stress the importance of the formation of capital, the encouragement of private investment and the development of a spirit of enterprise receptive to modern technical and commercial methods among workers conditioned in the past to traditional modes of life.

Adult Education in Britain

THE report of the Universities Council for Adult Education for the academic year 1960–61 refers to some studies of the character of students made in Nottingham, Newcastle and Leeds (Pp. 24. Liverpool: Universities Council for Adult Education. 1962). The Nottingham survey indicated that students were distinguished chiefly by their minority interests and were drawn predominantly from the professional middle class. Leeds reported a trend for women students to outnumber men considerably, and also that 43·1 per cent of students in grant-aided extension courses had received full-time education up to training college or university, while a further 31·7 per cent had stayed at school beyond the minimum leaving age. Extra-mural departments have arranged a growing number of courses for specific vocational groups, some of these being at post-graduate level and often in close collaboration with internal university departments. The Younghusband report has stimulated consideration of the part to be played by university extra-mural departments in the education and training of social workers, and, besides refresher courses of a similar type, departments have undertaken courses to meet special needs, including courses for Children's Officers, hospital administrators and officers of the National Assistance Board. Particular reference is made to the development of day-release courses, including liberal studies, and stress is laid on the importance of teaching being given by full-time university tutors. Besides the four weeks course for managers in industry arranged by the University of Cambridge at Madingley Hall, the "Background to Management" courses in Manchester continued and there are three established seminars for business men in Birmingham, while Edinburgh continued its seminars for management and at Sheffield an evening course in industrial relations led to a request for a course for management staff inside a factory. A survey of science in extra-mural studies in the Bristol area indicated that science represented 26 per cent of the programme in 1960–61 compared with 12 per cent 10 years ago. Eight departments referred particularly to local history, and in Belfast and Scotland language teaching has developed notably, for example, as a medium for studying a modern country. While there is evidence of the particular value of residential centres, non-residential centres still have their place and the importance of proper centres is emphasized, as is the need for more full-time tutors.

Sugar Beet Farming in Britain

IN replying for the Government in a debate on May 2 on a motion to annul the Sugar Beet (Research and Education) Order, 1962, the Joint Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, Mr. W. M. F. Vane, welcomed the debate as directing attention to the valuable research, covering diseases and sugar content, in which the Imperial College of Science and Technology and the University of Nottingham, as well as Rothamsted Experimental Station and other research stations, had participated. The industry had taken the initiative throughout, and between 1942 and 1961 the average yield of beet per acre had risen from 9·4 to 13·4 tons, while virus yellow had become much less of a menace and methods of control had improved. Disease and fertilizer experiments previously carried out by the Dunholme Field Station, Lincolnshire, of the Rothamsted Experimental Station were being transferred to a new field station at Broom's Barn, Higham, near Bury St. Edmunds. This would be a permanent station with more opportunities for research, which would be concentrated mainly on problems concerning sugar beet. The Order required the Minister in framing the research programme to consult the Sugar Beet (Research and Education) Order, which since 1942 had worked well. Mr. Vane said that the £14,000 going to the plant breeding station at Cambridge and other items in the Schedule were financed by the levy authorized by the Order, and the motion was withdrawn.

The Zoological Society of London: Annual Report

IN its annual report for 1961 (Pp. 56+8 plates. London: H.M.S.O., 1962), the Zoological Society of London directs attention to the continuing excellence of the health and welfare of the animals in the collections, and the new high level of attendances at Whipsnade. Problems of buildings are also discussed. Each new house has its own problems of structure and design according to the strength, behaviour and best methods of display of the animals it has to shelter. There is also the æsthetic problem to contend with. One of the most complicated buildings now to be built is a new Small Mammal House. To allow adequate time for research, the Council of the Society has commissioned Prof. Misha Black, professor of industrial design (engineering) at the Royal College of Art, to design a building in which nocturnal, jumping, burrowing and flying mammals can be suitably exhibited and studied. Dr. I. W. Rowlands has been appointed Senior Research Fellow in reproductive physiology, and will be in charge of the Wellcome Institute of Comparative Physiology. Five other Research Fellows and two Research Students in the physiology of animal reproduction were appointed during the year. These appointments have been made possible by the grant from the Ford Foundation.

Awards

NEW annual awards to be presented by the Society for contributions to zoology are: the Prince Philip Prize, to be awarded to a pupil of zoology in any school in Great Britain, Northern Ireland, the Channel Isles, and the Isle of Man, on the basis of an account of some practical work in the field of animal ecology; the Stamford Raffles Award, commemorating the Founder of the Society, to be awarded to an amateur zoologist for contributions to zoology, or to a professional zoologist for contributions which extend beyond his field of specialization; and the Thomas Henry Huxley Award, to be awarded to a postgraduate research student in any university of Great Britain and Northern Ireland, for outstanding original work in zoology submitted as a doctoral thesis. The prize and two awards will consist of sculptures by Mr. Henry Moore. Further information can be obtained from the Secretary of the Society,

International Commission on Zoological Nomenclature

NOTICE is given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in *Bulletin of Zoological Nomenclature* (19, Part 3 to be published on May 28, 1962): (1) Validation of the generic name *Doto* Oken, 1815 (Gastropoda). *Z.N.*(*S.*) 1006. (2) Validation of the specific name *caricae* (*Cynips*) Linnaeus, 1762 (Insecta, Hymenoptera). *Z.N.*(*S.*) 1047. (3) Validation of the generic name *Lystrophis* Cope, 1885 (Reptilia). *Z.N.*(*S.*) 1484. (4) Designation of a neotype for *Gryllus campestris* Linnaeus, 1758 (Insecta, Orthoptera). *Z.N.*(*S.*) 1485. (5) Validation of the specific name *alveofrons* (*Dasiops*) McAlpine, 1961 (Insecta, Diptera). *Z.N.*(*S.*) 1492. (6) Designation of a type-species for *Pisidia* Leach, 1820, and suppression of the specific name *istrianus* (*Cancer*) Scopoli, 1763 (Crustacea, Decapoda). *Z.N.*(*S.*) 1496. (7) Validation of the generic name *Stereomastis* Bate, 1888 (Crustacea, Decapoda). *Z.N.*(*S.*) 1497. (8) Suppression of the generic name *Arctopsis* Lamarck, 1801, and the specific names *lanata* (*Arctopsis*) Lamarck, 1801, and *tribulus* (*Cancer*) Linnaeus, 1767 (Crustacea, Decapoda). *Z.N.*(*S.*) 1498. (9) Validation of the generic name *Ophiophagus* Günther, 1864 (Reptilia). *Z.N.*(*S.*) 1500. (10) Suppression of Dahl's "Coleoptera und Lepidoptera", 1823. *Z.N.*(*S.*) 398. (11) Suppression of Kishida's pamphlet *Notes on the Family Trombidiidae of Japan*, 1909. *Z.N.*(*S.*) 400. Any zoologist who wishes to comment on any of the above cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7.

National Survey of Air Pollution

URBAN conditions do not necessarily preclude a high standard of purity of the air according to the Department of Scientific and Industrial Research Warren Spring Laboratory. Commenting on measurements of air pollution made in 1958–59, the Laboratory says that figures show that, even in large manufacturing towns, including London, the annual average of the smoke concentrations at certain sites was less than half the annual average for urban areas over Britain as a whole (about 200 millionths of a gramme per cubic metre of air). Sites in the centre of Wolverhampton and in Woolwich are among the places singled out for their freedom from smoke pollution. At a site in the centre of Wolverhampton, the average concentration during the year (expressed as millionths of a gramme per cubic metre of air) was 59; at a site in Woolwich it was 72, compared with an average of 219 over the whole of London. "Results such as these show the basic soundness of the policy of smoke-control areas. Improvements in one area are not nullified, though they can be affected, by smoke drifting in from neighbouring districts" (*Report of Warren Spring Laboratory* 1961—Report of the Warren Spring Laboratory Steering Committee and the Report of the Director of Warren Spring Laboratory for 1961. Pp. vi+34+8 plates. London: H.M.S.O., 4*s.* net, 1962). One in four of the towns in the North of England where measurements were made in 1958–59 had annual average concentrations of more than 300 millionths of a gramme of smoke per cubic metre of air. To obtain a really comprehensive picture of the distribution of air pollution throughout Britain, the Warren Spring Laboratory is organizing a National Survey of Air Pollution, with daily smoke and sulphur dioxide measurements being made at more than 1,000 sites in all parts of Britain, including a statistically chosen representative sample of towns of all sizes in England and Wales. Many of the local authorities co-operating in this Survey are already taking measurements and others are installing apparatus.

Guide to Careers

IN this year's edition of the National Union of Teachers' *Guide to Careers for Young People*, the bulk of the contents are devoted to information about particular careers and fields of employment, rather than to advice about aspects of careers guidance, preparation for employment and the sources of careers information (Pp. 84. London: National Union of Teachers. 3*s.* 6*d.* 1962). This is an experiment, undertaken after considering suggestions from a number of schools which use the *Guide*. There is an even greater need for this *Guide* now than there was at its inception. The emergence into employment of the children of the 'bulge' years means that there are many more young people to inform and advise about careers. An even more compelling indication of the need is the situation revealed by two recent surveys conducted among young people in their first year of employment. These have provided evidence of young girls and boys obtaining jobs generally bearing little relation to the jobs they aimed at, or being far from clear about what work was entailed in different occupations, of employers not giving any details of job offered, or giving inaccurate information or misusing the term 'apprenticeship'. No one concerned with the task of passing on careers information, least of all careers masters and careers mistresses, can be satisfied with this state of affairs. For individual children these surveys point to unhappiness, frustration, restlessness, smouldering discontent; for a nation which needs to utilize all the talent and skill in its citizens, they reveal untapped potential and wasted and half-used resources. The *Guide* should play a part in ensuring that the boys and girls shall be better prepared for their future employment.

British Section of the Society of Protozoologists

At the first meeting of the newly formed British Society for Parasitology, which was held at Cambridge during April 3–5, twenty-six protozoologists met to establish a British Section of the Society of Protozoologists. The first meeting of the Section will be held in the Department of Zoology, University College, London, on October 20. All those who are interested in Protozoa are invited to attend. The temporary administrative committee consists of: Mrs. K. M. G. Adam, Department of Zoology, University of Edinburgh; R. J. Cole, Department of Zoology, King's College, London; K. Vickerman, Department of Zoology, University College, London, from whom further information can be obtained.

The Institution of Mining and Metallurgy: Awards

The following awards have been made by the Institution of Mining and Metallurgy: *The Gold Medal of the Institution for* 1961, to Prof. F. A. Forward, in recognition of his contributions to the development of metallurgical processes, particularly that of pressure leaching; *Honorary Membership of the Institution*, conferred on Prof. W. R. Jones, past-president, in recognition of his distinguished services to the science of economic geology and to the Institution; *'The Consolidated Gold Fields of South Africa Limited' Gold Medal* for the session 1960–61, to K. C. G. Heath, for his paper entitled "Mining and Metallurgical Operations at Rhodesia Broken Hill—Past, Present and Future" (*Trans. Inst. Min. Metal.*, 70); *'The Consolidated Gold Fields of South Africa, Limited' Premium of Forty Guineas* for the session 1960–61, to Dr. J. A. E. Allum, for his paper entitled "Photogeological Interpretation of Areas of Regional Metamorphism" (*Trans. Inst. Min. Metal.*, 70).

Lederle Medical Faculty Awards

The Lederle Laboratories Division of the American Cyanamid Company is making available a number of medical faculty awards which will begin with the academic year 1963–64. These awards are intended only for individuals who share in all departmental responsibilities, particularly in the teaching of undergraduate medical students. They are not intended to be research fellowships, or for the support of individuals whose only or major function and interest is the conduct or supervision of research activities. They are also not intended for assistance of research departments or divisions of postgraduate medical schools, but are primarily for assisting medical schools in providing for their major clinical disciplines individuals who, having demonstrated interest and ability in teaching and research, will devote their major efforts to the teaching of clinical medicine with the view of integrating the knowledge and principles of the biological sciences, as applied to man. Further information can be obtained from Lederle Medical Faculty Awards, Office of the Secretary, Pearl River, New York.

Spectroscopy in the Metallurgical Industry

A symposium on "Spectroscopy in the Metallurgical Industry", arranged by the Spectroscopy Group of the Institute of Physics and the Physical Society in collaboration with the British Non-Ferrous Metals Research Association and the British Iron and Steel Research Association, will be held in Buxton, Derbyshire, during July 5–6. The programme will include: X-ray fluorescence analysis of copper alloys; X-ray fluorescence analysis in metallurgical research; mass spectroscopy; spectrographic control of nickel alloy production; control analysis in a steel works; control analysis for stainless steel production; analysis of high silicon steels with a vacuum spectrometer; spectroscopy in the assay of precious metals; spectroscopy in the German metallurgical industry. Further information can be obtained from Dr. L. Bovey, Building 329, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

Announcements

The twelfth world poultry science congress will be held in Sydney during August 13–18. Further information can be obtained from the Ministry of Agriculture, Fisheries and Food, Whitehall Place, London, S.W.1.

The thirteenth session of the International Commission for Uniform Methods of Sugar Analysis will be held in Hamburg during August 26–31. Further information can be obtained from Dr. D. Gross, Tate and Lyle Research Laboratories, Keston, Kent.

The International Federation of Information Processing Societies Congress, 1962, is to be held in Munich during August 27–September 1. An exhibition of computers and other information processing will be held during the congress. Further information can be obtained from Dr. A. P. Speiser, IBM Research Laboratory, Zurichstrasse 108, Adliswil-Zurich.

The second international congress of radiation research, sponsored by the International Committee for Organizing Congresses of Radiation Research and the Association for Radiation Research, will be held in Harrogate during August 5–11. Further information can be obtained from Dr. Alma Howard, Mount Vernon Hospital, Northwood, Middlesex.

The 1962 Summer School in Infra-red Spectroscopy, organized by Unicam Instruments, Ltd., will be held in Cambridge during July 15–28. Residence will be in Gonville and Caius College, and practical tuition in sample handling and interpretation will be given in the laboratory of Unicam Instruments, Ltd., at the newly opened Scientific Centre. Further information can be obtained from Mr. D. E. Sheppard, Unicam Instruments, Ltd., York Street, Cambridge.

An interdisciplinary conference on "Electromagnetic Scattering", sponsored by the Division of Colloid Science and Surface Chemistry of the American Chemical Society and the Office of Aerospace Research of the United States Air Force, will be held in Potsdam, New York, during August 13–15. The programme will include the following sessions: scattering by spherical particles; scattering by non-spherical particles; scattering by charged species in solution; interactions in solids and liquids as determined by electromagnetic scattering; multiple and incoherent scattering. Further information can be obtained from Dr. Milton Kerker, Clarkson College of Technology, Potsdam, New York.

Erratum. In the article "Hazards of Dust", appearing on p. 244 of the April 21 issue of *Nature*, Prof. R. C. Browne reviews *The Relation between Pneumoconiosis and Environmental Conditions: an Analysis of the Results of the First Series of X-ray Surveys in the National Coal Board's Pneumoconiosis Field Research*. The authors of this report are D. Hicks, J. W. J. Fay, J. R. Ashford and S. Rae, and not the two latter only as printed in the review.

GAS CHROMATOGRAPHY

Recent Literature

PUBLISHED volumes on gas chromatography fall into four fairly well-defined classes. The first, proceedings of symposia, has become a well-established format for the publication of original papers in the field, and a higher proportion of the significant literature of the technique is concentrated in such volumes than in most other subjects. Compilations of abstracts form an extremely valuable small second class, providing a compact source of information on the technique. Thirdly, handbooks with a practical bias intended mainly for the beginner have been available since early in the 'fifties, the first, by C. S. G. Phillips, being followed by a succession of others which have, in general, kept well abreast of practical developments. It is in the last class of what can be called authoritative comprehensive texts that the technique is most obviously deficient. Keuleman's book is about the only representative of this latter group and even the second edition is, in many aspects, out of date. Six volumes recently published[1-6] reflect this distribution, including two symposia proceedings, one compilation of abstracts, two handbooks and a text on theoretical aspects, unfortunately, at present, only available in German.

The publication of papers presented at symposia in the form of a bound proceedings rather than in the normal scientific journals would seem to me to be justified only on the basis that they appear quickly as a compact group, and that the generally valuable and interesting discussion which follows their presentation is included. Unfortunately, the proceedings of the Second International Symposium organized by the Instrumentation Division of the Instrument Society of America[1] meets neither of these two requirements, its publication having been delayed more than eighteen months after the symposium and even then it is without the discussions, which had been included so effectively in the proceedings of the first symposium. The increase of nearly 50 per cent in price over the latter is also somewhat surprising.

These deficiencies are even more regrettable as the thirty-four papers presented cover almost every aspect of the technique. There are fewer papers of a theoretical character than might have been expected, but a treatment of band broadening effects in large columns by M. J. E. Golay should provide a useful basis for the improvement of preparative scale columns.

Many new developments in apparatus and technique are described with some emphasis on ionization detectors, which were still something of a novelty at the time of the meeting. Unfortunately, neither the improved Ryce and Bryce ionization detector nor the radio-frequency discharge detector, which are described in some detail, seems since to have achieved the reliability and sensitivity of the hydrogen flame or argon ionization detectors. An attempt is made to clarify the difficult question of comparing detector performance on a general basis. Both high-temperature operation and temperature programming are discussed and the use of column back-flushing illustrated. Column packings receive some attention both from the point of view of the influence of the support and for achieving specific separations. Among the latter, the use of liquid-modified adsorbents to separate low molecular weight olefines and the resolution of permanent gas mixtures with molecular sieves and active carbon are noteworthy. Several papers deal with the problem of identification of peaks using, in addition to retention data, such methods as micro-chemical tests and mass spectrometry. Useful ancillaries, such as an automatic fraction cutter and a precision integrator, are described.

Applications include process monitoring and control, the water content of hydrazine, the analysis of ortho-para hydrogen and the determination of dissolved oxygen in petroleum liquids. Illustrations of the use of a commercially available apparatus for preparative scale work are also given.

Finally, on a lighter note: all aspiring pioneers in science should read the after-dinner comments by A. T. James on his association with A. J. P. Martin which resulted in the discovery and early development of gas-liquid chromatography. This should be prefaced by a perusal of Martin's account in the proceedings of the first symposium, given in similar circumstances.

The proceedings of the third symposium organized by the Society for Analytical Chemistry and the Gas Chromatography Discussion Group[2] contains the twenty-nine papers presented at that symposium together with the discussion which followed them. The proceedings are divided into three sections, entitled "Apparatus and Technique", "Theory and Application" and "General Application", each prefaced by an introductory lecture presented by well-known workers in the field.

In introducing the first section, R. P. W. Scott outlines most of the aspects of the technique which still require development or clarification. Several useful papers on detectors follow with interest centred mainly on the argon and flame ionization detectors. A novel method of producing very low concentrations of volatile substances in permanent gases by diffusion, for the calibration of the latter, is described. An improved integrator provides a versatile facility for automatic quantitative interpretation of chromatogram and, for rapid elution, details of apparatus by which the elution curve can be displayed repetitively on a large cathode-ray tube are given.

M. J. E. Golay introduces the second section with a stimulating discussion of the future prospects of the capillary columns he originated and of the trends in the development of large columns for preparative purposes. Several other papers also describe recent advances in these two fields. The limitations of organic stationary phases at high temperatures and methods for assessing their performance are covered.

Three other important contributions are concerned with an ingenious application of Doering's methylene insertion reaction to provide retention data for unavailable hydrocarbons, the use of transition metal complexes to separate unsaturated compounds and a demonstration of the feasibility of using clathrate complexes for separations according to molecular shape.

An interesting account of work in the U.S.S.R., mainly on gas–solid chromatography, by A. A. Zhukhovitsky, opens the last section on applications. These cover a very wide range from the separation of metal halides to anæsthetic research.

As with previous proceedings in these series, the book is well produced, and the editor is to be congratulated on its early publication and particularly for making the considerable effort to include an author- and subject-index. The steady increase in the cost of these volumes is regrettable.

The third compilation in the series, *Gas Chromatography Abstracts*, maintains well the high standard previously achieved. The volume contains 875 abstracts of papers which appeared in 1959 and the early part of 1960. The abstracts are usually short and compact, but quite adequate in most cases to decide what papers in the rapidly expanded literature on the technique are worth a more detailed study. By far the most valuable feature of these volumes, however, is the elaborate index which enables references on even the most obscure subject to be found if they exist.

The editor and his abstractors are to be congratulated for continuing to provide so consistently such an excellent service for all those interested in gas chromatography and its application.

Gas Chromatography[4], by Ernst Bayer, is an extremely useful laboratory hand-book for all those engaged in working with this fascinating technique. It seems to strike just the right balance between essential historical and theoretical background and the practical aspects with which it is, of necessity, largely concerned. The author is obviously one of the small number of enthusiasts who embraced the technique in its early stages and has since turned his hand to most of the new developments as they appeared. This outlook has, however, led him to cram too much material into too few pages, and although this is seldom confusing, the treatment of many topics is so brief that it often becomes irritating.

There is a wealth of information on almost every significant form of apparatus which has been used, but perhaps the most valuable section is that dealing with a wide range of practical applications. Small errors, many of which seem to spring from the translation, are fairly frequent; for example, on p. 37 the method of coating capillary column is vague if not misleading, and on p. 55 the term "ionization potential" is incorrectly used. These do not, however, seriously detract from the usefulness of the book as a handy source of information in the laboratory. Newcomers to the technique faced with the problem of getting on with the job as soon as possible will find it invaluable, but even the old hands will find it useful to have on the shelf.

Dr. D. Ambrose and Barbara A. Ambrose have prepared a volume[5] that is an interesting contrast to Bayer's book. The authors have set out to provide a compact balanced primer for the beginner, and to this end they have carefully selected the essential features of the techniques and discussed them with just sufficient material to make the treatment clear but not confusing. For this reason the book is interesting to read at a sitting in contrast to Bayer's book, which is best used to throw some light on a particular problem when it arises.

The chapters on separation and selective solubility, and qualitative and quantitative analysis are particularly good, but on the apparatus side it is a little disappointing that more emphasis has not been given to capillary columns and ionization detector, two of the more important recent developments. The text has not been cluttered by references to all the relevant papers, but a list of suggested items for further reading is included at the end of each chapter. It is a happy thought to include as appendixes a guide to the choice of commercial equipment and a list of addresses of manufacturers of complete instruments and ancillary apparatus.

The book is well produced with very clear diagrams, and its moderate price should encourage its appearance on the bookshelves of most laboratories in addition to its obvious place in chemical libraries.

Theoretische Grundlagen der Gas Chromatographie[6], by Von Géza Schay, is at present available only in German and therefore will be of restricted interest. Prof. Schay has attempted to fill a notable gap in the literature of gas chromatography by standing back, after the initial period of hectic development has subsided, in order to take a fresh look at the technique and in particular the basic physical chemistry on which it rests.

Not unexpectedly, there is a pronounced bias for gas–solid interactions and the gas chromatographic techniques which take advantage of such adsorption. The treatment of adsorption isotherms and the frontal analysis, displacement and chromothermographic procedures is excellent and will be stimulating to many workers in Britain less familiar with developments in gas–solid chromatography. On the other hand, the chapters dealing with the elution techniques on gas–liquid chromatography are less impressive, and the sections on capillary columns and ionization detectors are somewhat disappointing. An assortment of applications is given in the last chapter, but these seem out of place in a book of this type and could well be omitted in favour of an expansion of the chapters on gas–liquid chromatography and general techniques if an English edition is contemplated.

D. H. Desty

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[1] *Gas Chromatography*. Second International Symposium held under the auspices of the Analysis Instrumentation Division of the Instrument Society of America, June, 1959. Edited by Henry J. Noebels, R. F. Wall and Nathaniel Brenner. Pp. xvi+463. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 16 dollars.

[2] *Gas Chromatography 1960*. Proceedings of the Third Symposium organized by the Society for Analytical Chemistry and the Gas Chromatography Discussion Group of the Hydrocarbon Research Group of the Institute of Petroleum, Edinburgh, 8–10 June, 1960. Edited by R. P. W. Scott. Pp. xvii+466. (London: Butterworth and Co. (Publishers), Ltd., 1960.) 95*s*.

[3] *Gas Chromatography Abstracts 1960*. Edited by C. E. H. Knapman, assisted by C. G. Scott. Sponsored by the Gas Chromatography Discussion Group (Associated with the Hydrocarbon Research Group of the Institute of Petroleum.) Pp. xi+200. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 42*s*.

[4] *Gas Chromatography*. By Ernst Bayer. (Elsevier Monographs: Chemistry Section, No. 10.) Pp. xii+240. (Amsterdam: Elsevier Publishing Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 25*s*.

[5] *Gas Chromatography*. By Dr. D. Ambrose and Barbara A. Ambrose. (Newnes Practical Science Books.) Pp. vii+220. (London: George Newnes, Ltd., 1961.) 40*s*. net.

[6] *Theoretische Grundlagen der Gaschromatographie*. Von Géza Schay. (Physikalische-chemische Trennund Messmethoden, Band 3.) Pp. viii+267. (Berlin: Veb Deutscher Verlag der Wissenschaften, 1960.) 29.80 D.M.

Gas Chromatography Discussion Group

THE annual general meeting of the Gas Chromatography Discussion Group of the Institute of Petroleum Hydrocarbon Research Group was held in the Glazebrook Hall of the National Physical Laboratory on March 16 and was followed by an informal symposium attended by about two hundred members and guests. The report of the Committee for the past year records that the membership of the Group is now approaching five hundred, and the weight of work which this growth has imposed on the honorary officers has led the Committee to look favourably on an offer by the Institute of Petroleum to assume responsibility for a large part of the routine administration of the Group. On the recommendation of the Committee the meeting agreed that this offer should be accepted. Besides the two informal symposia, and preparations for the international meeting in Hamburg, the main activity of the Group during the past year has been the preparation of *Gas Chromatography Abstracts*, which is distributed quarterly to members; the bound volume for 1960 has been available for some time and that for 1961 will be published shortly.

Introducing the informal symposium which followed, Dr. J. S. Anderson, director of the National Chemical Laboratory, welcomed the meeting to Teddington and made some monitory remarks on the organization of scientific societies. In the first paper Dr. G. A. P. Tuey gave details of the improvements made in the preparative apparatus described at the Amsterdam symposium by Atkinson and himself. Metal columns had been substituted for the glass ones used originally, since the use of an all-metal apparatus made the elimination of leaks easier; Ermeto compression type joints had proved satisfactory. The 1-m. by 1-in. diameter columns were joined by capillary U-tubes which served the same purpose as the mixing washers recommended by Golay for use in large columns. A katharometer designed for analytical use had proved unsuitable as a detector because of the peak reversal which occurred at high flow-rates; attempts to pass part only of the effluent through the katharometer were also unsatisfactory because the proportioning did not remain constant for all peaks, and in addition the throttle was liable to cause condensation of vapour as a result of adiabatic cooling and consequently an unsteady base-line. The best form of katharometer for this application was one with transverse sensing wires able to accept the total gas flow, such as had been used in the University of Birmingham. It appears from various authors that, with appropriate sample size, an H.E.T.P. of 3–5 mm. may be obtained in columns up to 50-mm. diameter. Recent work on H.E.T.P. as a function of flow-rate in these preparative columns somewhat surprisingly yielded S-shaped curves, of which one limb tended towards the origin at low rates of flow, in place of the normal U-shape. When the results were used to compare the parameters H.E.T.P., Resolution and S (suggested by Purnell), the last was found to be independent of sample size and was therefore considered a better parameter than the other two for characterizing a column. In discussion Dr. H. Boer and Mr. D. H. Desty emphasized the value of the cross-section detector in preparative work because it had a linear response up to almost 100 per cent vapour. In reply to a question, Dr. Tuey stated that the highest sample recovery was obtained by scrubbing the gas with a solvent or a reagent; an electrostatic precipitator was liable to cause chemical change.

Dr. J. N. A. Ridyard discussed the linearity and response of the flame ionization detector. The upper limit of linearity is generally given as 10^{-8} amp., corresponding to a mean flow of about 2 μgm./sec. for a 1-mgm. sample on an ordinary analytical packed column. Ridyard investigated the effect of varying the operating conditions and used a computer to derive contour maps of the response at 1 and 10 per cent non-linearity as functions of the hydrogen and nitrogen flow-rates, the height of the probe above the jet and the diameter of the jet. There was generally an optimum position for the probe and a longer linear range was obtained if the probe was negative with respect to the jet. There were optimum rates of flow of both hydrogen and nitrogen, and this last point was confirmed in discussion by Dr. R. Kaiser.

Dr. E. Heine described the use of packed capillary columns, an interesting innovation in technique which is suitable for the separation of low-boiling hydrocarbons. The columns are made of glass drawn out in the usual way in the machine described by Desty. The tubing (2-mm. internal diameter) which is used, however, has first a clean wire 1-mm. diameter placed in it centrally and the annular space is then packed with powdered adsorbent (alumina, 0·1–0·15-mm. diameter, prepared by baking at 400° C. for 9 hr.) so that as the column is drawn the adsorbent moves with the glass and sticks to the inner wall of the capillary, which is about 0·3 mm. in diameter. Before use the column is flushed with dry hydrogen for a day at 120°. Heine showed chromatograms of high-speed separations of gaseous mixtures which he had obtained on these columns; in one example, obtained on a 2-m. column at 90° C., complete separation of the ethane/ethylene and propane/propylene peaks had been obtained; in another, 15 saturated and unsaturated hydrocarbons up to C_4 had been separated at 80° C. The method of aqueous deactivation to adjust selectivity, suggested by C. G. Scott, could be used with these columns; if carrier gas containing a suitable concentration of water was passed into the column, equilibrium was reached in a few hours. On close examination irregularities in the packing were obvious, but despite this the column performance was good, presumably because the sequence of narrow gas passages and empty spaces between the grains allowed quick mass transfer between the phases. In discussion Mr. C. G. Scott commented that he had tried to make similar columns but found them too fragile because of the strains set up in the glass by the adhering particles of alumina; there is evidently some know-how in question here which remained unexplained to the meeting. Dr. A. Goldup spoke as a protagonist of liquid-coated capillaries and showed a chromatogram obtained on a 2-m. column, 0·002 in. diameter, coated with squalane, in which a gaseous mixture, similar to one quoted by Heine, had been separated in 5 sec. Mr. D. H. Desty mentioned his attempt to make a regularly packed capillary column by drawing and twisting wire.

Dr. T. R. Phillips, in describing the separation and analysis of the isotopes of hydrogen, directed attention to the possibility that the displacement technique might yet find a use in modern gas chromatography, since the method offers the advantages of greater capacity for a given-sized column and that the sample is not contaminated by carrier gas. This last statement appears somewhat paradoxical because it is generally put in the opposite sense in favour of elution chromatography. It may, however, be justifiable in respect of preparative gas chromatography since in this technique one of the grestest problems has been the separation of vapours at low partial pressures from large amounts of carrier gas, and it may be advantageous to avoid the use of a carrier in some circumstances. Phillips has separated hydrogen and deuterium on columns containing molecular sieve 5 Å. in a method which is effectively one of self-displacement. The order of elution is first hydrogen, second deuterium (the opposite to that obtained by Glueckauf and Kitt in their separations on palladium black) and the method is useful for removing small quantities of hydrogen from deuterium-rich mixtures. Up to 300 ml. of sample was passed into the column cooled in liquid nitrogen; the Dewar vessel containing the liquid nitrogen was gradually lowered and the sample itself displaced the hydrogen, which was then followed by the deuterium. At the end of each run the column was filled with deuterium and there was no need to remove this before admitting the next sample; there was thus no need for evacuation of the column or for a carrier gas. Tests showed that molecular sieve had no tendency to promote the formation of HD molecules.

Dr. P. G. Jeffery described the application of gas chromatography to the analysis of gases obtained from rocks and minerals. The rocks he had examined were mostly silicates and these yielded small quantities of carbon dioxide when boiled with dilute phosphoric acid. The reaction had been carried out in a 10-ml. flask on powdered samples of up to 1 gm., and the gas evolved was analysed on columns containing silica gel or activated carbon. The method enabled concentrations as low as 5 p.p.m. to be determined, or, on the other hand, enabled analyses to be made on very small samples, as, for example, when rocks had been hand-sorted under the microscope, if the concentrations were higher. The same method had been used for determining the carbon dioxide content and nitrous oxide content of monoethanolamine solutions. Attempts were also being made to determine the water which is present to the extent of 2–20 per cent in most rocks, either on the surface of the mineral grains, chemically combined, or as hydroxyl ions held by weak forces between the layers of minerals with sheet structures. The water was driven off by fusing the sample in a silica tube with a suitable flux (for example. a 50/50 mixture of sodium tungstate and borax glass) at temperatures up to 700° C. In early experiments the water had been allowed to react with calcium carbide, but later it was found more satisfactory to determine it directly on a polyethylene glycol column. The method was calibrated by using samples of rocks containing known concentrations of water; in fact it had proved difficult to ensure the liberation of all the water from the samples and there were side reactions which removed water. Other gases, for example, helium from pitchblende, had also been determined by heating rock samples, and in this way hydrogen, helium, argon, oxygen, nitrogen and carbon monoxide had all been obtained from silicate rocks. The volume of the gases was greater than the free space and their presence could only be accounted for by some kind of adsorption. The novelty of this application of gas chromatography aroused considerable interest and many in the audience were intrigued by Jeffery's claim to have found hydrocarbons (even though only to the extent of a few p.p.m.) in granite.

The last paper of the meeting was given by Dr. R. P. W. Scott on resistance to mass transfer in capillary columns. Golay derived an expression for H.E.T.P. in terms of solute and column parameters, but experimentally it has been found that values of H.E.T.P. are significantly higher than those given by the equation Khan suggested a theory to explain the difference in which he postulated another effect, the interfacial resistance to mass transfer. Scott had tried to determine the reality of this effect; the experiments and their mathematical analysis were designed to distinguish between the effects of all the factors taken account of in the Golay equation. A column consisting of 100 ft. of nylon capillary 0·020-in. diameter was used, and H.E.T.P.'s were determined at different film thicknesses (from zero upwards) and gas velocities for samples of n-heptane on dinonyl phthalate with argon as carrier gas. In this system Khan's factor was not found to be significant, but it might well be when a more polar solute is in question What did appear to be a significant cause of departures from the Golay equation was a factor associated with the wall of the column, presumably adsorption.

D. AMBROSE

VEB CARL ZEISS, JENA

GOLDEN JUBILEE OF WORK ON AIDS TO VISION

FOLLOWING a long period of scientific preparatory work the Zeiss Works at Jena organized, fifty years ago, the 'Ophthalmo'-Department and began the manufacture of ophthalmic lenses, including specific types of aids to vision as well as ophthalmological instruments.

This article reviews the development of that Department during the past five decades and the considerable extent to which Jena instruments and aids to vision were able to share in the world's progress in ophthalmology and in the sphere of ophthalmic optics.

Despite their manifold drawbacks, the flat forms of spectacle lenses—bounded by like surfaces on both sides—actually retained that form for more than a period of six hundred years; and this in face of the fact that meniscus-shaped lenses were suggested as early as in the eighteenth century and that time and again after 1800, W. H. Wollaston and L. J. Schleiermacher in 1842 directed attention to the theoretical importance of rotation of the eye. A few firms produced plano-lenses and, in a few instances, even meniscus lenses on the strength of empirical experiments and, for the correction of astigmatic eyes, spectacle lenses with toric surfaces. However, mercenary motives proved strong enough to override any of these beneficial suggestions, thus permitting the inefficient equilateral lenses and cylinder lenses to hold their own.

Ernst Abbe, who himself wore spectacles with equilateral lenses, was very dissatisfied with this state of affairs concerning spectacle lenses. Yet, overburdened with other Zeiss problems, his illness and early death prevented him from devoting his full attention to the solution of the problem.

Moritz von Rohr, shortly after Abbe's death, energetically occupied himself with the problem. Aided by the suggestive and advisory assistance of Allvar Gullstrand, the Swedish ophthalmologist and Nobel Prize winner, known for his outstanding mathematical and optical talents, this work was considerably promoted. M. von Rohr, H. Boegehold and A. Sonnefeld provided the geometro-optical preliminaries for starting the production of the well-known 'Punktal' lenses of Carl Zeiss, Jena, and other aids to vision associated with them, including Katral lenses, telescopic spectacles, etc. (Although now retired, H. Boegehold and A. Sonnefeld are still active with the Jena Zeiss Works in a scientifically advisory capacity.)

The new point-focal depicting Zeiss spectacle lenses were for the first time put on the market on April 1, 1912. Unparalleled, the Zeiss 'Punktal' lens found open markets thenceforth throughout the world and earned the unqualified recognition of opticians and spectacle wearers. From that time on, often despite considerable visual afflictions, the 'Punktal' lenses afforded visual relief and improved vision to millions of people by helping to restore former working capacity up to old age. Rational and optically highly developed production methods initiated by R. Lincke provided a guarantee that, even on large-scale production, spectacle wearers could be sure of obtaining first-choice Zeiss spectacle lenses of an invariably high quality.

The Jena Zeiss Works also carried out pioneer work on spectacle frames ('Perivist' spectacles) and of specific types of visual aids (telescopic spectacles, reflector spectacles, contact lenses). After 1945, the Jena firm furthermore succeeded in crystallizing the scientific work on the aids to vision by the production of spectacles for kyphotic wearers, of 'Isopal' lenses, anterior-chamber lenses, corneal contact shells, etc. Work is thus still being carried out towards providing the spectacle-wearing public with still better and perfect aids.

Within the same fifty-year period (also as a result of Gullstrand's suggestions and of his co-operation with M. von Rohr), ophthalmologists were provided with new instruments, under the supervision first of O. Henker and, later on, of H. Hartinger and F. Fertsch. The new instruments rapidly became internationally recognized and elicited many professional suggestions, which again resulted in the establishment of close contacts with eminent ophthalmologists throughout the world and in the design, as a consequence, of other new instruments for professional purposes. The 'Gullstrand' slit-lamp, built and progressively modernized in Jena, may be said to represent an indispensable asset in any ophthalmological consulting room. Further instruments which originated in the Jena Zeiss Works include the following: 'Vertex' refractionometer for measuring the refractive power of spectacle lenses; retinal camera, for the photographic reproduction of the fundus oculi; projection perimeter, for examining the visual field ; coincidence refractionometer, for measuring imperfections in the refractive powers of the eye.

While in pre-war times Zeiss ophthalmological instruments held what may be claimed to be a monopoly in the world's markets, new production centres sprung up in many other countries afterwards and afforded to the Zeiss Works an opportunity of proving the worth of their instruments in sound competition. VEB Carl Zeiss, Jena, as a consequence, set up a production programme which practically covers the whole latitude of this particular class of instruments. In pursuance of this programme the Jena firm also developed the new retinal camera 'Retinophot', which permitted the colour-photographic reproduction of the fundus oculi, as well as the Hartinger coincidence refractionometer for measuring imperfections in the refractive powers of the eye, and the Hartinger recording adaptometer for determining the quantitative curve of dark-adaptation. The excellent performance of these instruments resulted in a considerable increase of production and exports to practically all countries in the world. Progressive research and development work justifies the hope that the VEB Carl Zeiss, Jena, will continue to contribute effectively towards the improvement of vision and will efficiently assist ophthalmologists and the optical trade in fighting eye diseases and visual defects.

The June number of the *Jena Review* will publish special articles concerning the fifty-year history of Jena aids to vision and ophthalmological instruments.

AUGUST SONNEFELD

SCIENTISTS IN GOVERNMENT SERVICE

THE ninety-fifth report of the Civil Service Commissioners covers the period April 1, 1960, to December 31, 1961, and future reports will cover the calendar instead of the financial year*.

Much of the detailed information has been relegated to statistical tables which in the present report cover both 1960 and 1961. Recruitment to the administrative class of the Home Service and the senior branch of the Foreign Service is regarded as being specially encouraging: since 1955–56 the numbers of candidates competing and of recruits appointed, especially by Method II, have been rising, and, in 1961, 447 candidates competed by this method, of whom 38 were successful for the administrative class and 27 for the Foreign Service; the indications are that this will continue to be the more popular method. During the period the upper age-limit for candidates in the normal competitions for the administrative group of appointments was raised from 24 to 28, and the Commissioners, in conjunction with the Treasury, have reviewed the means of publicity used in the modern universities to bring Civil Service posts to the notice of students of good quality. Besides continuing to give serious consideration to the problem of attracting more competitors from universities other than Oxford and Cambridge, the Commissioners have given much thought to the nature of the examinations and administrative tests used for selecting candidates for the higher classes of the Service, but for the majority of candidates a general written examination still seems to be the most objective test of intellectual powers and capacity for self-expression, and, in conjunction with their records, the fairest way of selecting for interviews and group tests.

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* Report of Her Majesty's Civil Service Commissioners for the period 1st April, 1960, to 31st December, 1961. Pp. v+50. (Ninety-fifth Report.) (London: H.M.S.O., 1962.) 4s. net.

On the scientific and engineering classes, the Commissioners comment on the distribution of successful candidates among the older and the newer universities and over different parts of the United Kingdom, no university having a share greatly out of proportion to its numbers. While the Commissioners appear to have only moderate success in filling established posts which are notified to them, many of these posts are, in fact, occupied by people in a temporary capacity. This is not a disadvantage to the Scientific Civil Service, as the Zuckerman Report noted, and it is often preferred by the scientist. The recruitment of engineers appears to be less satisfactory although a small number of very good candidates has obtained professional qualifications through departmental student apprenticeship and other schemes. It must be several years before this source contributes significantly to the strength of the professional engineering class and meanwhile in the Works Group, including architects and surveyors, a large proportion of the basic grade vacancies remains unfilled. However, most of the vacancies for posts in grades above the basic level have been filled satisfactorily from competition.

Encouraging results were obtained following the decision to hold separate competitions for scientific staff for the Patent Office, 59 out of 75 declared vacancies being filled by this means in 1961.

Since June 1960 some of the larger departments have had authority to recruit up to half their entitlement of Research Fellows directly, and of 96 Research Fellows recruited in 1960 and 1961, 18 were appointed through departments. During 1961, 10 permanent appointments were made to the scientific officer class through the Interviewing Board, acting jointly for the Civil Service Commission and the Atomic Energy Authority, which visited Canada and the United States to see British scientists who desired to return to the United Kingdom, and 9 Research Fellows were also appointed.

BRITISH UNIVERSITIES DURING 1957–61

UNIVERSITY GRANTS COMMITTEE INTERIM REPORT*

ALTHOUGH the interim report of the University Grants Committee on University Development in the years 1957–61* was published five weeks after the Chief Secretary to the Treasury made his statement on March 14 regarding the recurrent grants for the next quinquennium, it may be assumed that the contents of this report were available to the Government when its decision was taken, and the Committee indeed expresses the hope that the record will be of assistance in considering the provision to be made for the quinquennium 1962–67. The present quinquennium has been one of increasingly rapid development in the universities: student numbers rose by 20 per cent in 1956–61 compared with 2 per cent in 1951–56, and staff by 18·4 per cent. Although total university expenditure rose by 61 per cent compared with 46 per cent and recurrent Treasury grants by 72 per cent, from £28·2 million in 1956–57 to £48·4 million in 1960–61, and non-recurrent grants from £9·1 million to £22·1 million, or 143 per cent, the universities have not been able to achieve all that was hoped for when the grants for the quinquennium were announced in 1957. Besides relatively greater expansion in the pure and applied sciences and in research, the continued decline in the value of money has been an important factor. Building programmes have steadily increased in size since 1957, and although supplementary recurrent grants for the last three years of the quinquennium were approved in 1959 and other supplementary grants to cover the cost of increases in academic salaries in 1957 and 1960 the strain on university finances has been great.

At the beginning of the quinquennium, the Committee's grant list included 21 universities and 3 university colleges; to these the University of Sussex, which first appeared in the grant list in 1960–61, has been added, and the University College of North Staffordshire is applying for a Royal Charter as a university, while authority has been given for the establishment of six more universities. By October 1961 the number of students had grown from 50,000 in 1938–39 and 100,000 in 1958–59 to 111,385 compared with the 102,000 visualized at the time of the quinquennial settlement. Omitting the University of London, with 22,574 full-time students, the Universities of Oxford and Cambridge with 8,800 and 8,934, respectively, and counting separately the two divisions of Durham, the five constituent colleges of the University of Wales, and St. David's College, Lampeter, at October 1961, three of these institutions had less than 500 full-time students; one, 500–999; one, 1,000–1,499; ten, 1,500–1,999; four, 2,000–2,999; three, 3,000–3,999; two, 4,000–4,999; and four, 5,000 and more. Of the 31,671 students entering for the first time in 1961–62, 8,433 were in science, 5,118 in applied science, 2,530 in medicine, 663 in dentistry, 560 in agriculture, and 255 in veterinary science; for 1956–57 the corresponding figures were 5,888; 3,842; 2,441; 692; 562; and 227, respectively. For the distribution of all full-time students by faculties the corresponding percentages were 25·2; 15·2; 11·2; 2·7; 1·8; and 1·1 in 1961; and 22·2; 13·9; 14·4; 3·0; 2·1; and 1·3 in 1956–57, respectively. The Committee is basing its authorizations for building plans on the assumption that this tendency will continue and that two-thirds of the increase in student numbers in the coming years will be in pure and applied science.

Once again the Committee reports that generally the proportion of outstandingly good and outstandingly weak students remained lower, while that of good second-class students was higher than it was before the War. A further inquiry in 1958–59 into the success or failure of students who entered universities in October 1955 to study arts, pure science, applied science or agriculture and forestry showed that the proportion who left without success on account of academic failure was 9·9 per cent, compared with 11·3 per cent in a similar study for October 1952. The proportion of full-time advanced students continued to increase: in 1960–61 they numbered 17,836 compared with 13,379 in 1956–57, and the proportion of higher degrees to first degrees in pure science was 21·4 per cent, and in applied science 19·6 per cent. Of 11,608 (or 10·8 per cent) full-time students taking courses of a year's duration or longer in 1960–61 coming from homes outside the United Kingdom (compared with 9,907 in 1956–57 and 5,213 in 1938–39) about 60 per cent came from within the Commonwealth, while the British Post-graduate Medical Federation had a further 2,013 students from overseas out of 2,994 taking courses of less than a year.

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* University Grants Committee. University Development: Interim Report on the years 1957 to 1961. Pp. 28. (Cmd. 1691.) (London H.M.S.O., 1962.) 1*s*. 6*d* net

There has been little change in the proportion of full-time students who live in colleges or halls of residence; although the numbers increased, from 24,652 in 1956–57 to 29,474 in 1960–61, the percentage remained 27·4. On the other hand, both the numbers living in lodgings, and the percentage, increased: from 41,291 in 1956–57 (46·0 per cent) to 54,614 (50·7 per cent) in 1960–61. Moreover, the University Grants Committee points out that the supply of lodgings for university students is becoming exhausted and in some places is likely to contract. The position varies widely, and in 1960–61, 53·4 per cent of full-time students at Cambridge and Oxford lived in colleges or halls of residence and only 1·9 per cent at home; for Scotland, the corresponding figures were 12·4 and 49·3, and for London, 18·3 and 27·9; for other institutions in England and Wales they were 27·6 and 17·2. Nor do these figures indicate the full extent of the variations, from the University College of North Staffordshire, where virtually all students are residential to Birmingham, or the Manchester College of Science and Technology, where almost all students live in lodgings or at home. Lack of capital admittedly has so far prevented the building of more halls of residence, but the University Grants Committee has already pledged fullest possible support for more ample provision for halls of residence in the coming quinquennium.

On academic staff this report merely records an increase in full-time personnel in teaching departments from 10,485 in 1956–57 to 12,921 in the autumn term, 1961; for professors, the corresponding figures are 1,459 and 1,684; for readers, assistant professors and independent lecturers, 757 and 926; for senior lecturers, 4,903 and 1,604; for lecturers, 4,903 and 5,959; and for assistant lecturers and demonstrators, 1,176 and 1.559. These figures do not, however, include staff of the rank of senior lecturer or below at Oxford and Cambridge. The report gives the rates of academic salary current in 1954, 1957 and 1960, but makes no comment beyond indicating that further proposals were submitted to the Chancellor of the Exchequer in July 1961 and that the Chancellor approved a recommendation for revision of the existing scheme for supplementing superannuation benefits which came into effect on April 1, 1960. Incidentally, in a written answer in the House of Commons on April 19, Mr. H. Brooke estimated the cost of the new scales for lecturers and assistant lecturers under the 3 per cent increase in the total salary bill for university teachers at about £1 million a year. At the same time he repeated his view that the university financial position should be reviewed in about two years time.

On income and expenditure generally, the report records an increase in the aggregate income of the universities from £41·656 million in 1956–57 to £67·467 million in 1960–61, of which 69·8 per cent and 72·7 per cent, respectively, was from Parliamentary Grants; 10·8 per cent and 9·4 per cent from fees; 4·0 and 2·8 per cent from endowments; 3·1 per cent and 2·0 per cent from grants from local authorities; 6·5 per cent and 8·9 per cent from payments for research; and 1·2 per cent and 0·6 per cent from donations and subscriptions. Earmarked grants presented a minor feature in the quinquennium, but rose from £11,000 in 1959–60 to £39,230 in 1960–61 and £36,250 in 1961–62; of the latter figure, £8,000 is a contribution to the Institute of Criminology, Cambridge; £12,000 for postgraduate courses in radiobiology at Birmingham; £8,000 for postgraduate courses in conservation studies at University College, London; £4,000 for developments in management studies at Bristol (with a further £1,250 for developments at Cambridge); and £3,000 for postgraduate courses for youth leaders at Manchester. Proposals for increased fees were put to the universities which have proved generally acceptable and will take effect in 1962–63.

Expenditure rose from £41·841 million in 1956–57 to £67·163 million in 1960–61. Of this, administration represented 7·0 per cent and 6·7 per cent, respectively; for salaries and superannuation of teaching staff the corresponding figures are 43·2 and 44·2, respectively; for departmental wages (technicians and laboratory assistants) 11·4 and 11·4; for departmental and laboratory maintenance, 13·1 and 13·8; for repairs and maintenance of buildings, 6·3 and 3·1; and for rates, insurance, heat, light, water, porters' wages, 6·5 and 9·8. Special reference is made to rates, discussion of which was necessitated both by the decision to exclude universities and colleges in receipt of Exchequer grant from the mandatory relief of 50 per cent recommended by the Pritchard Committee and which was given effect by the Rating and Valuation Act, 1961, and also by the uncertainty as to the basis on which university property should be valued. The report does not refer in detail to the test cases which have been proceeding but merely notes the decision that the universities should be reimbursed for their expenditure on rates during the next quinquennium (estimated at £1·5 million in 1961–62) by earmarked grants.

Of the non-recurrent grants totalling £92·363 million for the four years 1957–58 to 1960–61, £69 million was approved for buildings and professional fees, £18·7 million for furniture and equipment and £4 65 million for sites and properties, the actual expenditure for these purposes being £54 million, £10·8 million and £4·7 million, respectively. Of the total, 7 per cent was for buildings associated with arts subjects, 62 per cent for buildings for pure and applied science and 31 per cent for general service buildings such as libraries, student unions, refectories, halls of residence and the like. A major departure from the normal practice of recommending grants only for the initial furnishing and equipping of new accommodation has been in the authorization of provision of about £2 million over a period of years towards the cost of new electronic computers. With the contributions the universities themselves will make, this will ensure that in due course practically all universities will have their own computers and the one or two others will have access to computers. Besides the action taken in accordance with the recommendations of the Gater Committee and of Sir Arthur Rucker, the University Grants Committee has suggested to universities standards of accommodation of various types on which they can base their applications for grants for new accommodation, and has adopted new methods, based on cost analyses, of assessing grants for halls of residence and for science buildings, which encourage economy and flexibility. Finally, the Committee refers to the new arrangements put into effect as a result of criticism from the Public Accounts Committee, under which the Comptroller and Auditor General has full access to the records maintained by the Treasury for their control purposes. These arrangements were considered by the Committee of Public Accounts for the Session 1960–61 to have worked satisfactorily and the Committee recommended they should remain in force.

DIAMONDS CONTAINING CONTROLLABLE IMPURITY CONCENTRATIONS

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THE presence of considerable quantities of impurities in natural diamond has recently been confirmed[1,2]. This led Frank[3] to remark that multiple techniques of examination must be used on such material. In view of the report of Yoneda[4] concerning possible effects of nitrogen on the X-ray diffraction patterns of diamond, it seems worthwhile to us to comment further on some of the results which we have gained by the deliberate introduction of a given impurity into laboratory-grown diamond. We shall limit ourselves to a qualitative examination of the electron spin resonance spectra of a few specimens, in the belief that the profundity of the effects suffices to establish that progress in this area is now limited by the composition variability of natural diamond.

Samples of diamond were prepared in the following way. A uniaxial press system[5] was used in conjunction with an indirectly heated sample holder[6]. The reaction space was filled with extruded rods of spectrographic graphite powder mixed with the powdered transition metal catalyst. The static pressure applied during extrusion was 500 lb./in.^{2}. The impurity to be introduced into the growing diamond was present either in elemental form in the mix or as an alloy component with the transition metal. Thus, in the series of experiments reported here, pure nickel was used as the catalyst, and pure boron or aluminium powder was added in the requisite amount to the reaction mixture. All the materials used in the reactions were pre-fired in vacuum and cooled in argon. Cell assembly was carried out under dust-free conditions. Diamond was produced by subjecting these samples to pressures in the vicinity of 57 kb. and temperatures in the range 1,400°–1,600° C. (slightly different temperatures and pressures are utilized with the different catalyst compositions, since the equivalent alloys melt at different temperatures).

The product diamond was recovered by acid dissolution of the metal and residual graphite, followed by extensive washing. The crystals made using pure nickel consisted of attractive, light-green octahedra, whereas the aluminium-containing diamond was colourless (using a 33 atomic per cent aluminium in nickel mix) and the boron-containing material was a deep indigo (using a 5 atomic per cent boron in nickel mix). Both the boron- and aluminium-doped diamonds were electrical conductors. Resistivities were between 5×10^0 and 5×10^8 Ω-cm., depending on the conditions of preparation.

Doped diamond was also prepared by high-pressure, high-temperature diffusion of the doping elements into existing man-made diamond, both in the presence and absence of a transition metal catalyst. Lightly coloured or colourless conducting diamond was recovered from these experiments. The concentrations of boron and aluminium introduced by these techniques ranged up to 10^{21} atoms/c.c., as determined by chemical analysis, for example, by alkali titration of boron trioxide resulting from combustion.

Samples of these materials were examined by electron spin resonance spectroscopy. All v-mode, derivative spectra were obtained using a Varian Associates *V*-4500 ESR spectrometer, a *V*-4007 6 in. electromagnet, a *V*-4547 Multi-purpose cavity system and a Moseley *X–Y* recorder. Spectrometer frequency was maintained at 9152 ± 5 mc.p.s. (field calibration was obtained with *V*-4210*A* NMR spectrometer equipped with water sample). Low-temperature spectra were obtained by maintaining sample temperatures (measured with a thermocouple) to ± 2° C. in a flow of pre-cooled, dry nitrogen gas. Any possible radio-frequency saturation was checked by varying the klystron output over the available 20 decibel range, during which no changes in paramagnetic susceptibility or line contours were observed.

The electron spin resonance spectrum of our commercially available General Electric 'Man-Made' diamond 'RVG' (nickel-based) is shown in Fig. 1. There are two major features of interest: the broad band *a* (curve I) is typically that to be expected from transition metal impurities: we therefore assign it to nickel. The size of the absorption is consistent with the estimated concentration of nickel, namely $< \sim 10^{20}$ atoms/c.c. The detail *b* is shown on curve II with × 2 dispersion and × 5 amplification compared with curve I. The main structure of *b* appears to be

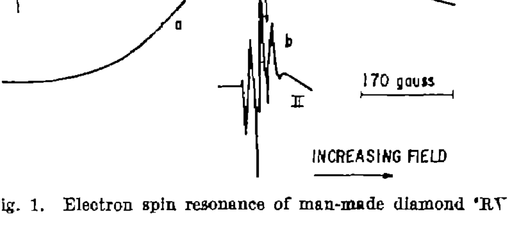

Fig. 1. Electron spin resonance of man-made diamond 'RVG'

a triplet, centred very close to a g-value (spin splitting factor) of 2·0: that is to say, whatever is producing b is behaving as though it possessed electrons of very nearly perfectly non-interacting spins with negligible spin-orbit coupling (it must be remembered that the specimen consists of many small crystals with random orientation in the sample tube).

Since weak fluorescence had been seen in this material, but not with nominally similar diamond of our Research Laboratory preparation, we examined the spectrum obtained from specimens in which the purity of the starting materials had been rigidly controlled. This showed the same major features as curve I; a specimen (3) consisting of material selected from their batch for its comparative clarity, showed less-developed resonance bands than that taken at random. Such triplets could conceivably arise from nuclear coupling with the two protons ($I = 1/2$) in $\cdot CH_2$, or nitrogen ($I = 1$). The second of these was already known to be a major impurity in natural diamond: the first was a possibility because residual hydrogen is known to cause poor diamond growth in the nickel system[7], or indeed to suppress growth completely if enough is present. Consequently, the radical $\cdot CH_2$ might be present in the crystals. Since some care had been taken to exclude hydrogen from the reactants, we considered the only possible source of hydrogen to be the water with which the sample holders[6] had been previously equilibrated. We therefore equilibrated six sample holders with deuterium oxide prior to preparing more diamond. The product from these runs, which was visually similar to the above specimens, was then examined by electron spin resonance. Once again the triplet detail (b) was seen. Had this previously been due to $\cdot CH_2$, we would have expected an expansion to a quintet due to the two deuterium atoms ($I = 1$) in $\cdot CD_2$. We therefore concluded the triplet b was due to substitutional nitrogen atoms in the diamond lattice. Further evidence was obtained by measuring the total width of the triplet (70 gauss) and the integrated intensity ratios of the lines (1 : 1 : 1). These values are consistent with the data of Smith, Sorokin, Gelles and Lasher[2]. Vacuum fusion analysis of a large sample of this diamond revealed a content of ~ 0·05 per cent nitrogen.

We next examined the diamonds prepared using an aluminium : 2 nickel catalyst (Fig. 3). The large wide band a in the nickel-based spectrum has disappeared (nickel–aluminium-based diamond is not strongly paramagnetic) and the detail at $g = 2·0$ is considerably reduced. Examination at higher gain showed that the latter was not exactly similar to the previous detail b, but poor signal-noise made it impossible to define the structure exactly. It seems likely that the detail is a highly suppressed nitrogen resonance. Some additional evidence in support of this view was obtained by examining nickel-based diamond into which aluminium had been introduced by high-pressure, high-temperature diffusion. The wide a band was lessened in conjunction with near extinction of the triplet b, indicating the considerable sensitivity of the nitrogen-atom resonance process to the presence of aluminium, or the loss of nitrogen from the crystal during incorporation of aluminium.

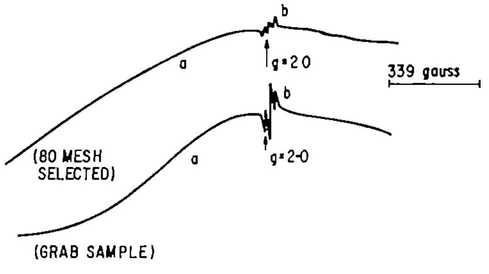

Fig. 2. Electron spin resonance of nickel-based diamond, laboratory preparation

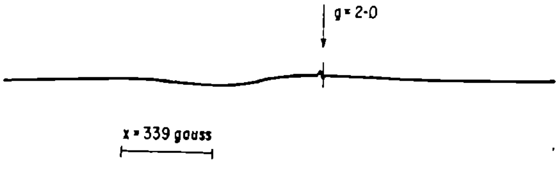

Fig. 3 Electron spin resonance of diamond from system using 2 nickel · aluminium catalyst

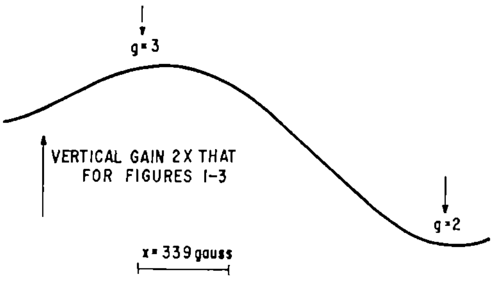

Fig. 4. Electron spin resonance of boron-doped diamond, growth-incorporated

Examination of boron-doped diamond (boron incorporated by growth) gave no sign of any absorption at $g = 2$ at room temperature, but a substantial broad resonance centred around $g \approx 3$ (Fig. 4). No hyperfine structure appeared when the specimen was cooled to 100° K., but the $g = 3$ band decreased in intensity, and a structure of unresolved detail began to appear at $g = 2$. We have no explanation to offer for these phenomena at present. It is apparent that the system is quite complex, since the nickel band is suppressed in these samples, but they are known to contain some nickel. Evidence for a nickel–boron electronic interaction was also derived from measurements on 'Man-Made' diamond into which boron had been introduced by diffusion. The spectrum was a horizontal straight line.

It is readily apparent from these results that differences of kind may be demonstrated in the electron spin resonance spectra of diamond by the introduction of quite small amounts of impurities. A careful examination of the X-ray parameters of these materials showed that there was no difference in the observed a_0 values down to the fourth decimal place. It is surprising that this should be so when up to 10^{21} impurity atoms/c.c. are present. In addition, diffraction spikes were seen in abundance only with those diamonds obtained from the 2 nickel : aluminium catalyst, in which nitrogen is presumably present only to a very small extent.

It seems to us, therefore, that not only are multiple techniques required in the examination of diamond, but also that it is necessary to have available specimens of known and reproducible composition.

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RESOLVING POWER WITH TRITIUM AUTORADIOGRAPHS

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THE penetrating power of β-particles from tritium is very low, and an autoradiographic image of a labelled structure to which the sensitive emulsion is applied therefore shows very little blurring due to spreading of the β-particles away from their source. The sharpness of the image has been calculated[1], but it was thought desirable to make a practical direct test with a radioactive source of known configuration.

'Araldite' epoxy resin, type *CY*212 (CIBA (A.R.L.), Ltd.) was made radioactive by combination with tritiated succinic acid. The reaction is similar to that taking place with dodecenyl succinic anhydride hardener (type 964*B*). The resin (2·7 gm.) was mixed with the ^{3}H-succinic acid (25 mc., 32·4 mgm.; Radiochemical Centre), and heated at 120° C. for 3 hr. The resin was then cured in the normal way, in gelatine capsules, with 964*B* hardener and 964*C* accelerator. The radioactivity of the product was 4,290 μc./gm. Its specific gravity was 1·12. The blocks were cut on a Huxley ultramicrotome (Cambridge Instrument Co.).

Two methods were used. In the first, a block of the radioactive 'Araldite' was cut to a smooth face on the microtome, and it was then re-embedded in non-radioactive 'Araldite'. The composite block was sectioned at right angles to the original face, and the sections, 0·75μ thick, were set up with Kodak '*A.R.*10' autoradiographic film. In these preparations a straight edge of radioactive material presented itself to the emulsion. After exposure for 165 hr. the autoradiographs were developed (Kodak '*D.*76', 5 min., 20° C.). The region of the radioactive straight edge was photographed under the microscope, and prints were made at a magnification of about 6,000. The spread was recorded by passing a narrow slit (240 mm. long, 0·1 mm. wide) over the print, by means of a micrometer, and measuring the amount of light transmitted from a lamp on one side of the slit to a photomultiplier on the other; the dark images of the silver grains caused a reduction of the light when they passed the slit. The average was taken for nine prints. The maximal grain density was about 400/100μ², and at this density there is appreciable loss of yield on account of 'over-hitting'; a correction was made for this. Each point on the record (Fig. 1) represents the contribution from several hundred grains. The spread of an autoradiographic image on either side of a narrow disk source is the differential of the curve in Fig. 1. The parameter which is chosen to characterize the spread is the width of the band, centred on the disk, which would contain 50 per cent of the whole grain count. This is equal to the width (Fig. 1) of the part of the observed record from 25 per cent to 75 per cent of the maximum. In this case it is 0·38μ. In emulsion '*A.R.*10' the grains are of diameter 0·2–0·4μ, and it is estimated that by pin-pointing the centres of the grain images the spread should be reduced from 0·38 to 0·34μ.

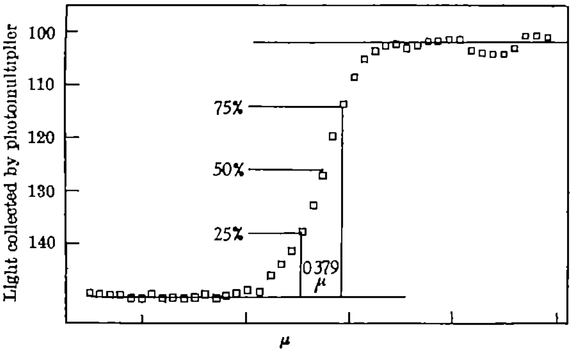

Fig. 1

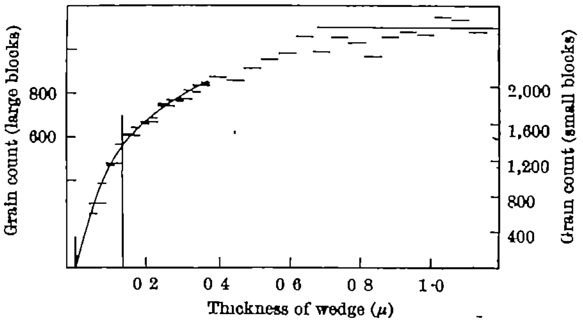

Fig 2

The second method was less direct in its approach. A face was cut on a block of the radioactive 'Araldite', and the angle of cutting was then altered by 0·5° so that subsequent sections were in the form of wedges, tapering in thickness. The autoradiographs, after times of exposure varying from 23 to 95 hr., were photographed, and the grain densities at varying distances from the tip of the wedge were counted on a number of prints. A correction was made for over-hitting. The extreme tip of the wedge breaks away during sectioning, and in Fig. 2 the curve is extrapolated beyond the region showing grain yield in the autoradiograph. The tip of the wedge appears to have broken away at a thickness of 0·031μ.

It is not difficult to prove that the form of this curve (Fig. 2) should be identical with that of each half of the symmetrical distribution over a thick section of a straight-edge source, as found in the first method. The solution for a disk source is therefore the differential with respect to the thickness of the wedge. The band containing 50 per cent of the total grain count from such a source is 0·226μ wide. This does not take account of the size of the grains and the corrected value for the band width, assuming pin-pointing of image centres of the grains, is found to be 0·24μ.

The discrepancy between the results by the two methods is possibly accounted for by inadequate allowance for grain size. This would mean an overestimate of the band-width by the first method and an underestimate in the second. If this is so, it may be concluded that a disk source will produce an image in which 50 per cent of the grains, when pin-pointed, lie in a band of width somewhere between the two estimates, 0·24–0·34μ; that is, 50 per cent of the grain centres lie within 0·12–0·17μ of the disk. By making use of the fact that the distribution is similar in form to a Gaussian probability curve it may be shown that the frequency of distribution descends to half its peak value at 0·21–0·30μ from the source. This is about the figure suggested earlier[1] on indirect data.

It should be noted that the results apply to sections of material which are thicker than about 0·5μ, that is, to sections in which self-absorption limits the contribution of deeper layers. Although the resolution could be improved by using extremely thin sections, it is generally not practicable with light microscopy to do so.

I thank Prof. A. F. Huxley and Dr. G. Winfield (CIBA (A.R.L.), Ltd.) for helpful discussion. This work was aided by a grant from Muscular Dystrophy Associations of America, Inc.

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AN INTERNAL WAVE IN LAKE NYASA AND ITS PROBABLE SIGNIFICANCE IN THE NUTRIENT CYCLE

By D. H. ECCLES

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LAKE NYASA is extremely oligotrophic and shows very marked nutrient depletion above the thermocline which is formed about October[1-5]. In December it lies at about 20–40 m., but is driven down to about 150 m. by July and often breaks down in August. Despite these break-downs high nutrient-levels have never been recorded in the upper 40 m. There appears also to be a more permanent thermocline at about 200 m. (refs. 2 and 5) and the Lake probably represents a 3-layered system comparable with Lake Victoria[6]. Large short-term fluctuations in the level of the upper thermocline are well known at Nkata Bay[1-5] and have been interpreted as components of a uninodal seiche[2]. Series of temperature readings taken on voyages based on Nkata Bay have shown large displacements of this thermocline. In 1958 a difference of level of 30 m. was recorded between Nkata Bay and Mbamba Bay 70 km. to the north-east and intermediate stations showed a uniform gradient[3]. In December 1960 a large internal wave was observed in a voyage from Nkata Bay to Kota Kota returning up the other side of the Lake[5]. This wave was too short to represent a uninodal seiche.

During January–February 1961 a voyage was made from Nkata Bay to Fort Johnston and back. Series of temperature readings were made using a thermister thermometer, reading to 0·02° C., at the stations indicated in Fig. 1. Readings were taken every 5 or 10 m. in the region above the upper thermocline, but when this was approached the intervals were reduced to 2·5 m. and increased to 5 m. once it had been passed. The positions of isotherms at 0·2° C. intervals were obtained graphically from the results. The thermocline occurred in the region of the isotherms for 26°–27° C. and the area between these isotherms is plotted graphically in Fig. 2, which also shows the time and date of the readings. Considerable fluctuations in level were observed on the south-bound trip (Fig. 2*A*) between Nkata Bay and Monkey Bay with regular peaks at stations 2, 9 and 13 (possibly 5) separated by about 70 km. These were not so apparent in the northward trip (Fig. 2*B*), where fluctuations were less regular, although there is some indication of peaks at stations 23, 26–27 and 29. Unfortunately it was not possible to obtain any readings between stations 31 and 32 due to bad weather. The abrupt step between stations 7 and 8

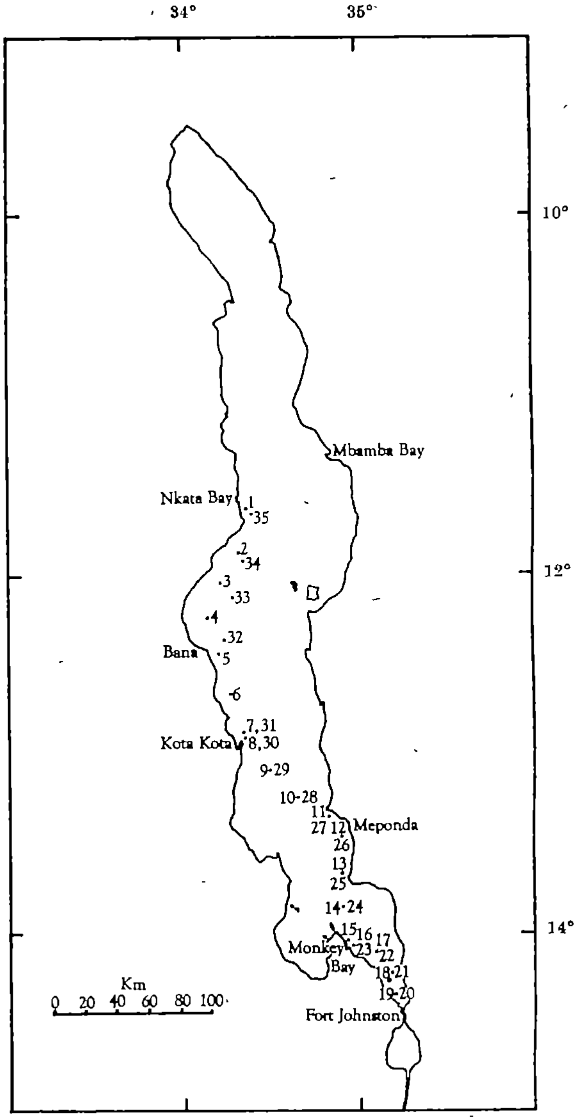

Fig. 1. Lake Nyasa showing positions of sampling stations

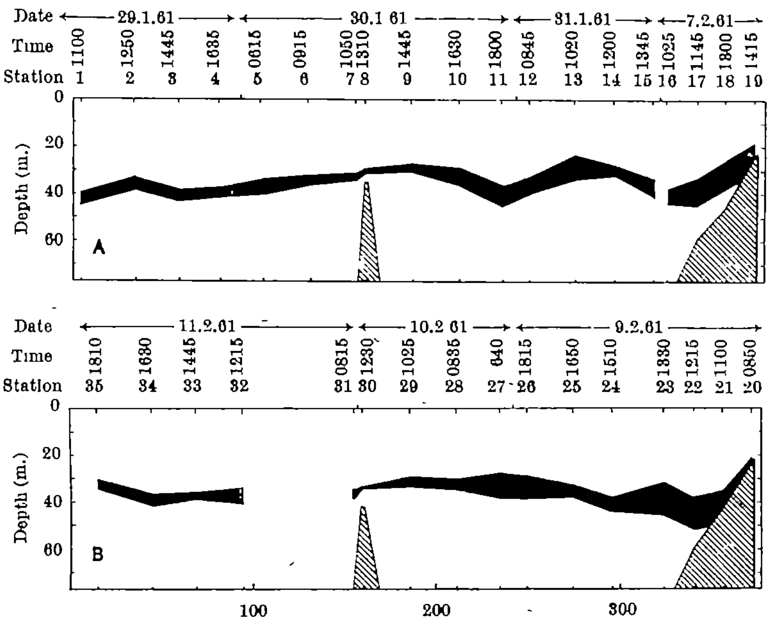

Fig. 2. The region between the isotherms for 26° and 27° C. in Lake Nyasa in January and February 1961. *A*, Southward voyage; *B*, northward voyage. Hatched areas indicate level of the Lake bottom

and 30 and 31 is undoubtedly the effect of a sand bar which extends from the end of the spit at Kota Kota. In the south-east arm, between Monkey Bay and Fort Johnston (Figs. 2*A* and *B*, stations 16–23), the amplitude of the fluctuations was increased and the isotherms became more widely spaced. In this region the thermocline meets the bottom, which rises gently to the south, and comparatively small changes in level of the thermocline must involve considerable horizontal displacements of water with resultant turbulence bringing about wider spacing of the isotherms. The extent of horizontal displacement can be judged from Fig. 2; on February 9 the isotherm for 26° C. met the bottom about 10 km. farther north than on February 7. Movements of this magnitude must also result in disturbance of the upper layers of the bottom deposits, and thus accelerate the release of the nutrients to the water.

The south-east arm of the Lake supports a much richer fishery than any other area[7,8] and it has long been considered by members of the Joint Fisheries Research Organization that this is partly due to tilting of the thermocline and upwelling of nutrient-rich water under the influence of the strong southerly winds that blow during May–August. Recent observations tend to support this view. The fertility of this area may also be influenced by the fact that annually the thermocline falls below 100 m., which is the maximum depth of the south-east arm, and nutrients can be returned directly to the epilimnion while some parts of the area are so shallow that the bottom never falls below the thermocline and nutrients are not lost. The internal waves described here suggest a further mechanism whereby the fertility of the south-east arm is maintained, and this is operative at the period when the thermocline is most marked and winds are northerly.

The headquarters of the Joint Fisheries Research Organization in Nyasaland will be transferred to Monkey Bay within a year and it will then be possible to undertake more detailed work on the hydrology of the southern portion of the Lake.

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R_M VALUES IN THE GAS-LIQUID CHROMATOGRAPHY OF STEROIDS

By B. A. KNIGHTS and Dr. G. H. THOMAS

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CLAYTON[1] has shown that the retention time ($r_{(n+a+b+c+\cdots)}$) of a sterol methyl ether on gas-liquid chromatography may be expressed in the form:

$$r_{(n+a+b+c+\cdots)} = r_n \times k_a \times k_b \times k_c \times \cdots$$

where r_n is the retention time of the unsubstituted nucleus and $k_{a,b,c}$ . . . are group retention factors for a series of non-interacting substituents at positions *a,b,c*, etc. Quantitative investigations (to be published) have also been made by us, where we have considered the logarithm of the retention time of a steroid as being made up of the additive contributions of the individual substituents together with that of the nucleus to which they are attached.

We wish now to relate these observations to the theoretical studies which have formed the basis of similar quantitative work on the behaviour of compounds in paper chromatographic systems.

Bate-Smith and Westall[2] defined a function, R_M, for paper chromatography as:

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

R_M is a function of the free energy of the compound, which in turn can be regarded as being made up of the sum of the free energies of the various groups of which the molecule is composed. It follows that R_M values for components of a molecule contribute additively to its chromatographic mobility.

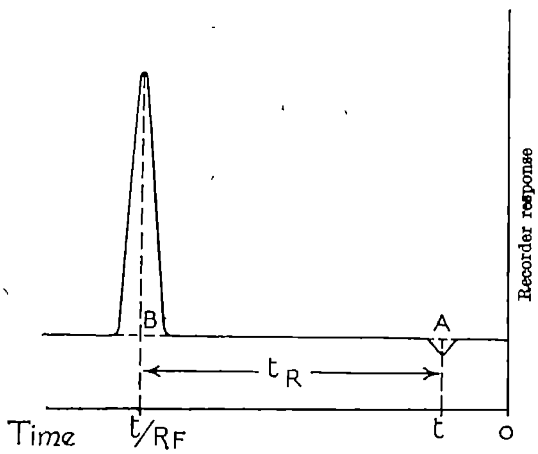

Fig. 1. Elution diagram. O, time of injection, *A*, air peak

The application of these concepts in the structural analysis of steroids has been reviewed by Bush[3].

The theory of gas-liquid chromatography differs from liquid-liquid chromatography only by virtue of the added complication arising from the compressibility of the mobile phase[4]. R_F has the same chromatographic meaning in both systems. In gas chromatography, the flow-rate of the mobile phase increases continuously as it advances down the column; since there is a corresponding increase in the rate of movement of the compound, the R_F remains constant[5]. In the chromatogram shown in Fig. 1 the time taken for the gas front to travel the length of the column is given by the time-interval (t) between the start of the chromatograph (O) and the appearance of the negative air peak (A). The retention time (t_R) of the substance is given by the interval AB and can be expressed as:

$$t_R = \frac{t}{R_F} - t = t\left(\frac{1}{R_F} - 1\right)$$

The relative retention time (r) using a standard x run under the same chromatographic conditions is:

$$r = \frac{\left(\frac{1}{R_F} - 1\right)}{\left(\frac{1}{R_{Fx}} - 1\right)}$$

Therefore:

$$\log r = R_M - R_{Mx}$$

It follows then, that the logarithm of the relative retention time of a substance is made up of the additive contributions of the individual groups (g) together with the log contribution for the relative retention time of the nucleus (N):

$$\log r = \Sigma \Delta R_{M_g} + \log^r N$$

The above relationship is implicit in our previous work. The group retention factor (k) described by Clayton can be related to R_{M_g} by the expression:

$$\log k = \Delta R_{M_g}$$

In order to conform to the existing nomenclature in paper chromatography we feel that there is some advantage in retaining the terms ΔR_{M_g} and ΔR_{M_r} to denote changes in log resulting from differences in structure of the type defined by Bush[3], that is, ΔR_{M_g} − $A\,\Delta \log r$ value due to the simple substitution of another group for a hydrogen atom; ΔR_{M_r} − $A\,\Delta\log r$ value due to a change in molecular structure other than the simple substitution of a hydrogen atom, for example, epimerization of a hydroxyl group, etc.

The work presented here was carried out with the object of testing with what degree of accuracy ΔR_{M_g} values for substituents could be determined by gas chromatography. We are indebted to Prof. W. Klyne for providing samples of mono-substituted steroids from the Medical Research Council Steroid Reference Collection. Compounds were chromatographed on 'Celite 545' (85–100 mesh) coated with 6 per cent '*QF*-1-0065' (a fluorinated alkyl silicone[6]). The running conditions were as follows: temperature, 250°; inlet pressure, 22 p.s.i.; flow-rate, 58–62 ml./min. The time of emergence was measured from the negative air peak and the relative retention times were calculated using cholestane as standard.

Table 1. RELATIVE RETENTION TIMES OF SOME STEROID HYDROCARBONS ON '*QF*-1-0065'

Steroid	r	$\log^r N$
5α-Androstane	0·18	− 0·75
5β-Androstane	0·17	− 0·78
5α-Pregnane	0·28	− 0·55
5β-Pregnane	0·25	− 0·61
5α-Cholestane	1·00	0·00
5β-Cholestane	0·90	− 0·05

The $\log^r N$ values of some androstanes, pregnanes and cholestanes are presented in Table 1. It can be seen that the order of elution is $C_{19} < C_{21} < C_{27}$, and that for pairs of hydrocarbons differing only in their configuration at C–5, the 5β-steroid is eluted ahead of its 5α-isomer. In Table 2 are given ΔR_{M_g} values for ketone and hydroxyl substituents at selected positions of the steroid molecule. These values were determined by subtracting, from the $\log^r$ of the compound listed in Table 2, the log r value of the appropriate steroid devoid of the functional group. The results serve to illustrate the degree of accuracy with which these values can be calculated and, further, to show that the contribution of a substituent depends not only on its chemical nature but also on its position in the molecule and its configuration. Such differences are of considerable importance in providing constitutional information about the structure of unidentified compounds.

A detailed analysis of ΔR_{M_g} values of functional groups in the steroid molecule is being undertaken and the results will be discussed fully elsewhere.

Table 2. ΔR_{M_g} CONTRIBUTIONS OF KETONE AND HYDROXYL GROUPS FOR COMPOUNDS CHROMATOGRAPHED ON '*QF*-1-0065'

Group	Steroid*	ΔR_{M_g}
3-Ketone	5α-Androstan-3-one (1·02)	0·76
	5β-Androstan-3-one (0·93)	0·75
	5α-Androstane-3,17-dione (4·72)	0·77
	5β-Androstane-3,17-dione (4·27)	0·77
	5α-Pregnan-3-one (1·60)	0·76
	5β-Pregnan-3-one (1·51)	0·79
	5α-Cholestan-3-one (5·46)	0·74
	5β-Cholestan-3-one (5·04)	0·75
3-OH (equatorial)	5α-Androstan-3β-ol (0·54)	0·48
	5β-Androstan-3α-ol (0·50)	0·48
	5β-Androstan-3α-ol-17-one (2·23)	0·49
	5α-Pregnan-3β-ol (0·83)	0·47
	5β-Pregnan-3α-ol (0·77)	0·50
	5α-Cholestan-3β-ol (3·03)	0·48
	5β-Cholestan-3α-ol (2·88)	0·50
17-Ketone	5α-Androstane-17-one (0·80)	0·65
	5β-Androstan-17-one (0·72)	0·64
	5α-Androstane-3,17-dione (4·72)	0·67
	5β-Androstane-3,17-dione (4·27)	0·66
	5α-Androstan-3α-ol-17-one (2·18)	0·66
	5α-Androstan-3α-ol-17-one acetate (3·29)	0·66
	5β-Androstan-3α-ol-17-one (2·23)	0·65
	5β-Androstan-3α-ol-17-one acetate (3·04)	0·65
20-Ketone	5β-Pregnan-3α-ol-20-one (2·76)	0·55
	5β-Pregnan-3α-ol-20-one acetate (3·84)	0·59
	5β-Pregnane-3,20-dione (5·37)	0·58
	5α-Pregnane-3,20-dione (5·90)	0·57

* The relative retention times (cholestane used as standard) of the compounds are given in brackets.

This investigation was supported, in part, by a grant made by the Ford Foundation to Sir Solly Zuckerman, to whom we are indebted.

We thank the Caroline Harrold Fund for a grant to cover the purchase of a Pye argon gas chromatograph and the London Rubber Co. for funds to support a university research fellowship for one of us (B.A.K.).

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USE OF THE COMPLEXES OF THIOL CARBOXYLIC ACIDS WITH NICKEL IN CHEMICAL ANALYSIS

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DETERMINATION of nickel in microquantities with dimethylglyoxime and its higher homologues is well known[1]. Besides these nitrogen and nitrogen co-ordinating ligands, other ligands have been suggested for its determinations. These are nitrogen and oxygen co-ordinating: 8-hydroxy quinoline[2], and nitrogen and sulphur co-ordinating: dithioxamide (rubeanic acid)[3a] and thioxine[3b]. Sulphur and oxygen co-ordinating ligand, for example, β-thiol propionic acid[4], has also been used recently for the determination of nickel; and in the following the results with similar ligands, namely, α-thiol acetic acid and *o*-thiol benzoic acid for determining nickel spectrophotometrically, are briefly reported. The use of the former in polarographic determination of nickel[5,6], and also in spot detection of chromium[7,8] in presence of titanium, has been described already by me.

Nickel (spec. pure), 0·2979 gm., was dissolved as perchlorate to make a 500-ml. solution. α-Thiol acetic acid (B.D.H.) and *o*-thiol benzoic acid (m.p. 162°–164°) were used.

Beckman's *p*H meter model *H*2 and Hilger Watts Uvispectrophotometer No. 700,308 were used. The two reactants were allowed to react together and the *p*H was set with ammonia or caustic soda solution as desired. The solution was then made up to volume.

The following observations were made:

(1) Nickel gives coloured complexes with thiol acetic acid. In aqueous centimolar solution of nickel the colour of the complex is yellowish-green in acidic solution showing pink at a *p*H of about 5 and is violet at *p*H 7 and above. The absorption curve of the coloured complex at a *p*H of 8·5 is shown in Fig. 1.

(2) Similar absorption curves are obtained whether one uses ammonia or caustic soda for setting the *p*H.

(3) There are two absorption maxima in the curve, one at 515 mμ (referred to later as position '*X*') and the other a broad, rather flat absorption band in the region 600–640 mμ (referred to later as position '*Y*').

(4) The plot of the absorbance at *X* with the change of *p*H is shown in Fig. 2 (curves *A* and *B*). The absorbance measured at *X* becomes essentially constant between *p*H 7–9. When ammonia is used for setting of *p*H, the absorbance falls off rapidly after *p*H 9 (curve *A*). But in case caustic soda is used for the same purpose, the absorbance increases after *p*H 9 and once again becomes constant between *p*H 11–12; at still higher *p*H's the absorbance increases again (curve *B*).

(5) At these regions, of *p*H 7–9 and of *p*H 11–12, the molar absorbancy indexes at *X* are 98 and 126, respectively, and Beer's Law has been found to obey for either of these regions of *p*H's for nickel concentrations varying between 2·5–50 μgm. nickel/ml. by using 40 mm. light path, or by using Ilford filter OG_1 with a Spekker absorptiometer. Therefore, either of these ranges of *p*H, that is, 7–9 and 11–12, may be usefully exploited for analytical purposes. The anions normally met with in chemical analysis, namely, sulphate, phosphate, pyrophosphate, tartrate, chloride, perchlorate, citrate and oxalate, do not essentially interfere in this determination.

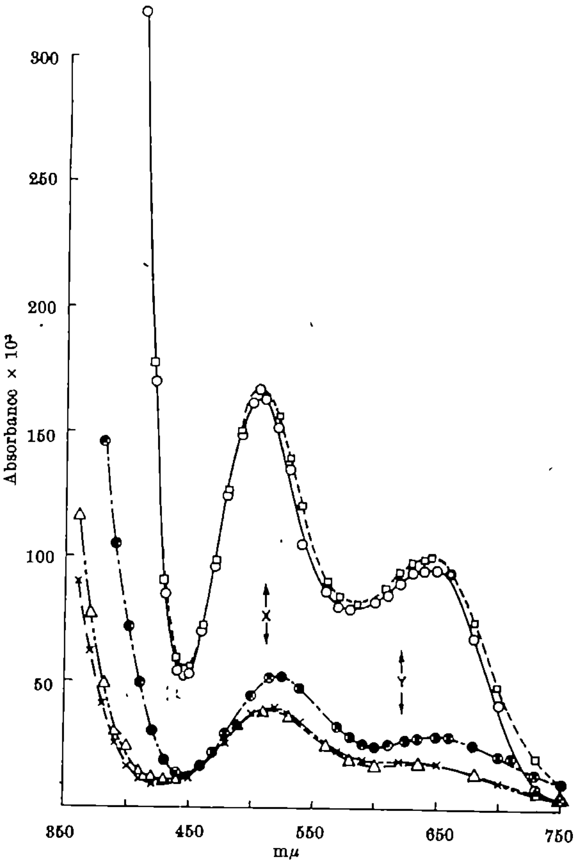

Fig. 1. Absorption curves of nickel solutions containing 23·95 μgm. nickel/ml. ($4{\cdot}08 \times 10^{-4}$ *M*) complexed with·
A (△), α-thiol acetic acid (excess), *p*H 8·5 set with ammonia;
B (×), α-thiol acetic acid (excess), *p*H 8·5 set with caustic soda;
C (○), α-thiol acetic acid (excess), *p*H 11·5 set with caustic soda;
D (○), *o*-thiol benzoic acid (excess), *p*H 8·5 set with ammonia;
E (□), *o*-thiol benzoic acid (excess), *p*H 8·5 set with caustic soda

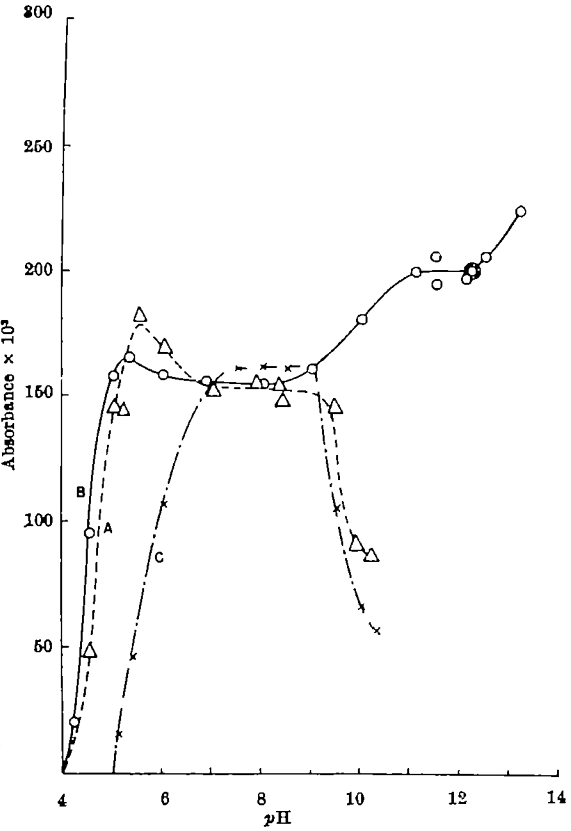

Fig. 2. Change of absorbance at 'absorption maximum' at *X* with varying *p*H of the solution containing 23·95 μgm. nickel/ml. (that is, 4·08 × 10^{-4} *M*) complexed with: *A* (Δ), a large excess of α-thiol acetic acid using ammonia for setting *p*H, *light path 40 mm.*; *B* (○), a large excess of α-thiol acetic acid using caustic soda for setting *p*H, *light path 40 mm.*; *C* (×) (7 molar) excess concentration of *o*-thiol benzoic acid using caustic soda for setting *p*H, *light path 10 mm.*

(6) This reagent can be used for determination of a few ions, namely, chromium, tungsten, uranium and nickel[8], molybdenum[9] and cobalt[10]; and is not specific to nickel. Manganese and indium do not interfere. The results of these determinations are being communicated elsewhere.

Observations regarding determination of nickel spectrophotometrically with *o*-thiol benzoic acid are also represented in Figs. 1 and 2. It may be noted that the *o*-thiol benzoic acid complex has molar absorbancy indexes 404 and 196, respectively, at 505 mμ and 635 mμ, and that analytically this complex is four times more sensitive than the α-thiol acetic acid complex.

No attempt is made here to offer any constitutional formula of the complex. The following observations in this respect, however, may be of interest.

The variation of the absorbance with the change of *p*H (Fig. 2) offers an interesting study. The coincidence of the inflexion of curve *B* at *p*H 5 and the rise of the curve *C* at the same *p*H is significant. The absorption curves at *p*H's greater than 5 have similarity of forms, especially for incidence of an absorption band at position *Y* for comparative points on curves *B* and *C*. The behaviour in case of thiol–acetic complex (curve *B*) may be explained by suggesting that more than one complex co-exist at *p*H's 5–9. Mention may be made here of the suggestion of Leussing *et al.*[11] regarding the co-existence of both monomeric and polynuclear species of complexes in solutions of nickel complexed with α-thiol acetic acid. The behaviour at higher *p*H's than 9 is again complicated. The comparative examination of these is already under study and will form the subject of another communication.

As stated earlier, in case of the thiol–acetic complex the molar absorbancy index between the region of *p*H 11–12 is about 30 per cent more than that in the region of *p*H 7–9. In this respect my observations are at variance with those of Christopher and Hamme[12] inasmuch as the latter have depicted that the absorbance between *p*H 7–12·25 remains constant. This is not borne out by Fig. 2 (curve *B*).

Attention may probably be directed to Fig. 1 in that in the case of thiol–acetic complex the position of absorption maximum at *X* between *p*H 11–12 shifts by 7 mμ bathochromically to 522 mμ, and also there is a change in the shape of the absorption spectrum at *Y* at *p*H's higher than 9.

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NON-DISSOCIABILITY OF THE METAL COMPONENT OF D-α-HYDROXY ACID DEHYDROGENASE BY CHELATING AGENTS

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SINCE the simultaneous finding in two laboratories[1,2] that ethylenediamine tetraacetic acid (EDTA) and other chelating agents reversibly inhibit the D-α-hydroxy acid dehydrogenase of yeast (DHAD, 'anaerobic lactic dehydrogenase'), the mechanism of this effect has been the subject of considerable controversy. Reduced to its essentials, the question is whether chelating agents act by resolving this and related metal-flavoproteins to inactive flavoprotein, as asserted by Curdel *et al.*, or whether they act by forming an enzymatically inactive holoenzyme chelate, as suggested by Tubbs[5] and maintained by us[6–8]. As more fully discussed else-

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where[3], a definitive answer to this question transcends in importance the particular observations involved, since a clear understanding of the action of chelating agents is essential for any interpretation based on their use as diagnostic agents in the field of metal flavoproteins. The purpose of this article is to present evidence incompatible with the assumption that the action of chelating agents on DHAD is the removal of its metal component.

We have previously reported that both DHAD and the closely related zinc-flavoprotein, D-lactic cytochrome reductase, which undergoes reversible inhibition by chelators in the same manner, may be extensively reactivated following inhibition by chelators without added metals[4]. This was accomplished by the removal of uncombined chelator on 'Sephadex', followed either by dialysis in the cold to dissociate protein-bound chelator or by incubation at 10°–45°. The latter technique, as applied to the reductase, resulted in the finding that both the rate and the extent of this 'spontaneous' reactivation depend on the temperature. These experiments were interpreted to show that the main reason for the inhibitory effect of chelating agents on these two enzymes is the formation of a chelated holoenzyme which is unable to bind substrate. Owing to the instability of the chelated enzyme full reactivation was not reached in these studies. Thus the possibility remained that a part of the inactivation was due to secondary, irreversible changes in the chelated protein or to removal of the metal or both.

Recently, conditions have been found to stabilize the chelated forms of both DHAD and the reductase[8]. By means of these full reactivation of the reductase was attained without the use of metals. Unambiguous proof that removal of the metal plays no part in the inhibition of the reductase was obtained following the isolation of the enzyme in homogeneous form and the analytical demonstration that it is a zinc-FAD enzyme[10]. It was found that the zinc content of the enzyme remained constant throughout the cycle of reversible inactivation but the inhibited form contained 2 moles chelator (o-phenanthroline) per gm. atom of zinc present[11].

Notwithstanding these facts, in a recent article dealing with the effect of acid ammonium sulphate on DHAD, Iwatsubo and Curdel[12] maintain that their original hypothesis of the mode of action of chelators is correct, although the data presented do not concern the action of chelators. In the hope of settling this question, we wish to present some data which clearly show that under the conditions used by these workers chelators do not dissociate the metal component of DHAD.

Following treatment with EDTA, the pH stability curve of the inactive DHAD is sharper and different from that of the untreated enzyme. The pH of maximum stability is 6·8. Dialysis at this pH results in full reactivation of the EDTA-inhibited enzyme (Fig. 1). Since no added metals are used in this experiment, reconstitution of the holoenzyme is precluded as a mechanism of the reactivation, and, hence, inactivation occurs solely because of chelation, not because of removal of the metal.

Although the lability of DHAD has prevented its isolation in sufficiently pure form to provide additional evidence from analytical demonstration that the inactive enzyme contains stoichiometric amounts of metal, zinc analyses are meaningful in showing that the reactivation by dialysis is not due to spurious contamination of the dialysing fluid with zinc. It was found that the zinc content of the preparation is the same before (4·8 mμmoles/mgm. protein) as after reactivation (4·7 mμmoles/mgm.).

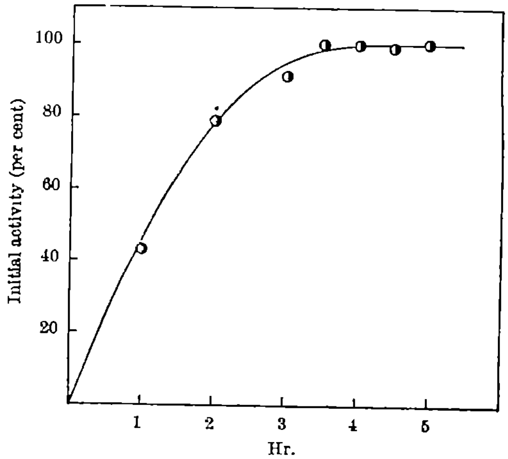

Fig. 1. Reactivation of DHAD by dialysis without added metals. Conditions: inactivation with 0·8 m*M* EDTA, *p*H 6·8, for 15 min. at 30°, reactivation, following passage through a column of 'Sephadex *G*-25' (equilibrated with 50 m*M* phosphate, *p*H 6·8) at 0°, by dialysis at 0°, under nitrogen, against 50 m*M* phosphate – 1 m*M* D-lactate, *p*H 6·8

Further evidence in support of the conclusion that EDTA inhibits DHAD by chelation is shown in Fig. 2. As demonstrated earlier for the reductase[6], both the rate and the extent of the 'spontaneous' reactivation depend on the temperature of incubation, and the fraction of enzyme in the active form may be readily varied by the mere readjustment of the temperature. Analytical studies in the case of the reductase have shown that the reversible equilibria are[11]:

$$\text{En}-\text{Zn} + \text{chelator} \rightleftarrows \underset{\textit{inactive}}{\text{En–Zn–chelate}} \rightleftarrows \underset{\textit{active}}{\text{En–Zn–chelate}} \rightleftarrows \text{En–Zn} + \text{chelator}$$

Thus a temperature-dependent intramolecular shift of the chelator occurs from catalytically inactive to active form. In the case of DHAD the same intramolecular shift of the chelator is indicated by the fast inactivation elicited by cooling a reactivated

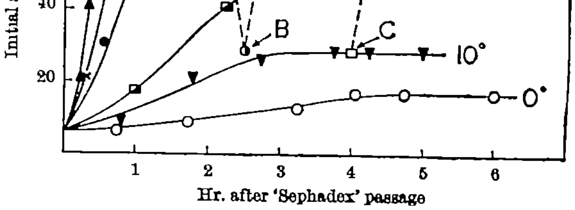

Fig. 2. Reactivation of DHAD at various temperatures. Samples of the inactivated, 'Sephadex'-passed enzyme (Fig. 1) were incubated in 50 m*M* phosphate – 1 m*M* D-lactate, *p*H 6·8, under nitrogen, at a concentration of 32 mgm. protein/ml. at the temperatures indicated. Aliquots were removed for assay as shown. At time *A* an aliquot was cooled from 38° to 10°; at point *B* it was reheated to 38°. At *C* an aliquot was heated from 10° to 38°

sample (point *A*, Fig. 2). A corresponding rate of inactivation at 10° would occur only at very high concentrations of *free* EDTA, whereas the actual concentration of EDTA in this experiment was very low, since only protein-bound EDTA was present.

The two reasons which led Curdel and Labeyrie[3] to postulate and maintain that the inhibition of DHAD by EDTA proceeds by removal of the metal were as follows. (1) These authors, when testing reactivation without prior removal of unreacted chelator, found that only Zn^{++} and, to a small extent, Co^{++} reactivated the enzyme. This apparent specificity was interpreted to show that (*a*) zinc is the prosthetic group, and (*b*) EDTA acts by dissociating zinc from the enzyme. They further observed that the Zn^{++}-reactivated enzyme has the same kinetic constants (K_M and $V_{max.}$) for D-lactate as the untreated one, while the constants of the Co^{++}-reactivated enzyme are different[3]. This was interpreted as confirming both of the conclusions mentioned. As to the first observation, Boeri *et al.*[2] reported two years ago that a series of divalent metals reactivate the EDTA-inhibited enzyme, provided that uncombined chelator is removed before adding the metal. The following results show that Zn^{++} is only slightly more effective than other divalent cations and that, therefore, the presumed specificity does not exist.

Samples of the EDTA-treated enzyme used in the experiment of Fig. 1, following 'Sephadex' passage, were incubated for 5 min. at 30° as follows: 1·89 mgm. protein in 0·1 *M* phosphate, *p*H 6·8, 0·15 ml. total volume, containing added metal at concentrations varied from 1×10^{-4} *M* to $8{\cdot}3 \times 10^{-4}$ *M*. Aliquots were assayed by the ferricyanide method[2]. Activity was calculated at infinite metal concentration from double reciprocal curves. The reactivation reached with different metals, expressed as percentage of the activity of the untreated enzyme, with *apparent* K_M for the metal in parentheses, were: Zn^{++}, full reactivation ($1{\cdot}25 \times 10^{-6}$ *M*); Co^{++}, 88 per cent ($1{\cdot}3 \times 10^{-6}$ *M*); Mn^{++}, 84 per cent ($1{\cdot}75 \times 10^{-6}$ *M*); Fe^{++}, 74 per cent ($1{\cdot}66 \times 10^{-6}$ *M*). The apparent K_M values show that the results cannot be interpreted as being due to traces of Zn^{++} in the other salts used.

As to the second reason, the apparent dependence of the constants of the reactivated enzyme on the metal used has also been observed by the authors and presented a paradox until recently. It has now been established, however, that if precautions are taken to stabilize the enzyme, the same K_M values are obtained in untreated, 'spontaneously' reactivated, and metal-reactivated samples, regardless of the metal used. When differences occur, they are the consequence of secondary changes in the protein and are as evident with Zn^{++} as with any other metal used for reactivation, provided that substrates other than lactate are tested. With lactate only minor differences are observed[7].

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SEROLOGICAL HETEROGENEITY OF NORMAL HUMAN HÆMOGLOBIN

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In work reported previously[1] the reactions of anti-adult-hæmoglobin sera with hæmoglobin prepared from adult blood and from umbilical cord blood were studied by Oakley and Fulthorpe's gel-diffusion method[2]. In this method antiserum is allowed to diffuse from one side and antigen preparation from the other into a central column of gel. A band (really a disk) or bands of precipitate form in this layer; the number of bands of precipitate is an indication of the least number of antigen–antibody systems concerned in the reaction. The gel used was 1 per cent agar and the antisera showed one band of red precipitate with normal adult hæmoglobin and two bands of red precipitate with cord-blood hæmoglobin. This result was given by a considerable number of the anti-adult-hæmoglobin sera, but among those examined later three behaved differently: one showed two bands of red precipitate with adult hæmoglobin and three red bands with cord-blood hæmoglobin; one showed one red band with adult and three with cord-blood hæmoglobin; the third showed only one red band both with adult and with cord-blood hæmoglobin.

At the suggestion of Dr. C. G. Pope, gelatine was substituted for agar in diffusion experiments and was found to increase the resolution of bands of precipitate: for example, with cord-blood hæmoglobin, the antiserum which had shown one red band in agar showed four red bands in gelatine.

Glass tubes of 20-cm. length and 3-mm. calibre were bent into the form of the letter J; the narrow calibre allowed the economical use of antisera and the shape enabled the central column of plain gelatine to be introduced without contamination by the reactants. 0·16 per cent hæmoglobin was mixed with an equal volume of 6 per cent gelatine at 37° C.; 0·6 ml. of the mixture was introduced into the short limb of a tube and left for 2 hr. in a refrigerator to solidify.

Three per cent gelatine at 37° C. was added to the long limb of the tube to give a column of 20 mm. and, after this had solidified, 0·2 ml. undiluted antiserum was added to the long limb of the tube above the plain gelatine. The tubes were stoppered and stored at 1° C. Gelatine and hæmoglobin preparations were dissolved in a solution which contained 0·71 per cent sodium chloride and 0·2 per cent sodium bicarbonate; 1 in 10,000 merthiolate was present in each reagent.

All antisera described in this and the previous article[1] had been obtained from rabbits after injection of samples of the same preparation of hæmoglobin from a single bleeding of one adult (C. W.). Five preparations of hæmoglobin were examined in gelatine diffusion experiments: that used for the injection of rabbits, two obtained from the blood of adults, J. P. and R. N., and two from the umbilical cord-blood of babies E. A. and V. C. Antisera and antigens were stored at − 20° C.

With good precipitating antisera, which contained about 5 mgm. antibody per ml., bands of precipitate appeared in gelatine diffusion experiments in 7–17 days; the bands were not coloured when they first formed, but as they increased in intensity the red colour became obvious. Changes in appearance continued for more than 2 months.

In agar diffusion experiments[1], antiserum 23*D* had shown one band of red precipitate with adult hæmoglobin and two with each of four preparations of cord-blood hæmoglobin. In gelatine diffusion experiments, this antiserum showed two red bands with adult hæmoglobin and more than two with cord-blood hæmoglobin (Fig. 1: Nos. 1, 2 and 3). Antiserum 52*E*, which had shown one red band in agar both with adult (C. W.) and with cord-blood hæmoglobin (E. A.), showed in gelatine diffusion experiments two or more red bands with hæmoglobin (C. W.) and four red bands with hæmoglobin (E. A.) (Fig. 1: Nos. 5 and 7).

In comparisons between the reactions in gelatine of antisera from different rabbits with the same preparation of hæmoglobin, differences were observed in the number of red bands, their distance apart, their width and the position of the most intense band relative to the others (Fig. 1: Nos. 6 and 8; Nos. 2 and 7). This diversity was also observed when using antisera obtained from the same rabbit after different numbers of courses of injections (Fig. 1: Nos. 1 and 4: antisera 23*D* and 23*F* were obtained from rabbit 23 after 4 and after 6 courses of injections respectively).

In comparisons between the reactions of the three preparations (J. P., C. W., R. N.) of adult hæmoglobin with the same antiserum, differences in the number of red bands, their distance apart and their width were observed with some antisera (Fig. 1: Nos. 5 and 6); this diversity may suggest differences in the relative proportions of antigenically distinct hæmoglobins in the blood of different adults. In similar comparisons between the reactions of two preparations of cord-blood hæmoglobin with the same antiserum, differences were also observed with some antisera (Fig. 1 : Nos. 2 and 3); the two preparations of cord-blood hæmoglobin E. A. and V. C. contained respectively 87 and 83 per cent of fœtal (alkali-resistant) hæmoglobin estimated by the method of Jonxis[3].

With a given antiserum, a greater number of red bands was generally observed with preparations of cord-blood hæmoglobin than with preparations of adult hæmoglobin; with cord-blood hæmoglobin the distance between the upper and lower limits of precipitation in the plain gelatine layer was always greater than with adult hæmoglobin (Fig. 1: Nos. 1, 2 and 3; Nos. 5, 6 and 7).

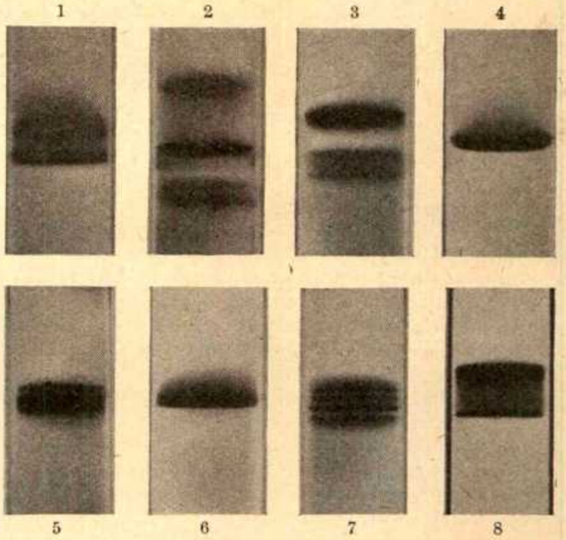

Fig. 1. Precipitates formed in gelatine when four anti-adult-hæmoglobin sera (23*D*, 23*F*, 52*E*, 35*F*) react with preparations of hæmoglobin from three individual adults (J. P., C. W., R. N.) and from two individual babies (E. A., V. C.). (1) 23*D* with J. P.; (2) 23*D* with E. A.; (3) 23*D* with V. C.; (4) 23*F* with J. P.; (5) 52*E* with C. W.; (6) 52*E* with R. N.; (7) 52*E* with E. A.; (8) 35*F* with R. N.

In experiments with seven antisera, the number of red bands observed with the three preparations of adult hæmoglobin ranged from 1 to 4 and with the two preparations of cord-blood hæmoglobin from 2 to 5. In some reactions the maximum number of bands was not easy to determine because one or more bands had diffuse margins and were incompletely resolved. To reduce the risk of artefacts which may be caused by temperature changes, the tubes were observed infrequently and more bands might have been visible at some stage. Wilson[4] has emphasized that the number of bands may diminish during the course of a diffusion experiment: bands may not be stationary and, if moving at different rates, can become superposed; increase in width can cause bands which have been distinct to overlap.

After the experimental tubes had been stored at 1° C. for 11 months, most of the bands of precipitate were still bright red. In a few experiments with adult hæmoglobin as the antigen, one of two red bands had become brown; this may indicate differing degrees of stability[5] in antigenically distinct hæmoglobins.

In a few experiments, a band which was not detectably coloured was seen in addition to red bands. Bands in which colour was not visible during several months of observation were narrow and faint; they may have been due to a reaction involving an antigen from the red cell other than hæmoglobin or to a reaction between a hæmoglobin and its antibody in which the reactants were present in low concentration.

Fractions of adult hæmoglobin (J. P.) of low and high R_F value were obtained from an 'Amberlite *IRC* 50' column after a single elution at 1° C. with a buffer of *p*H 5·84 which contained 0·07 *M* sodium chloride, 0·09 *M* trisodium citrate and 0·01 *M* citric acid. The fractions were compared using nine antisera. With seven antisera, both fractions showed two or more bands of red precipitate and with five antisera the number of bands shown by the two fractions differed. After 11 months, bands formed in experiments with the fraction of high R_F were still bright red, but those shown by the fraction of low R_F had become brown[6].

Analyses of precipitates formed by mixing undiluted antiserum with hæmoglobin solutions have shown that the anti-adult-hæmoglobin sera were capable of completely precipitating adult hæmoglobin and a cord-blood hæmoglobin which contained as much as 87 per cent of fœtal hæmoglobin[7]. Two possible explanations are suggested for the complete precipitation of cord-blood hæmoglobin: antibodies to adult hæmoglobin cross-reacted with fœtal hæmoglobin; or, the adult hæmoglobin preparation (C. W.) used for injection of rabbits contained traces of fœtal hæmoglobin[8,9] which had stimulated antibody production. These two explanations are not mutually exclusive.

Recent physico-chemical investigations have indicated that normal human hæmoglobin is to be regarded as a group of related substances rather than a single substance. By column chromatography, six hæmoglobins have been demonstrated in blood from one normal adult and seven in one cord-blood[9,10].

The results obtained in gelatine diffusion experiments with adult and with cord-blood hæmoglobin as antigen could be provisionally explained by the presence of antigenically distinct hæmoglobins in a single sample of blood from one individual, the relative proportions differing from person to person. The diversity of results obtained with different antisera (Fig. 1: Nos. 1 and 4; Nos. 2 and 7; Nos. 6 and 8) might be partly due to differences in the concentration of antibodies to these different hæmoglobins in antisera obtained from individual rabbits, or from the same rabbit after different numbers of courses of injections. In an antiserum prepared against a mixture of antigenically distinct hæmoglobins, some or all of the antibodies to one hæmoglobin may cross-react with one or more other hæmoglobins and the proportion of antibodies which cross-react may differ in different antisera. Such differences of proportion may contribute to the diversity of the results obtained with different antisera.

In the reactions of some antisera with adult hæmoglobin two, narrow, red bands of great and similar intensity and colour developed close together. Such reactions are not easily explained by the presence of one major (79–83 per cent[11]) and several minor hæmoglobin components in adult blood demonstrated by physico-chemical methods. The antisera were not reacting solely with minor components because they were able to precipitate adult hæmoglobin completely.

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ISOMERIC FORMS OF HÆMOGLOBIN H

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IN the course of an investigation of the functional properties of hæmoglobin H[1], it was observed that starch granule electrophoresis of the hæmolysate from patients with hæmoglobin H disease occasionally showed two fast components instead of one. It is the purpose of this article to report that hæmoglobin H will migrate electrophoretically either as one or as two components, depending solely on the ionic strength and the *p*H of the medium.

Blood was obtained from the two patients previously studied[1] and the methods used were those described earlier[1]. The hæmoglobin was applied to the starch blocks either as oxy- or as carbon monoxy-hæmoglobin without any influence on the results.

The electrophoretic behaviour of hæmoglobin H is illustrated in Fig. 1 and Table 1. A single fast component is observed on starch granules, on starch gel or on paper at an ionic strength of 0·1 at *p*H 7·5. When the ionic strength was decreased, separation into two components occurred in such a way that the amount of the slower component increased at the expense of the faster with decreasing ionic strength (cf. Table 1). When the two components which appear at an ionic strength of 0·033 were eluted and re-subjected to electrophoresis at an ionic strength of 0·1, only a single component with the original mobility was obtained (Fig. 2).

Table 1. INFLUENCE OF IONIC STRENGTH AND *p*H ON STARCH BLOCK ELECTROPHORESIS OF HÆMOGLOBIN H

(*A*) *p*H = 7·5

Ionic strength	Per cent of total hæmoglobin 'slow' H	 'fast' H
0·033	20·0	12·5
0·066	5·5	30·0
0·100	none	33·4

(*B*) Ionic strength = 0·033

*p*H	Per cent of total hæmoglobin 'slow' H	 'fast' H
7·5	20·0	12·5
7·0	7·0	28·5
6·5	none	38·7

The relative amounts of 'slow' and 'fast' H vary somewhat with different lots of starch.

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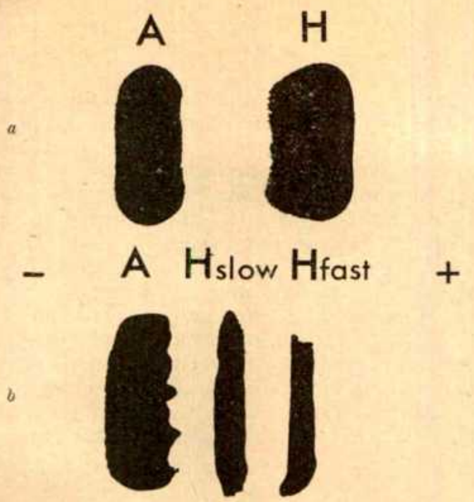

Fig. 1. Starch-block electrophoresis of whole hæmolysate. *a*, Phosphate buffer pH 7·5, $\mu=0{\cdot}10$, 16 hr.; *b*, phosphate buffer pH 7·5, $\mu=0{\cdot}033$, 16 hr.

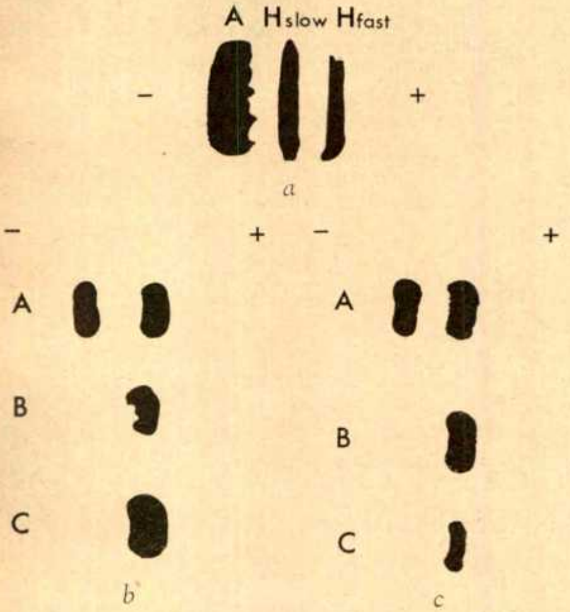

Fig. 2. Reversal of transformation. *a*, Starch-block electrophoresis of whole hæmolysate, phosphate buffer $\mu=0{\cdot}03$, pH 7·5, 16 hr.; *b*, single starch block, phosphate buffer $\mu=0{\cdot}03$, pH 6·5, 16 hr. *A*, Whole hæmolysate; *B*, fraction 'H slow' eluted from block of Fig. 2*a*; *C*, fraction 'H fast' eluted from block of Fig. 2*a*; *c*, single starch block, phosphate buffer $\mu=0{\cdot}10$, pH 7·5, 16 hr. *A*, Whole hæmolysate; *B*, fraction 'H slow' eluted from block of Fig. 2*a*; *C*, fraction 'H fast' eluted from block of Fig. 2*a*

A similar relationship was observed with respect to changes in pH. Thus, only a single component was present at pH values less than 7 even at an ionic strength as low as 0·03. Above pH 7 the slower component appears and its relative amount increases with increasing pH at constant ionic strength (Table 1). As in the case of the ionic strength-dependent transformation, the process was found to be reversible, since the two components obtained at pH 7·5 travelled with the same mobility when they were re-subjected to electrophoresis at pH 6·5 (Fig. 2).

In summary, therefore, it appears that hæmoglobin H can exist in two forms. That which is stable at high ionic strength has the greater mobility towards the anode and therefore a larger negative charge. Under the influence of low ionic strength and increasing pH, a progressive transformation to a slower and therefore less negatively charged species takes place. The rate of transformation is evidently slow enough to permit the separation of the two species during electrophoresis.

The nature of the two components was further investigated by total amino-acid analysis and molecular weight determinations. Complete amino-acid analyses on the Spinco amino-acid analyser model 120 revealed no significant differences in the composition of the two components or between them and the single component of hæmoglobin H which results from electrophoresis in high ionic strength. The analysis of the latter material was reported previously[1].

As can be seen from Table 2, neither the molecular weight nor the sedimentation constant showed any significant difference at low and high ionic strength.

Table 2

	Ionic strength = 0·033	Ionic strength = 0·10
Mol. wt.	71,000 ± 5,000	68,000 ± 4,000
$S_{20,w}$	4·55	4·55

The hæmoglobin H was eluted from the starch with phosphate buffer pH 7·5 of the indicated ionic strength and concentrated by ultrafiltration as described previously (ref. 1). The data were obtained on a Spinco model *E* analytical ultracentrifuge, using a Wratten filter No. 29 and Kodak 1 *N* spectroscopic plates. Molecular weights were determined by the Archibald method (ref. 16). The sedimentation velocity of the hæmoglobin at the two ionic strengths was determined simultaneously in a double cell rotor using a 1° positive wedge. In this way a difference of 0·1 sedimentation unit could have been detected.

The two electrophoretically distinguishable components must therefore be regarded as identical in primary structure and molecular weight.

The occurrence of two fast components in hæmoglobin H disease has been encountered in a number of laboratories[2-4]. Silvestroni *et al.*, for example, described five such cases. The only difference found between the faster component (H_1) and the slower component (H_2) was a "fetal type of U.V. spectrum" in the latter. Their claim that hæmoglobin H_2 is Bart's hæmoglobin and therefore consists of γ-chains[5], whereas hæmoglobin H_1 consists of β-chains is unacceptable in the absence of amino-acid analyses, especially since neither fraction was alkali-resistant. Bart's hæmoglobin would be particularly easy to detect by isoleucine analysis alone, since 4γ-chains contain 16 isoleucine residues[6], in contrast to α- and β-chains, which do not contain this amino-acid. (No significant amounts of isoleucine were found in any of our fractions and neither component was significantly alkali-resistant.) It is therefore possible that Silvestroni *et al.* were dealing with the same type of isomerization as described here. In general, it would seem advisable in all such cases to investigate the effect of ionic strength and pH and to exercise caution in using mobility as an absolute criterion for the identification of hæmoglobins.

Transformations of the kind described here are not without precedent in protein chemistry. Perhaps the most thoroughly investigated example is the case of serum albumin. This protein has been shown to undergo a reversible transformation in the pH-range of 3·5–4·5 to a form with a greater electrophoretic mobility towards the cathode[7,8]. Within this range two components are observed, the proportion of the faster increasing with decreasing pH. The transformation of the slow isomer (*N*) to the fast one (*F*) was found to involve the binding of three additional protons. It is noteworthy that a species with a mobility slower than *N* was observed on the alkaline side of the isoelectric point, that is, between *p*H 10 and 11 (ref. 9). Related phenomena were also encountered with ovalbumin and γ-globulin by Phelps and Cann[10], although here the interpretation is complicated by buffer ion effects.

Wishnia *et al.*[11] observed a *p*H-dependent transition between two species of conalbumin near *p*H 4, which is reversible and which they were able to correlate with proton binding by the rapid flow titration technique[12].

A sharp discontinuity in the mobility versus *p*H curve of tobacco mosaic virus protein takes place between *p*H 5 and 7 (ref. 13). In contrast to the other examples, profound changes in molecular weight, which occur in the same *p*H range, have to be taken into account in this case.

The interpretation of our observations on hæmoglobin H is analogous to those given in the investigations of these proteins. Hæmoglobin H, which is a tetramer of 4 β^A-chains, is known to be far less stable than hæmoglobin A, the tetramer of 2 α^A- and 2 β^A-chains[14]. (The stability of hæmoglobin H also seems to vary from patient to patient. Thus, the hæmoglobin H from patient N. H. was considerably more stable than that from L. G. Nevertheless, the hæmoglobin H from both sources showed the isomerization.) It is therefore not surprising that it can exist in more than one isomeric form. More specifically, the transformation of the fast to the slow component can be pictured as follows.

At a *p*H sufficiently above the isoelectric point to allow the accumulation of enough negative surface charges, a decrease in ionic strength results in repulsion between these charges and therefore in some unfolding. Some previously inaccessible basic sites thus become available for proton binding which leads to a decrease in the net negative charge on the protein and therefore a slower mobility towards the anode. This change can be reversed either by an increase in ionic strength, that is, decreasing the forces between the existing charges on the surface of the molecule, or by actually reducing the number of such charges, that is, a lowering of the *p*H towards the isoelectric point.

The presence of inaccessible proton binding sites in hæmoglobin is a well-established fact, discovered by Steinhardt and Zaiser with the rapid flow titration technique[15]. The lesser intrinsic stability of hæmoglobin H apparently permits the liberation of such groups in the neutral *p*H range, in contrast to hæmoglobin A, where they become available only in acid solution.

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ELECTROPHORETIC AND IMMUNOLOGICAL STUDIES ON STRUCTURAL SUB-UNITS OF HUMAN CERULOPLASM

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SINCE the isolation of the copper-binding protein ceruloplasmin by Holmberg and Laurell[1] in 1948, chemical investigations of this protein have been limited. Curzon has envisaged a structure in which each copper atom provides a strong link between side-chains of four parallel peptide chains which are further stablized by disulphide bridges and hydrogen bonds[2]. Scheinberg, Aisen and Morell suggested that 16 free carboxyl groups, probably in aspartyl or glutamyl residues, are the sites of the copper binding[3]. The presence of three *N*-terminal amino-acids[4] would indicate at least the same number of polypeptide chains present.

Methods for the electrophoretic separation of structural sub-units of proteins have been developed and applied successfully to the study of haptoglobin[5], α-2 macroglobulin[6] and human γ-globulins[7]. The methods applied in this work on human ceruloplasmin were essentially the same as those described in ref. 6. Ceruloplasmin was obtained from two large-scale preparations and was estimated to be 94–96 per cent pure by Grabar's immunoelectrophoresis, starch-gel electrophoresis according to Smithies and two-dimensional zone electrophoresis[8]. Both samples contained a small amount of another copper protein of lower mobility in the starch-gel[9]. When dissolved in 6 or 8 molar urea in alkaline buffers the ceruloplasmins lost their blue colour and on electrophoresis in starch-gel (prepared in 8 molar urea and formate buffer) several protein zones were obtained.

The pattern for ceruloplasmin treated with urea alone suggests that at least one fast-migrating sub-unit and three slower ones can be dissociated as shown in Fig. 1*A*. It must be emphasized that the multiple slow-migrating zones may represent aggregation and isomerization and not necessarily separate sub-units[10]. On reduction and alkylation, by a method previously described[6], the number of slower

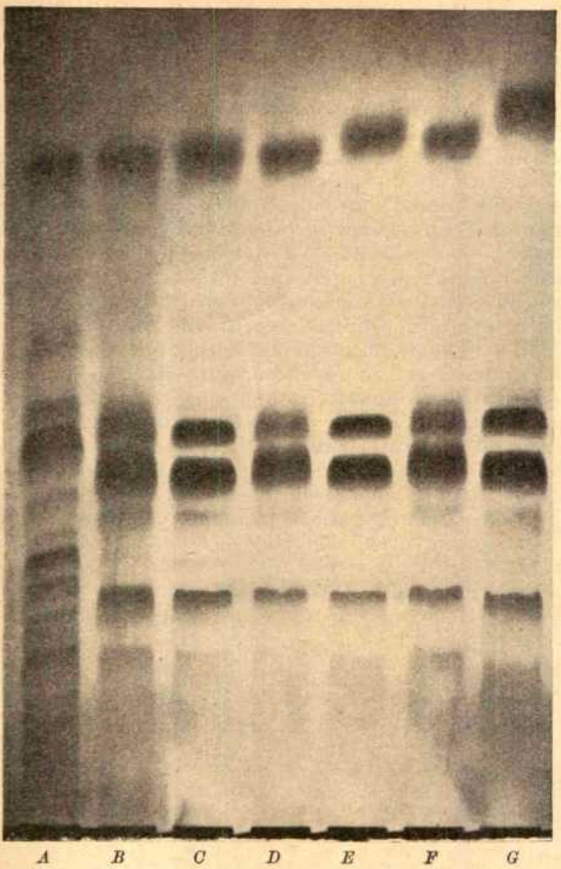

Fig. 1. Effect of reduction and alkylation on urea starch-gel electrophoretic pattern of ceruloplasmin. Ceruloplasmin treated with 8 *M* urea (*A*); ceruloplasmin reduced with varying concentrations (0·01 *M*, 0·1 *M* and 0·2 *M*) of mercaptoethanol in 8 *M* urea (*B*, *D*, *F*). Ceruloplasmin reduced with the above concentration of mercaptoethanol and then alkylated with 0·05 *M*, 0·25 *M* and 0·5 *M* iodoacetamide (*C*, *E*, *G*)

migrating zones was considerably reduced, and a simpler pattern emerged as shown in Fig. 1*B–G*. Performing the electrophoresis in urea starch-gels containing 0·02 *M* mercaptoethanol yielded identical patterns. Essentially similar patterns were obtained with four different batches of ceruloplasmin. However, when heterogeneous (possibly polymerized) ceruloplasmin[8] showing several bands by staining for oxidase activity and protein was used for similar experiments a more complicated pattern resulted. The simple pattern observed (Fig. 1*G*) would be consistent with the existence of several non-identical sub-units. Treatment of ceruloplasmin with mercaptoethanol alone (0·01–1·0 *M*) does not effect the electrophoretic and oxidase patterns in starch-gel (prepared in absence of urea). The amount of copper, determined after treatment with urea and subsequent dialysis, does not seem to be appreciably decreased. The same holds true for reduced and alkylated ceruloplasmin.

Further evidence of the dissociation of the ceruloplasmin molecule into several sub-units was obtained by ultracentrifugation in the presence of urea and by immunological methods. The apparent *S* rates of ceruloplasmin which had previously been reduced and alkylated in the presence of urea, and of ceruloplasmin treated with urea alone were found to be lower than that of untreated ceruloplasmin.

Moreover, material treated with urea (with or without reduction and alkylation) remained soluble after removal of the urea by dialysis. This behaviour may be contrasted with that of normal and abnormal γ-globulins which, after reduction and alkylation in the presence of urea, become insoluble on dialysis. When the material which had been treated with urea and afterwards dialysed was analysed by the Ouchterlony method, using highly specific rabbit anticeruloplasmin sera (the generous gift of Dr. J. Thorbecke-Hochwald, New York University) the presence of at least two immunologically active components could be detected as shown in Fig. 2. Since spurring occurs one may speculate that at least three antigenic determinants are present on the native molecule; however, additional lines of precipitation were observed, too faint to be reproduced. The results obtained were substantiated by immunoelectrophoresis in agar-gel. Identical results were obtained also with the reduced and alkylated materials. Furthermore, the immunological results were unaffected if both the Ouchterlony analysis and the immunoelectrophoresis were conducted on undialysed materials. A full report of these investigations will be given elsewhere. The immunological results described so far are suggestive of the existence on the protein molecule of several antigenic determinants which may be exposed by suitable treatment. Thus, enzymatic degradation of γ-globulin or albumin will expose new determinants[11–13]. A chemical method of degradation was used by Pope and Stevens[14] to uncover new antigenic groups of toxin of *Corynebacterium diphtheriae* and the results reported here suggest that chemical methods can, indeed, be used for such a purpose.

Electrophoretic results obtained by treatment with urea alone indicated that partial dissociation of the native ceruloplasmin occurred and that some of the

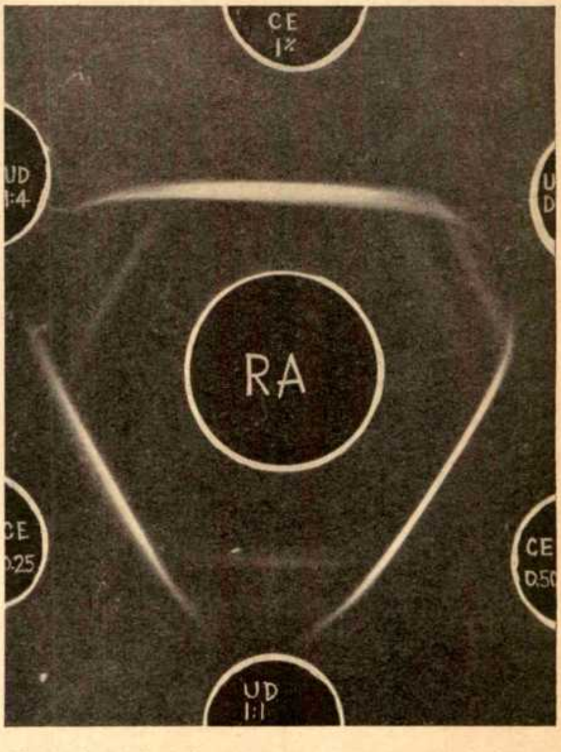

Fig. 2. Ouchterlony plate showing precipitin lines obtained with native and treated ceruloplasmin. *CE*, native ceruloplasmin (1, 0·5, 0·25 per cent); *UD*, ceruloplasmin treated with 8 *M* urea which was afterwards dialysed against normal saline (0·95 per cent) for 24 hr. at 4° C. and suitably diluted; *RA*, specific anticeruloplasmin rabbit serum

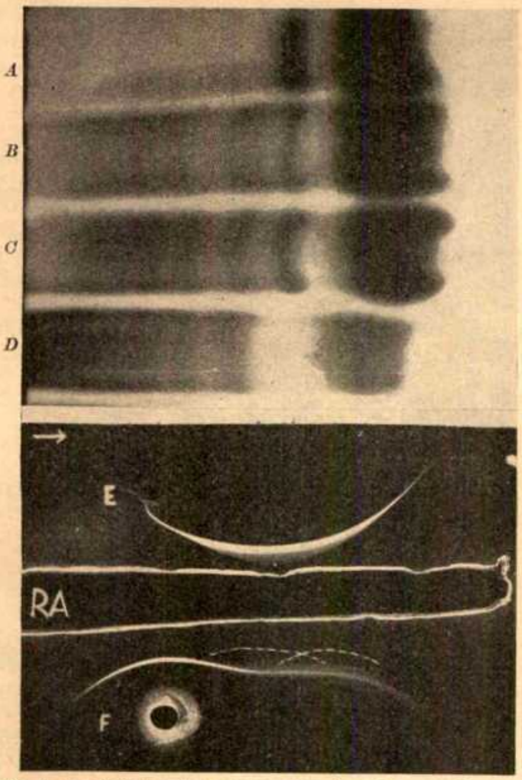

Fig. 3. Vertical starch-gel electrophoresis and agar-gel immunoelectrophoresis of ceruloplasmin treated with acetate, acetate-ascorbic acid and acetate–EDTA

Starch-gel patterns of ceruloplasmin: *A*, dialysed against saline; *B*, dialysed against 0·1 *M* acetate buffer (*p*H, 4·0); *C*, dialysed against 0·1 *M* acetate buffer in the presence of ascorbic acid (5 mgm./ml. of buffer); *D*, dialysed against 0·1 *M* acetate buffer in the presence of 0·1 *M* EDTA. Agar-gel immunoelectrophoresis patterns of ceruloplasmin: *E*, dialysed against saline; *F*, dialysed against 0·1 *M* acetate buffer in the presence of 0·1 *M* EDTA; *RA*, a specific rabbit anticeruloplasmin serum

sub-units are probably held together by weak forces. When the native ceruloplasmin was dialysed against 0·1 *M* acetate buffer (*p*H 4·0) or against either ascorbic acid or ethylenediamine tetraacetic acid (EDTA) in presence of the acetate buffer, profound changes of the electrophoretic patterns were noted. Thus, filter paper electrophoresis and agar-gel electrophoresis have shown two components instead of the usual one. Vertical starch-gel electrophoresis uncovered several new slower-migrating protein zones in addition to the two usually present as shown in Fig. 3. Using two-dimensional zone electrophoresis, the new slower-migrating zones were identified with the faster filter paper electrophoresis component. These results also show that ceruloplasmin can be rendered heterogeneous by such methods of treatment[9]. Furthermore, several new antigenic components were demonstrated by agar-gel immunoelectrophoresis, especially in the material treated with acetate–EDTA (Fig. 3*F*).

These preliminary results suggest that native ceruloplasmin can be dissociated into several non-identical structural sub-units. Investigations of the active side of the oxidase activity and the mode of copper binding should be facilitated once these sub-units are isolated. It is possible that genetic and pathological variants of this protein may be found at the level of its structural sub-units.

I am indebted to Dr. Oliver Smithies and Dr. G. A. Jamieson for their help in the preparation of this manuscript.

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LOCAL MEMBRANE CURRENT IN THE OUTER SEGMENTS OF SQUID PHOTORECEPTORS

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WHEN a quantum of light is absorbed by any of the millions of rhodopsin molecules in a dark-adapted human retinal rod there is a high probability that the cell will become excited[1]. Other photoreceptors may be equally sensitive to single photons[2]. The mechanism linking each of such a large number of photopigment molecules to the sensory output of one cell is not yet known; but two physical processes have been suggested as playing a part in conveying information about absorbed photons from the light-absorbing organelles of photoreceptors to the distal neurones of the visual system. The first is diffusion of light-created electrons and holes through the ordered structures in which photopigments are usually concentrated. It has been shown that such migration in the form of excitons (coupled electron-hole pairs) is improbable[3]; but photoconductivity may exist in rods under some conditions[4] and might contribute to the spread of excitation within outer segments (or to the flow of current through them). Its importance in living cells is still to be shown. A second suggestion is that light might release locally a chemical transmitter substance which diffuses to the dendrites of nearby neurones[5]. But the slow speed of molecular diffusion limits its range to a few microns during the short latency of vision, while the distal neurons of the visual system are often separated from the sites of light absorption by the much greater lengths of the receptor cells. Therefore, some additional mechanism seems required to account for signal transmission in long photoreceptors. A likely possibility is displacement of the cell membrane potential by light-induced flow of local membrane current, for such local currents are believed to produce the graded receptor potentials often seen in other kinds of sensory cells[6] and, in one case, to account for spread of excitation from sites where stimuli act to regions of the receptors where nerve impulses are generated or transmitter substances are released[7]. There is, in fact, evidence that some photoreceptor cells do depolarize when illuminated, although the source of the depolarizing current was not located[8].

This article reports some experiments which suggest that such locally originating receptor currents are capable of transmitting signals along both cell bodies and outer segments of squid photoreceptors.

The squid retina, like that of the octopus, is a packed array of long cells of which the photopigment-bearing processes (here called 'outer segments' by analogy with vertebrate rods and cones) are covered with piles of microvilli[9]. The outer segments are attached to nucleated cell bodies which lie just outside a dense layer of black pigment. From the cell bodies axones are given off which pass through a plexus and enter the optic lobe situated behind the eye[10]. Apart from the photoreceptors, only a few very small glial and pigment-bearing cells are present.

When live retinæ were isolated from dark-adapted animals (*Loligo pealii*) and sliced into strips about 100μ thick by cuts parallel with the long axes of the receptors, the strips survived and gave good electroretinograms for several hours in cold moist air (5°–10° C.) on the stage of an inverted microscope. In order to avoid light adaptation of the slices, all manipulations and observations were made in infrared radiation of wave-lengths 0·9–1·0μ rendered visible with the aid of photoelectric image converters. Micropipettes were inserted into the slices and zones in the layer of outer segments were stimulated with movable spots of red light 30–40μ in diameter. The positions of receptors, stimulus, and electrodes were recorded continuously with a motion picture camera photographing the screen of an image converter attached to the microscope. One frame from a record is shown in Fig. 1. The slice lies with the long axes of the receptors in the plane of the page. The tip of pipette *A* lies at the inner limiting membrane *L* while that of *B* has been thrust into the retina so that its tip (arrow) is surrounded by the proximal parts of the same group of outer segments which end at *A*. The cell bodies and axons lie below the pigmented layer *P*. The stimulus *S* enters the slice perpendicularly to the plane of the page, passing through parts of the outer segments of about 200 receptor cells.

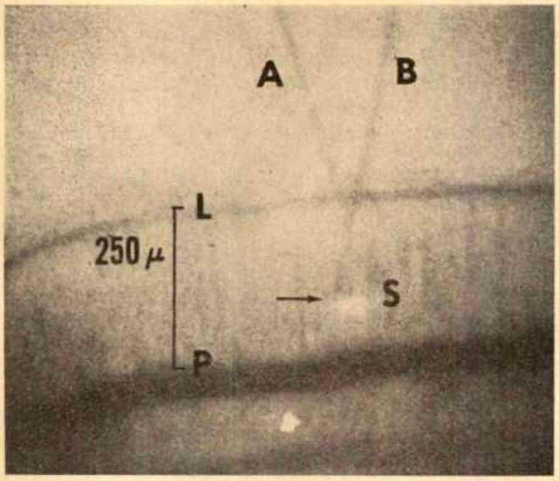

Fig. 1. Slice of living squid retina photographed with light of wave-lengths 900–1,000 mμ. Electrode *A* at inner limiting membrane *L*. Tip of electrode *B* at arrow in layer of outer segments just above pigmented zone *P*. Stimulus spot *S* consisted of wave-lengths 630–650 mμ

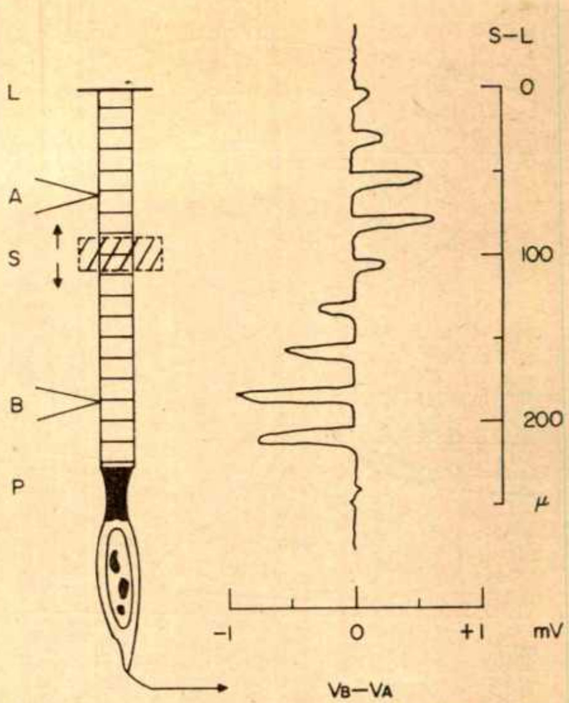

Fig. 2. Extracellularly recorded potentials in a retinal slice with stimuli at various depths in the layer of outer segments. Ordinate, distance of stimulus *S* from internal limiting membrane *L*. Abscissa, potential difference between electro tips. Stimulus intensity equivalent to 3×10^{11} photons cm.$^{-2}$sec.$^{-1}$ at 500 mμ. See legends of Figs. 1 and 3 for explanation of symbols

When two pipettes filled with sea-water and with tips 1–5μ in diameter were placed as shown in Fig. 1, no resting potential difference was recorded between them. This was not surprising, since the receptor outer segments are not more than 4–8μ in cross-section and the electrodes would be expected to create new extracellular spaces or enlarge existing ones by destroying some photoreceptors during penetration of the slice. However, when an intermittent light stimulus was moved along a line connecting the two tips, small graded slow potentials were seen which resembled the externally recorded electroretinogram[11]. The sign and amplitude of the potentials depended on the position of the spot, the tip nearest the light going negative to the one more distant (Fig. 2). An intermediate position was always found where the stimulus produced no potential difference. At this point the stimulus caused both electrodes to go equally negative relative to a distant indifferent electrode.

Detailed two-dimensional plots of the potential field in a locally illuminated slice showed a minimum confined to the illuminated region and a maximum spread out over the cell bodies the outer segments of which were in the path of the stimulus. Fig. 3 shows this for a stimulus placed near the tips of the outer segments. When the stimulus was moved deeper, the minimum remained centred on it, but the maximum did not shift in position. The topography of the field

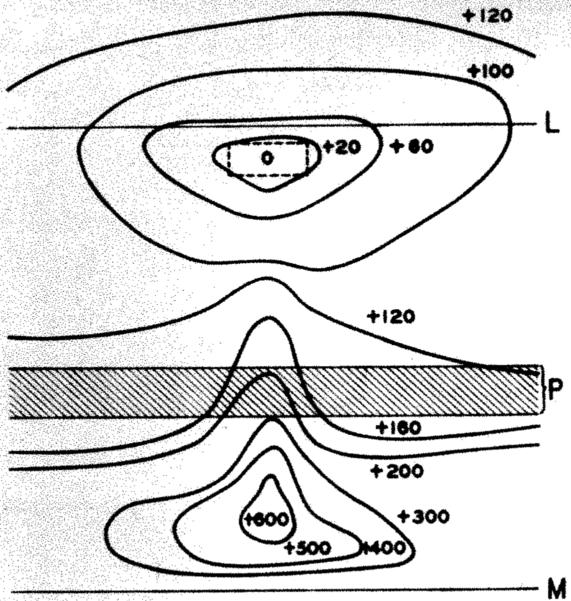

Fig. 3. Isopotential contours in a retinal slice illuminated near the tips of the outer segments. Internal limiting membrane at *L*, stimulus indicated approximately by dashed square. Cell bodies of receptors lie between pigment layer *P* and glial membrane *M*. Contours indicated in microvolts relative to potential at centre of stimulus. Plot was made by inserting a microelectrode into the slice in the direction from *L* to *M* and recording responses relative to an electrode at *L* as the stimulus was swept along a line parallel to *L*. Stimulus intensity equivalent to 6 × 10^{10} photons cm.$^{-2}$ sec.$^{-1}$ at 500 mμ

was independent of stimulus intensity below 10^{11} photons cm.$^{-2}$ sec.$^{-1}$ (500μ equivalent) because the amplitudes of the responses, like those of the electroretinogram[11b], were directly proportional to light intensity in this range. However, the region of negativity around the light became relatively larger when stimuli more intense than 10^{13} photons cm.$^{-2}$ sec.$^{-1}$ (500 mμ equivalent) were applied. This effect was believed due to saturation of the current-producing mechanism of the directly illuminated outer segments combined with excitation of surrounding cells by stray light scattered from the primary beam.

The fields observed were those to be expected if positive membrane current arose in the cell bodies of the illuminated group of receptors, flowed through the intercellular spaces and entered the outer segments where photons were actually being absorbed. Such a current should change the membrane potentials of the cells which produce it. Attempts were therefore made to record these changes directly with intracellular electrodes.

If a pipette with tip diameter less than 0·2μ and filled with potassium chloride solution (resistance 50–100 megohms in 3 *M* potassium chloride) was substituted for electrode *B* of Fig. 1 and carefully tapped into a slice, many regions were encountered in all retinal layers which had negative resting potentials of 10–50 mV. When an electrode was in such a region, illumination of the outer segments in line with its tip produced graded potentials of the usual form but much larger than those recorded with large pipettes filled with sea-water and always positive in sign. In bright light, the responses nearly abolished the resting potential but did not reverse it. Within 2 min. after penetration, the resting potential and light response usually disappeared, declining together until only a small but typical extracellular response from surrounding cells remained. Since the electrode resistance never increased more than 5 megohms when it was inserted into a region giving large responses, it is likely that the tip was in a photoreceptor, because their interiors formed the largest and most numerous spaces present. On this assumption, the space constants of the outer segments were estimated by measuring the amplitudes of the large responses elicited by stimuli at varying distances along the cells from the electrode tip. Values of 400–600μ were usually obtained. No intracellularly recorded action potentials were seen, perhaps because the photoreceptor axons were too severely depolarized by the injury associated with dissection and penetration; but it seems clear that light does produce local currents which markedly depolarize both cell bodies and outer segments and would be capable of initiating or modifying trains of spikes at the origins of the receptor axons.

The preceding results suggest that the action of light is to produce some local electrical change which allows current to flow into the outer segments within a few microns of the point where the photons are absorbed, but they say little else about what the change might be. However, some further information was obtained by light-adapting zones in the outer segments. When the tips of the outer segments the responses of which are shown in Fig. 2 were exposed to a flash containing 7·4 × 10^{14} photons cm.$^{-2}$ of wavelength 550 ± 10 mμ, the responses of the tips to subsequent test flashes were initially reduced more than tenfold, recovering their original responsiveness gradually over a period of 15 min. The unilluminated deeper parts of the same outer segments were not detectably affected by the adapting light. Adaptation of deeper zones produced corresponding effects. Intracellular recordings from the adapted cells showed no prolonged changes in their resting potentials, so that it is unlikely that their internal ionic composition was much affected by the light. Moreover, the adapting exposures were not great enough to activate more than 5 per cent of the rhodopsin in the illuminated zones, since the extinction coefficient of the squid photopigment is less than 20,000 cm.^{2} m.mol.$^{-1}$ at 550 mμ (ref. 12). This result is reminiscent of the drastic reduction in responsiveness of vertebrate retinæ by adapting exposures whose photochemical effects are minor[13].

Two alternative explanations can be offered for the ability of light to adapt zones in outer segments without depolarizing the receptors or destroying their photopigment. First, adaptation might alter the intercellular concentration of some ion which carries current into the receptors. The intercellular spaces seem very small in electron micrographs[9d] and their ionic composition could be greatly affected by a flow of charge that would leave the cytoplasm of the receptors almost unchanged. The 15-min. period required for an adapted zone to recover its sensitivity would then be due to diffusion of ions into the illuminated zones from neighbouring intercellular spaces. However, the diffusion of most small ions in water seems to be nearly two orders of magnitude too fast for this, and it would be necessary to suppose that diffusion is greatly retarded in the spaces between the outer segments. Local adaptation can also be explained in a second more abstract way. If the photopigment molecules were organized into functional units in which single absorbed photons inactivated at least 20 chromophores for a time, the observed adaptation would result. Further work will be necessary to assess the value of these suggestions; but it is clear, nevertheless, that the flow of the receptor current depends to a remarkable degree on highly localized changes induced by light in the outer segments of the receptors. The roles of electron-hole migration and diffusion of transmitter substances thus seem restricted to signal transmission through distances of only a few microns from the sites of light absorption, if indeed they play any part at all in visual excitation in the squid retina.

A detailed account of these experiments and of the electrochemical basis of the receptor current of the squid retina will be published separately. We thank the staff of the Marine Biological Laboratory, Woods Hole, Massachusetts, for their help during this work, and Mr. M. W. Klein of the Engineering Research Development Laboratories, U.S. Army Corps of Engineers, Fort Belvoir, Virginia, for the loan of infrared optical equipment. The opinions expressed herein are ours and are not to be construed as representing those of the U.S. Navy or the naval service in general.

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EXCYSTATION OF *EIMERIA ARLOINGI* (MAROTEL, 1905): STIMULI FROM THE HOST SHEEP

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ALTHOUGH excystation of various species of *Eimeria* has been investigated for many years, no completely satisfactory explanation of the mechanism has been advanced. In some avian species free sporozoites may be obtained by first mechanically rupturing the oocyst wall to release the sporocysts and then treating these with a trypsin solution (*E. tenella*[1]) or a trypsin-bile solution (*E. acervulina*[2]). Such treatments fail to produce free sporozoites of *E. bovis*[3] or of the species infecting sheep.

Initially in this work numerous attempts were made to produce *in vitro* excystation using mechanical means and also various enzymatic preparations, both natural and synthetic, in different combinations and *p*H-levels. In no instance did excystation occur.

As a possible explanation of these negative results, it was thought that at least two separate stimuli might be required, these occurring in sequence in the rumen or abomasum, and the small intestine. With this in mind sporulated oocysts were treated successively in fluid collected from the rumen, abomasum and small intestine. This produced a low percentage of excystation. Further work showed that a high percentage of excystation could be obtained by first introducing oocysts into the rumen enclosed in a 'Cellophane' sac via a fistula (following Sommerville's technique[4]), then incubating them in a solution containing trypsin and sheep bile. It was not shown that a stimulus from the abomasum was required.

These two stimuli, the first provided by the rumen and the second by the trypsin-bile solution, were then further examined.

Stimulus from the Rumen

Attempts to provide this stimulus *in vitro* by using ruminal fluid gave variable and generally unsatisfactory results unless incubated under an atmosphere of carbon dioxide. This suggested a close similarity to the stimulus for larval exsheathment described by Rogers and Sommerville[5] and Rogers[6]. After application of this ruminal stimulus the only visible change that could be seen in the oocysts was a lifting or splitting of the cap over the micropyle. That a further change had occurred was demonstrated by their ability to excyst if afterwards incubated in a trypsin-bile solution. Other evidence of change include: the collapse of the oocyst contents when placed in a hypertonic solution, for example, of sodium chloride, normally used for floating oocysts; the staining of the inner wall (but not the sporocysts) a characteristic purple with methylene blue; the destruction of the oocyst and contents when placed in a solution of sodium hypochlorite. It is postulated that the stimulus activates an enzyme system within the oocyst which chemically alters both the inner wall of the oocyst and a supposed outer coat of the sporocyst.

The nature of this ruminal stimulus was investigated *in vitro* using methods similar to those described by Rogers[6]. The results in Table 1 show some of the features of the stimulus. All preparations were incubated at 39° C.

These results clearly indicate that carbon dioxide is the essential part of the stimulus. The addition of bicarbonate does not enhance its action, but anaerobic and reduced conditions do.

It was observed that when isotonic buffer solutions were used, subsequent attempts to excyst the oocysts resulted in the liberation of the sporozoites from the sporocysts, but although actively motile they were unable to escape from the oocyst. With dilute buffers the sporozoites were capable of leaving the oocyst through the micropyle. Sodium bicarbonate solution of 0·1 *M* and above acted similarly to isotonic solutions. Solutions more dilute than this resulted

Table 1. INDICATING THE ABILITY OF SOME GAS MIXTURES UNDER DIFFERING BUFFERED AND REDUCED CONDITIONS TO PROVIDE THE RUMINAL STIMULUS

Buffer	Gas phase	pH	Reducing agent	Oocysts activated per cent
Bicarbonate	Air	8·3	–	0
Phosphate	N_2	6·8	–	0
Bicarbonate	N_2	8·3	+	0
,,	N_2	8·3	–	0
,,	15 per cent CO_2: air	6·8	–	16
Phosphate	15 per cent CO_2: N_2	6·8	–	49
Bicarbonate	,,	6·8	–	47
,,	,,	6·8	+	64

in free sporozoites, the effect being augmented with increasing dilution. Complete excystation of those oocysts incubated in isotonic buffers could be obtained if they were washed in water prior to incubation in the bile-trypsin solution. The significance of these observations is discussed below.

Oocysts used in the following experiments were allowed to sporulate in distilled water. Oocysts which sporulated in a potassium dichromate solution produced somewhat different results as will be described here. Unsporulated or incompletely sporulated oocysts do not react to the ruminal stimulus.

(1) *Effect of carbon dioxide.* Fig. 1 indicates the increasing activity of the stimulus with increasing concentration of carbon dioxide in the gas phase. The type of buffer does not appear to alter the result significantly; anaerobic and reduced conditions do. Results for carbon dioxide and oxygen mixtures were essentially similar to those given for carbon dioxide and air. Although this action was first investigated as a stimulus from the rumen, it is possible that conditions in the small intestine may also be adequate to initiate the stimulus. Very high concentrations of carbon dioxide were not inhibitory.

(2) *Effect of temperature.* The control that temperature has on the stimulus is shown in Fig. 2. No activity occurred at 30° C. With temperatures above 46° C. the stimulus was effective, but viable sporozoites were not obtained.

(3) *Effect of reducing agents.* The addition of a reducing agent to the buffer solution generally increased the effectiveness of the stimulus as shown in Fig. 1. Three chemically unrelated agents—sodium dithionite, cysteine and ascorbic acid—were used. All were effective.

(4) *Effect of sodium chloride.* The addition of sodium chloride in increasing amounts from 0·01 to 0·2 *M* to the buffer solution did not significantly affect the carbon dioxide stimulus.

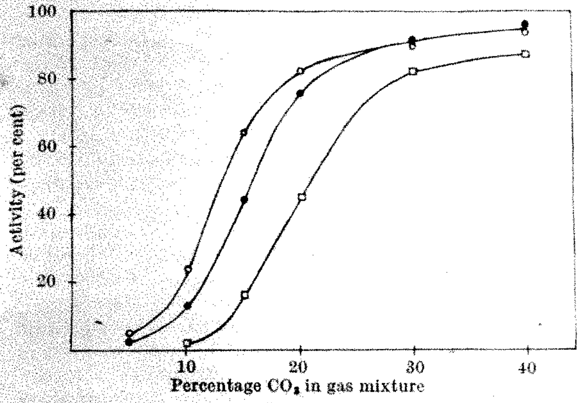

Fig. 1. Effect of increasing concentrations of carbon dioxide under aerobic, anaerobic and reduced conditions on the proportion of oocysts activated by the stimulus. Experiments were in sodium bicarbonate buffer at pH 6·8. Cysteine–hydrochloride 0·02 *M* was used as the reducing agent. Incubation at 39° C. for 8 hr. The measure of 'activity' was determined by the ability of oocysts to excyst when incubated in a trypsin-bile solution. □, CO_2 : air ; ●, CO_2 : N_2 ; ○, CO_2 : N_2 + cysteine

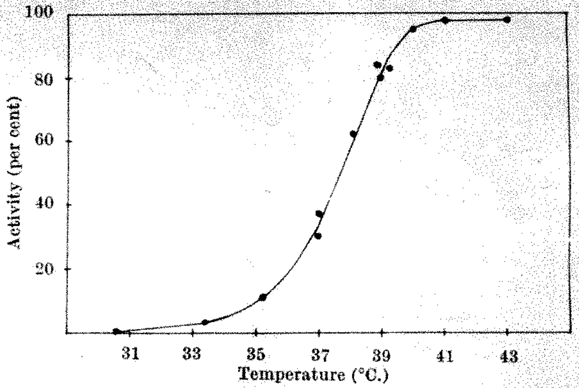

Fig. 2. Effect of temperature on the proportion of oocysts activated by the stimulus. Experiments were in sodium bicarbonate buffer at pH 6·8 with 20 per cent CO_2 : N_2 mixture incubated at 39° C. for 12 hr. The measure of 'activity' was determined by the ability of oocysts to excyst when incubated in a trypsin-bile solution

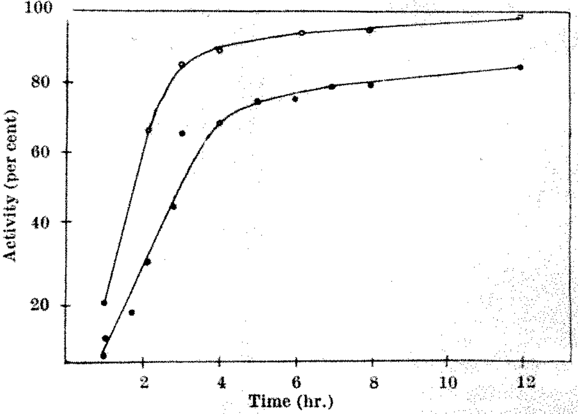

Fig. 3. Effect of time on the proportion of oocysts activated by the stimulus. Experiments were with CO_2 : N_2 gas mixtures and sodium bicarbonate buffer to give pH 6·8. Incubation at 39° C. The measure of 'activity' was determined by the ability of oocysts to excyst when incubated in a trypsin-bile solution. ○, 40 per cent CO_2 ; ●, 20 per cent CO_2

(5) *Time required for the stimulus to act.* Fig. 3 shows the effect of time on the proportion of oocysts activated by the stimulus. Most were affected after 6 hr., but with continued incubation the proportion was slowly increased.

(6) *Effect of potassium dichromate.* Oocysts which sporulate in potassium dichromate and are then subjected in turn to the ruminal stimulus and to incubation in a trypsin-bile solution show a lower excystation-rate than oocysts sporulated in distilled water. It is noteworthy that although a lower percentage excyst, and also a lower percentage show the characteristic purple staining with methylene blue, the destructive action of sodium hypochlorite affects a similar proportion of oocysts which have sporulated in potassium dichromate to those which have sporulated in distilled water. This would suggest that potassium dichromate does not affect the oocyst's ability to be influenced by the ruminal stimulus, but does reduce its permeability to the enzyme solution providing the second stimulus, possibly by an effect on the cap or micropyle.

Stimulus from the Small Intestine

The essential components of this second stimulus are trypsin and bile. In the foregoing experiments an excysting fluid of 0·25 per cent trypsin 1 : 250 and 10 per cent sheep bile in a phosphate–buffered balanced salt solution at *p*H 7·4 was used. Oocysts previously subjected to the ruminal stimulus will begin to excyst when incubated in this medium. The sporozoites become motile within the sporocysts, and, with the disappearance of the Stieda body, escape fairly rapidly into the cavity of the oocysts. They then proceed to move actively round the oocysts, their anterior end frequently making contact with the micropyle. In oocysts treated in water or buffers of low molar strength, the sporozoites push their way through the micropyle and escape. Following incubation the first free sporozoites are found after about 0·5 hr., the process continuing for some hours.

Both trypsin and bile must be present for the second stimulus to be effective. The stimulus was effective if the oocysts were added to a bile solution first, then, following washing, to a trypsin solution. It was not effective, however, if this procedure was reversed.

Trypsin may be replaced by papain. Sodium taurocholate (British Drug Houses) and 'Tween 80' had an action similar to bile. To ensure viability of the sporozoites the osmotic pressure and *p*H of the excysting fluid must be kept within physiological limits.

It is considered that the *in vitro* stimuli described here are essentially those which *in vitro* result in excystation. It is most probable that the initial stimulus in which carbon dioxide is the essential factor is effected within the rumen because relatively high levels of carbon dioxide are required. This is comparable with the stimulus for exsheathment of *Haemonchus contortus* larvæ[6]. Bile and trypsin, which constitute the second stimulus, would normally be encountered by oocysts in the small intestine. *In vitro* treatment with buffers of low osmotic pressure or washing in water is essential if escape of sporozoites from the oocysts is to be effected. Such treatment presumably causes the rupture of the thin membranous inner wall underlying the micropyle following its chemical alteration by the ruminal stimulus. Additional enzymes such as pepsin, pancreatin and lipase were not effective in replacing this step. It is considered that *in vivo* this may be accomplished by the physical conditions encountered by the oocysts within the gastro-intestinal tract, such as changes in hydrostatic pressure. It was noticed that oocysts within a 'Cellophane' sac in the rumen would start to break up after some hours. Hammond[3] reported the same observation on oocysts in 'Cellophane' sacs in the large intestine of the calf.

A limited number of non-quantitative observations were made on other species of *Eimeria* infecting sheep including *E. parva*, *E. intricata*, *E. faurei* and *E. nina kohl yakimovae*. It is probable that the sequence of excystation is essentially the same in all these species.

Limited observations on *E. tenella* from the fowl and *E. stiedae* from the rabbit suggest that the process of excystation in these species may be similar to those infecting the sheep. It would be interesting to compare quantitatively the stimuli required by species infecting different animal species.

I thank Prof. H. R. Carne and Mr. Sommerville for advice; and Prof. W. P. Rogers for helpful discussion.

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ÆTIOLOGY OF 'WET-BELLY' IN RANCH-BRED MINK

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THE external symptom of 'wet-belly disease' in mink is denoted by an area of wet, matted fur, surrounding the opening of the prepuce in male mink. This may vary in extent from 1 in. in diameter to an area extending over the whole abdominal surface. This is caused by a dribbling of the urine into the fur (Fig. 1). Because of the anatomical differences in the sexes, wet-belly is not common in female mink.

'Wet-belly disease' has become of considerable economic importance because of its increased incidence and the marked devaluation it causes in mink pelts. The latter results from the unprime, pigmented state of the leather (Fig. 2) underlying the matted, and frequently stained, fur in the affected area of skin over the abdomen.

This 'disease' has been attributed to a number of causes, including acidosis[1], nutritional anæmia[1], urinary calculi[2], and biotin deficiency[3]. Wet-belly was not correlated to ration composition[4], but to feeding of whole fresh-water fish[5], or feeding rations high in fat content[6,7], and to stress caused by handling[8]. Methods as being effective in preventing the condition include feeding choline[6,7] and feeding cooked potatoes[9].

Ranchers also noted that when high proportions of chicken waste, fish and tripe were used to replace horse meat in mink rations, 'wet-belly disease' became a major problem, with the number of defective pelts reaching 20–30 per cent of the kit male crop.

In 1960, experimental work was initiated at the Experimental Fur Ranch, Summerside, Prince Edward Island, Canada, on this condition. It was readily produced in 40–70 per cent of kit male mink by feeding rations containing high proportions of raw chicken waste, whole fish and beef tripe from August to December. (The ingredients of the rations were (per cent): chicken waste, 28·75; fish (small flounders and tom cods), 28·75; tripe, 5; horse meat, 15; cereal, 7·5; liver, 10; brewers' yeast, 0·5; milk powder, 0·5; canned tomatoes, 3·75; cod liver oil, 0·25.) The occurrence of the 'disease' coincided with the furring season and the onset of cold weather in the month of November. The incidence of the disease among 144 female mink receiving the wet-belly-inducing rations was less than 1 per cent.

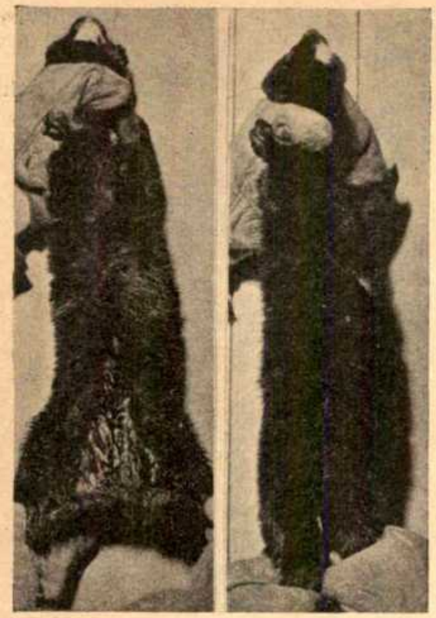

Fig. 1. Left, wet-belly disease; right, normal

The average body-weights of 33 male mink with 'wet-belly disease' and 39 normal mink on the same rations were not significantly different in the early stages of the disease on November 21 (1·58 and 1·61 kgm., respectively).

In two other experiments, using similar rations, the addition of 10 per cent fat (lard) caused no significant increase in the incidence of wet-belly among 144 male kit mink (1960 and 1961 seasons). The incidence was 27 cases of 'wet-belly' on the fat-supplemented rations and 31 cases on the rations without added fat.

Attempts to prevent 'wet-belly' among the above-mentioned mink, by the addition of a supplement containing buffering minerals, vitamins (including choline), lipotrophic factors, coenzymes and an anti-oxidant (supplement: dicalcium phosphate, sodium and potassium bicarbonate, magnesium sulphate, fumarate, aspartic acid, asparagine, cysteine, adenylic-5-acid, calcium pantothenate, choline, betaine, inositol, cyanocobalamin, bonemeal, milk powder, bran and wheat germ) were not effective. Attempts to cure the condition after its onset by the use of an antibiotic (chloromycetin), a sulphonamide (sulpha methoxypyridazine) and a chemical genito-urinary antiseptic (furadantin) gave inconclusive results with 82 cases of 'wet-belly disease'. It was noted in this investigation that the 'disease' had a high spontaneous resolution factor, and that frequent recoveries and relapses occurred among the male mink maintained on the 'wet-belly'-inducing ration throughout the winter and spring seasons.

The pathological findings were chiefly confined to an inflammation of the glans penis, terminal urethra and prepuce or sheath of the affected animals. In conjunction with these findings, it was also observed that unsheathing of the inflamed penis through the inflamed and constricted opening of the prepuce was painful. This is thought to cause the mink to allow the urine to dribble from the prepuce cavity through its opening on to the belly fur, giving rise to the external manifestation of the 'disease'. No bladder stones were found in the mink with 'wet-belly disease' on autopsy.

Examination of the urine showed no differences in acidity (pH 6·2 and 6·3) between 'diseased' and normal males. Negative findings were also obtained for the presence of significant amounts of glucose and albumin in urine. Histopathological sections showed no evidence of cystitis, but some kidney damage was present in the kidneys of the 'diseased' mink.

Bacteriological investigation of male mink with active 'wet-belly disease' showed that nine of ten animals examined were infected with *Proteus* organisms in the prepuce cavity, whereas ten normal male mink gave negative findings. Other bacteria of the coliform group, *Staphylococcus*, *Streptococcus fecales* and *Salmonella* organisms were variably present in normal and 'diseased' mink.

The bacterial smears from the prepuce cavities in mink with active 'wet-belly disease' were usually significantly more heavily infected with bacteria than corresponding smears from normal mink or from those which had recovered from the disease. In the latter, coincident with the diminution of contamination by bacteria, there was a significant disappearance of *Proteus* organisms and inflammation.

To determine whether the *Proteus* strains isolated from the prepuce cavity had infected the mink, agglutination tests were carried out with the blood sera of mink which were known to have had 'wet-belly disease' and to have recovered from it. Blood sera from seven of ten of such mink showed significantly high antibody titres (160–1,280) to a strain of *Proteus mirabilis* recovered from the prepuce cavities of the above mink. Conversely, all the sera from the above male mink gave negative agglutination tests to a strain of *Proteus vulgaris* recovered from one of them. As controls, blood sera were taken from six female mink which had received the 'wet-belly'-inducing rations for a period of six months, but which had not developed 'wet-belly' condition. These gave completely negative agglutination tests to the pathogenic strain of *Proteus mirabilis*.

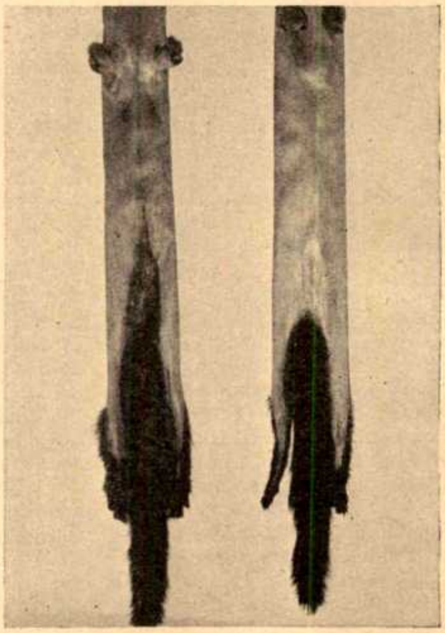

Fig. 2. Left, pigmented leather from wet-belly disease; right, normal leather

To learn the route of transmission of the *Proteus* organisms to the prepuce cavity, a bacteriological examination was made of the fæces of mink that had recovered from 'wet-belly disease'. *Proteus mirabilis* was found in the fæces of these mink, suggesting that the 'disease' originates via the intestinal tract. The fæcal strains of *Proteus mirabilis* gave the same morphological, cultural and biochemical tests and agglutinated to the same titre (with the same mink sera) as the original strain of *Proteus mirabilis* obtained from the prepuce cavities of these male mink.

Further examination was then made of the different meat and fish products of the 'wet-belly'-inducing rations for the presence of these organisms. *Proteus mirabilis* was found to be present in relatively high concentrations in the raw chicken waste, whole fish and tripe used in these rations. Also, it gave the same bacteriological tests, and was agglutinated by the sera taken from the male mink which had recovered from the 'disease'. On the other hand, five different samples of horse meat, which had not caused 'wet-belly disease', gave negative tests for the presence of *Proteus* organisms.

In another experiment, 48 kit mink (24 males and 24 females) were fed the above-mentioned 'wet-belly'-inducing ration (control); a second similar group was fed the same ration, except that the chicken waste, fish and tripe had been cooked; a third similar group was fed the same ration, except that the chicken waste, fish and tripe had been deep frozen to −10° to −20° F.

The incidence of 'wet-belly disease' was decreased 50 per cent by cooking and 44 per cent by freezing, when compared with the number of cases found on the control ration. No cases of 'wet-belly disease' occurred in the 72 females on the experiment.

A further experiment was carried out with 32 male mink kits. These were given a standard horse-meat ration, which could be expected to produce no 'wet-belly disease'. The rations of 16 of these mink were heavily contaminated daily with *Proteus mirabilis* organisms during August–December. 'Wet-belly disease' had occurred by December in eight of the 16 mink receiving the infected feed, but in only one mink of the 16 receiving the same but non-infected ration. Bacteriological smears showed that in 50 per cent of these mink with 'wet-belly disease', *Proteus mirabilis* could be readily recovered from the prepuce cavity.

Because at this Station the onset of visible external symptoms of this disease were not found to occur until the month of November, coincident with the onset of colder weather and furring of the mink, it would therefore appear that these may be stress factors which predispose the mink to infection by lowering their resistance. However, the mink at this Station had been caught and weighed weekly or bi-weekly during a period of 11 years (prior to the feeding of a 'wet-belly'-producing ration which resulted in 40–70 per cent occurrence of the 'disease' in 1960 and 1961) with less than 1 per cent incidence of this 'disease' among kit males.

Although further work is in progress, the experimental findings to date suggest that the 'disease' is a monovalent or polyvalent infection caused by *Proteus* organisms or/and other potential pathogens present in chicken waste, whole fish or tripe. These organisms have been found in high concentrations in such feedstuffs and are thought to infect (under suitable environmental conditions) young growing mink which may have a relatively low resistance to such potential pathogenic bacteria.

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IMMUNOLOGICAL PROPERTIES OF PHOTODYNAMICALLY INACTIVATED TUMOUR CELLS

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THE oxidation of biological substrates as mediated by the action of visible light and certain dyes has been termed 'photodynamic action'[1]. The photodynamic inactivation of tumour cells results in their inability to produce tumour growth on subsequent transplantation[2]. As an extension of this work it was of interest to inquire whether it is possible to induce an immune state by the inoculation of tumour cells thus attenuated by the action of light and dyes.

In *C3H* mice which have received an injection of viable *6C3HED* tumour cells, and which therefore harbour an actively growing tumour, a challenging dose of the same tumour at a different site is invariably rejected. Such a second-set reaction cannot be demonstrated in *C3H* mice which were originally injected with photodynamically inactivated tumour cells. Table 1 illustrates the results obtained in a representative experiment (for details as to materials and method of procedure see ref. 1). Four groups of twenty male *C3H/Hej* mice were injected subcutaneously on the right side, each with 500,000 *6C3HED* tumour cells. Prior to injection the cells had been treated as shown in Table 1. As expected, the cells which had been illuminated with white light (from a

Table 1. IMMUNOLOGICAL PROPERTIES OF *6C3HED* CELLS

Day 1	Day 12*	Day 19		Day 21		Day 25	
Cells injected subcutaneously on right side:	No. of mice in which tumour palpable on side						
	R	R	L	R	L	R	L
Saline, white light 15 min.	20	20	0	20	1	all dead	
10^{-5} *M* acridine orange, dark	20	20	0	20	0	all dead	
10^{-6} *M* acridine orange, white light 15 min.	0	0	0	0	18	0	20
10^{-5} *M* acridine orange, white light 15 min., cells suspended in Freund's adjuvant	0	0	0	0	17	0	20

* Twenty mice in each group; on day 13, 750,000 viable *6C3HED* cells injected subcutaneously on left side.

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* Public Health Service Fellow of the National Cancer Institute.

slide projector) and those which had been treated with dye in the absence of light were viable, and induced tumour growth at the site of injection in all animals. Those cells which had been exposed to both dye and light failed to induce tumour growth, as did similarly treated cells which had been re-suspended in Freund's adjuvant. Thirteen days after the original injection, all the mice received a second implantation, this time on the left side, consisting of 750,000 viable tumour cells. Eight days later only one of the animals in the group harbouring actively growing tumour on the right side had developed additional growths on the left (at autopsy this one growth proved to be an extension of the original tumour), whereas most of the animals which had no primary tumour had developed growths on the left side. The use of Freund's adjuvant did not enable photo-inactivated cells to induce immunity in the host.

Swiss mice which bear sarcoma 180 in the tail are about 60 per cent immune to a challenging dose of viable tumour cells injected a week after the tails have been amputated[3]. This immunity cannot be demonstrated in similarly treated mice which originally received tail injections of photodynamically inactivated tumour cells. Table 2 summarizes data on such an experiment. Ninety Swiss mice (obtained from the pathogen-free colony at the State University of New York, Downstate Medical Center, males and females equally divided among all three groups) were injected subcutaneously in the tail with 0·1 c.c. of a slurry of *S*180 cells, which had previously been treated as indicated in Table 2. Tumours did not develop in the tails of those mice which had received photo-inactivated tumour cells. Eleven days after injection the tails of all animals were amputated, and the stumps were allowed to heal. On day fourteen all mice received 500,000 viable tumour cells intraperitoneally. It can readily be seen that those animals which had received photodynamically inactivated cells were not immune to the challenging injection.

Table 2. IMMUNOLOGICAL PROPERTIES OF SARCOMA 180 CELLS

Day 1	Day 11*	Day 14	Day 64	
Cells injected subcutaneously into tail:	No. tumours in tail	developed ascites	No. mice surviving	Immune (per cent)
Saline, white light 15 min.	23/26	11/26	15/26	59
10^{-5} *M* thionine, dark	24/30	13/30	17/30	57
10^{-5} *M* thionine, white light 15 min.	0/30	30/30	0/30	0

* All tails amputated on day 11; on day 14, 500,000 viable *S*180 cells injected intraperitoneally.

Other workers[4-6] have shown that tumour cells can retain the power to immunize, even though they are no longer capable of progressive growth. Thus sarcoma 180 cells which had been irradiated *in vitro* with moderately heavy doses of X-rays (2,000 r.) could no longer induce tumour growth on transplantation, but conferred immunity to a challenging dose in 95–100 per cent of the animals. When irradiated with doses of 60,000 r. the tumour cells lost this immunizing ability as well[4]. Slightly higher figures were found[5] for achieving comparable effects in the case of a *C3HB* tumour in *C3H* mice. Irradiation for ten minutes with white light from a 500-W. lamp in the presence of 10^{-5} *M* thionine is sufficient to inactivate tumour cells[2]. During this time approximately 30 joules are absorbed per ml. of the suspension. This dose is sufficient to impair both the viability (as shown by lack of tumour growth on transplantation) and the immunizing ability of the cells, and is thus equivalent in action to a dose of 60,000 r. needed to achieve the same result with X-rays.

Tumour cells inactivated by photodynamic means are not able to induce immunity in the host-line in which they originated. This presumably indicates that under the above conditions the photodynamic process produces qualitative changes in the tumour cells which makes them antigenically different from viable tumour cells. It is interesting to note that most of the dyes which cause photodynamic inactivation of tumour cells have a strong affinity to RNA and DNA, and it is tempting to speculate that the photodynamic alteration of the antigenic properties of these cells was specifically mediated by their inactivation of protein-synthesizing systems in the cell. It would be of interest to investigate the (possibly selective) inactivation of the enzyme formation in bacterial cells by selected dyes in order to test this possibility.

These investigations were performed in the laboratory of Dr. S. C. Mohos at the State University of New York, Downstate Medical Centre. I thank him for his helpful suggestions.

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A SEX DIFFERENCE IN EFFECT OF TEMPORAL-LOBE NEUROSURGERY ON DESIGN PREFERENCE

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THE human brain has a pronounced asymmetry of function: one hemisphere, generally the left, is clearly 'dominant' for speech[1], and the other is possibly more important for perception[2,3]. The present investigation, part of a project on the psychological changes following temporal-lobe surgery, was concerned with a test of artistic aptitude and the impairment that should occur after removal of cerebral tissue from the non-dominant side. The neurosurgery produced asymmetrical effects of this kind; but, unexpectedly, they were found only in men; the lateralized effects in women were reversed.

Twenty-two patients with temporal-lobe epilepsy were tested on the Graves design judgment test before and again 13–31 (mean 17) days after undergoing unilateral temporal-lobe surgery for the relief of their epilepsy[4]. Forty-two additional cases were tested for the first time 1–8 (mean 4·2) years after such surgery. The age at surgery for the 64 cases was 11–57 (mean 31) years. In one man and two women

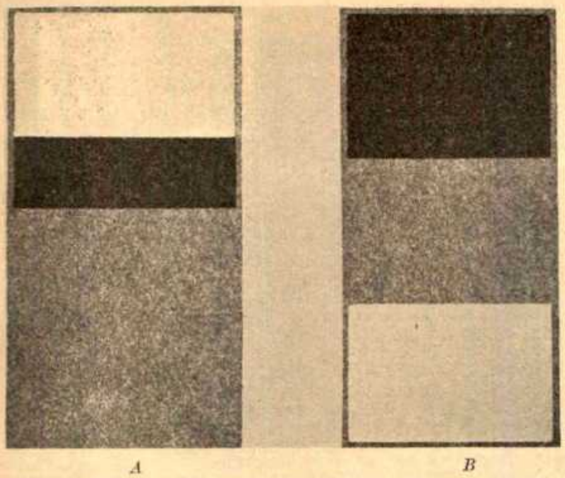

Fig. 1. Representative item (No. 46) from the Graves design judgment test. *A* is the more artistic design

the dominant hemisphere for speech was on the right side according to the 'carotid "Amytal" test'[5]; their operations were on the left. One of these women was in the group tested only after surgery.

The Graves test was designed to measure "certain components of aptitude for the appreciation or production of art structure"[6]. The test consists of 90 items in a booklet; for each item, the subject is required to choose the preferred design from two, occasionally three, printed on a page (an example is shown in Fig. 1). The designs are abstract with little or no 'meaningful' content; the inartistic ones contravene principles of æsthetic order and are often symmetrical designs or nearly so. The only colour used, and not in all items, is a greyish green. Preferences are marked on a standard answer sheet. The test is not timed.

The effect of the surgery on the patients' preferences appeared to be negligible until the sex of the patients was taken into account (Table 1). The group of men with resections in the non-dominant hemisphere showed a drop in mean aptitude score after the operations, whereas the women with non-dominant operations showed a rise. Conversely, with operations in the dominant hemisphere, the men showed a rise in score while the women showed a drop. An analysis of variance showed that this interaction of sex, side and change with operations was significant ($P < 0·01$). Since the rank order of the four means after the operations was the same as before, it might appear that the operations accentuated differences which were already present. However, separate analyses of the pre- and post-operative scores indicated a significant interaction of sex and side only after operation ($P < 0·01$).

Three patients had changes in score which were opposite to the general pattern described, and two patients showed no change. For three of these five cases the atypical nature of the operations seems to be pertinent to their inconsistent behaviour. The ablation on the non-dominant side in a man who showed no change in score was done in two stages nine months apart; his score might have dropped had he been tested after the first stage. The operations on the dominant side in two men who showed a drop in score were also unique: one was performed six days after a simultaneous exploration of both temporal areas, the other was interrupted by a laryngospasm and the brain bulging for a few minutes through the cranial opening. Possibly these operations entailed some disruption of functions on the non-dominant side.

Table 1. PRE- AND POST-OPERATIVE MEAN SCORES ON THE GRAVES TEST

Sex and temporal lobe operated	No. of cases	Pre-operative mean	Post-operative mean
Men, non-dominant	8	35·4	28·0
Women, non-dominant	5	41·8	47·2
Men, dominant	7	41·0	46·9
Women, dominant	2	31·5	26·5

A chance score would be 44; the published means for the test range from 41·0 for 106 non-art high school students to 77·3 for 54 students in their third year in an art school (ref. 6).

The amount of tissue removed in the 22 cases was often less than that usually reported for this type of operation, measuring about 3–5½ cm. from the tip of the lobe on the right and about 2½–4½ cm. on the left, and sometimes gyri or the tip of the lobe were spared. The relation between estimates of the amount removed and the amount of change in scores tended to be inverse although not statistically significant.

The low scores following some of the operations indicate a marked preference for designs which are symmetrical or otherwise too simply structured. These patients could be displaying the concreteness and simplicity in perception often reported in patients with gross brain damage. The better scores after other operations might be a result of the removal of disruptive epileptogenic tissue from the other side. An interpretation of the better scores as a result of practice seems unlikely because no general tendency for improvement was found in nine other cases that had exploratory operations with no removal of tissue.

The changes on the Graves test do not appear to be 'intellectual' in so far as they were not found to be related to changes in the scores on the Wechsler–Bellevue intelligence tests. There was an expected significant drop[2] in the mean of the total weighted scores for the six 'verbal' sub-tests after the operation in the dominant hemisphere ($P < 0·02$, one-tail test).

Although the post-operative changes on the Graves test are clear, they appear to be transitory. No significant relation to the surgery was discernible either for sex or side of operation in the group of 42 patients tested a year or more after their operations. The mean score for the 15 women (42·7) was slightly, but not significantly, higher than the mean for the 27 men (38·2). Furthermore, 11 of the original group of 22 patients have been tested a third time, with a mean lapse of 17 months since their operations. Ten showed a change opposite to that shown on the first post-operative test; three patients who got better scores on their first post-operative test dropped on the third testing, and the other seven dropped, then rose. A woman whose score was unchanged after a non-dominant operation scored lower on the last test. Finally, combining the data from these 11 cases on their third testing with the data from the other 42 cases also yielded no significant differences. That the test still did measure something in the latter group of 53 cases is indicated by a low, but significant, correlation with intelligence ($r = 0·47$).

The results obtained with the women, unlike those with the men, do not conform to the hypothesis that an impairment in some aspect of human visual perception occurs after removals from the temporal lobe of the non-dominant hemisphere. A similar failure of results for women to agree with those for men has been found in an examination of the effect of dominant temporal-lobe operations on an objective test of proverbs: the women were unaffected and the men dropped in score after operation[7]. The varying proportion of women to men in previous investigations may have been partly responsible for the varied success in demonstrating lateralized effect of temporal-lobe surgery[8].

The effects of the operations suggest that some physiological mechanisms underlying artistic judgment and verbal ability may overlap in the female brain but are in opposite hemispheres in the male. Some data in the psychometric literature could be interpreted in the same way: girls show positive correlations between their level of artistic interest and their competence on tests of verbal reasoning and language usage, whereas boys do not[9].

Although the Graves test is considered to be an aptitude test, it is not like the usual speed test which measures efficiency of perception; it is not timed and the subject is not asked to choose 'correct' answers. The Graves test is probably measuring a factor called 'liking for simplicity versus complexity' which has been demonstrated in several experiments on form preferences[10]. Temporary changes in an 'interest' factor as a result of neurosurgery might have been expected. But it is surprising to find that the function of each temporal lobe in regard to this trait appears to be opposite in each sex.

I thank Dr. Maitland Baldwin, clinical director of this Institute, for encouraging psychological research on his patients, and Dr. E. J. Laskowski for supplying the results of the carotid 'Amytal' tests.

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NEW RESULTS ABOUT THE MECHANISM OF PHOTOSYNTHESIS

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A REACTION scheme of photosynthesis derived from analysis of short-timed absorption changes has already been devised[1-3]. Further flashing light measurements lead to an extended scheme as presented in Fig. 1.

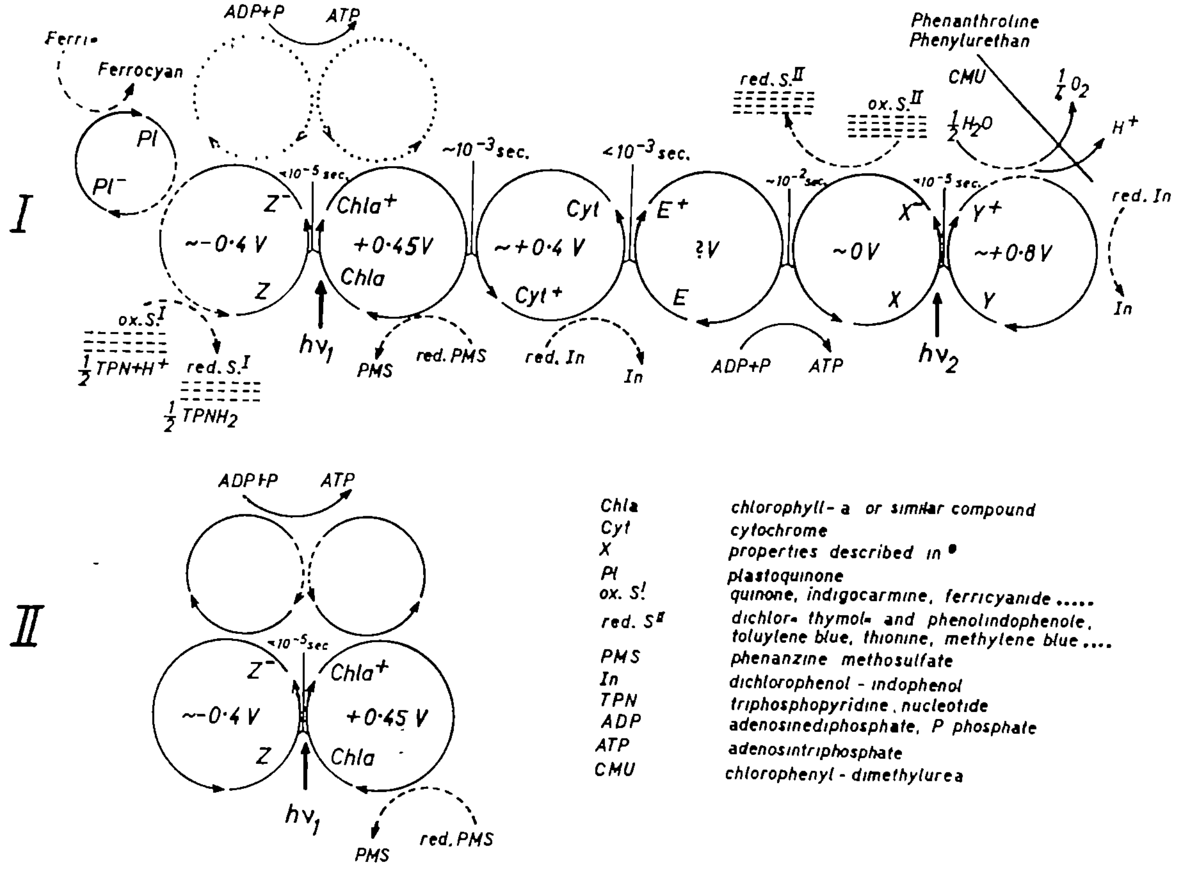
Cytochrome oxidation Cyt → Cyt$^+$ is characterized by changes in absorption around 405, 420 and 555 mμ. In green plants under normal conditions corresponding changes are observable only at 420 mμ. It was supposed that these are caused by cytochrome and that the smaller ones at 405 and 555 mμ are incidentally compensated by changes caused by $X \rightarrow X^-$. Extended measurements between 400 and 700 mμ give no evidence that $X \rightarrow X^-$ causes changes other than those around 475 and 515 mμ. Therefore, the changes at 420 mμ are obviously not caused by cytochrome. However, we found that typical changes (at ~ 405, ~ 420, ~ 555 mμ) caused by Cyt→Cyt$^+$ are always observable when the reduction of Cyt$^+$ is delayed. Such a delay can be caused in different ways: (*a*) By exciting *Chlorella* or chloroplasts only with $h\nu_1$-light (far red). In this case only small amounts of X^- are formed for reduction of Cyt$^+$ (ref. 1). (*b*) By exciting chloroplasts at $-150°$ C. At such temperatures the reduction of Cyt$^+$ is completely stopped[3]. (*c*) By addition of oxidized dichlorophenol-indophenol and small amounts of reduced dichlorophenol-indophenol which uncouple Cyt from X in chloroplasts (see Fig. 1). (*d*) By using aged chloroplasts. In such chloroplasts X and Y are inactive. Added small amounts of red. In or red. PMS ($< 10^{-5}$ M/l) are used as *e*-donor for Cyt$^+$.

Cyt→Cyt$^+$ becomes observable when the reduction time Cyt$^+$→Cyt is delayed more than 10^{-3} sec. This suggests that under normal conditions the reduction time must be shorter than 10^{-3} sec. Then the reduction is faster than the time of oxidation, which was estimated at ~ 10^{-3} sec.[3]. From these figures it is plausible that in normal conditions no accumulation of Cyt$^+$ occurs. (In ref. 1 we reported that Cyt$^+$ is reduced in ~ 10^{-2} sec. This was derived from the kinetics of the changes which appear under normal conditions at 420 mμ and from which we now have to suppose that they are not caused by cytochrome.)

The unknown agent reducing Cyt$^+$ in $< 10^{-3}$ sec. is called *E*. This is reduced directly or indirectly by X^-. As opposed to ref. 3, in Fig. 1 of this article: (*a*) the experimentally estimated redox-potential of cytochrome[1] is depicted; (*b*) *Chla* is used instead of *C*.

Bishop[4] has shown that plastoquinone *Pl* has a function in the *e*-transport during photosynthesis. From the changes in absorption in the ultra-violet[5] we have to assume that *Pl* is at least engaged as an *e*-acceptor of Z^- (Fig. 2). But from the redox potential of X and the reversible extraction experiments with petroleum ether[1] another part of *Pl* seems to be engaged with the reaction of X.

In ref. 3 it was shown that the light-induced changes in absorption at 430 and 703 mμ[3,6] are caused by one and the same substance—by photo-oxidation of a chlorophyll *a* or a similar compound (*Chla* → *Chla*$^+$)—because both changes show identical kinetic behaviour. These investigations were extended. Different *p*H values (2–12), temperatures (5–35° C.), and concentrations of added reduced phenazine methosulphate (10^{-6}–10^{-4} M/l.) exert a strong influence on the decay time or magnitude of the changes; but in any event the behaviour at 430 mμ is exactly the same as at 703 mμ. Detailed analysis of the changes at 430 and 703 mμ by one of us (B. R.) shows that these changes are caused by photo-oxidation of *Chla* in two completely different reaction systems I and II (Fig. 1). This means that at least three different light reactions are engaged in photosynthesis. 50 per cent of *Chla* is engaged in the non-cyclic electron transport of system I; the other 50 per cent of *Chla* reacts in system II, which probably operates only by cyclic electron transport. This can be shown in the following way.



Chla	*chlorophyll-a or similar compound*
Cyt	*cytochrome*
X	*properties described in* ●
Pl	*plastoquinone*
ox. S.I	*quinone, indigocarmine, ferricyanide .....*
red. S.II	*dichlor- thymol- and phenolindophenole,* *toluylene blue, thionine, methylene blue ....*
PMS	*phenanzine methosulfate*
In	*dichlorophenol - indophenol*
TPN	*triphosphopyridine, nucleotide*
ADP	*adenosinediphosphate, P phosphate*
ATP	*adenosintriphosphate*
CMU	*chlorophenyl - dimethylurea*

Fig. 1. Reaction pattern in photosynthesis at 20° C. ●, Witt, H. T., and Muller, A., *Z. phys. Chem.*, *N.F.*, 21, 1 (1959). Cyt^+ can react also with red. PMS. At higher concentrations (≮ 10^{-5} μ/c.) red. PMS by-passes Cyt^+ and reacts with $Chla^+$

By exciting with flashing light the 50 per cent *Chla* in I as well as the 50 per cent *Chla* in II are oxidized and afterwards reduced (causing negative changes at 703 mμ, see *a* in Fig. 2). By using continuous background illumination of low intensity with only $h\nu_1$-light (708 mμ), the 50 per cent of *Chla* in system I, Cyt and *E* should be enriched in oxidized form because nearly no X^- is produced for its reduction. Vice versa, the other 50 per cent *Chla* in system II should not be enriched at low intensity because of the cyclic *e*-transport. Therefore, the $h\nu_1$-light of a supplementary flash (with $h\nu_1$- and $h\nu_2$-lights) can oxidize only the 50 per cent *Chla* in system II with following reduction (*b* in Fig. 2). The $h\nu_2$-light of the same flash produces X^- (causing changes at 515 mμ (see *c* in Fig. 2)). X^- should reduce the 50 per cent *Chla* in system I, already oxidized by the continuous background light, via Cyt and *E*. This can be followed by the *positive* changes at 703 mμ (*d* in Fig. 2)[7].

The simultaneous oxidation of X^- can be followed at 515 mμ (*e* in Fig. 2). The rate-determining step for the reduction of already oxidized $Chla^+$, via E^+ and Cyt^+, is given by the oxidation time of X^- ($\sim 10^{-2}$ sec.). Therefore, the time dependence of the changes at 515 mμ and of the *positive* ones at 703 mμ should be the same (compare *d* and *e* in Fig. 2). Different conditions such as different *p*H (6–8), temperatures (0–35° C.) or ageing (0–7 days) have strong influence

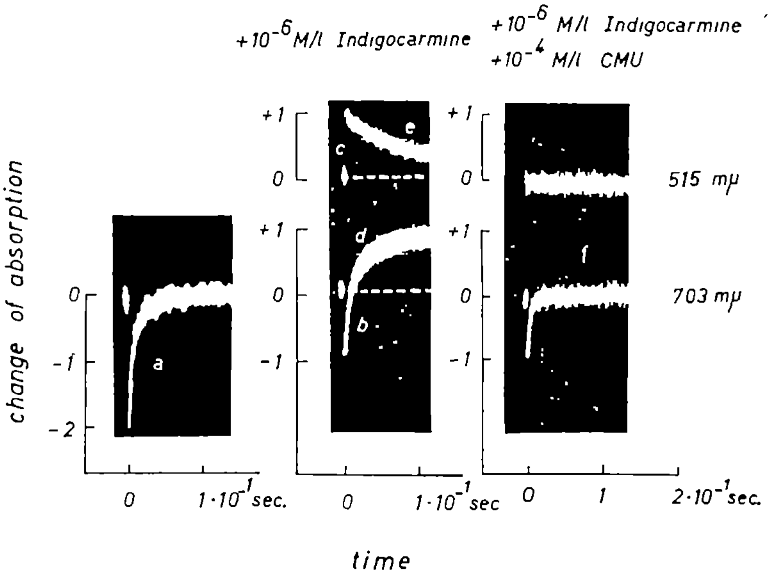


Fig. 2. Relative changes of absorption on chloroplasts (spinach) caused by a flash of light. 20° C., *p*H = 7. The flash was turned on at *t* = 0; flash duration = 10^{-4} sec.

on the changes at 515 mμ (*e*) and the positive ones at 703 mμ (*d*). But in any event the influence is—as expected—exactly the same at both wave-lengths. This shows quantitatively the co-operation of *Chla* and X via Cyt and E in system I. Comparing the positive changes of *Chla* in I with the negative ones of *Chla* in II (*d* and *b* in Fig. 2) in dependence of poisons or ageing (0–7 days) or *p*H (6–8) it results for both a quite different dependency. This confirms that the *Chla*-reactions operate in two different systems, I and II. For example, chlorophenyl dimethylurea (CMU) stops non-cyclic *e*-transport by blocking oxygen production. Therefore, addition of chlorophenyl dimethylurea should stop all reactions of system I but none in system II because of the cyclic *e*-transport in II. Fig. 2*f* shows the result. As expected, there remain only the negative changes caused by the 50 per cent of *Chla* operating in system II. (With high concentrations of chlorophenyl dimethylurea, $\geqslant 10^{-3}$ *M*/l., as used in ref. 3, all changes vanish.)

Further analysis shows that also in system I a cyclic *e*-transport is possible: $\overset{\longrightarrow}{Z \leftarrow \mathit{Chla}}$. But while the probably only cyclic *e*-transport in II is always operating, cyclic *e*-transport in I is only possible when no *e*-acceptor, ox.S.$^{\mathrm{I}}$ (Fig. 1) or *Pl* (Fig. 1) is available for Z^- in system I. We found out that in chloroplasts oxidized indigocarmine, quinone and ferricyanide are compounds which react with Z^- preventing cyclic *e*-transport in system I (in the experiments of Fig. 2 oxidized indigocarmine was used). Further evidence for the probability of the scheme in Fig. 1 is given by the separation of the mechanism into seven partial reactions (see ref. 10).

From the redox potentials it follows that phosphorylation[8] (ADP + P → ATP) can be coupled with reactions as indicated in Fig. 1. A non-cyclic phosphorylation can take place in system I between Cyt and E or E and X. It may be that the function of system II is to generate a cyclic phosphorylation. With ATP and $TPNH_2$ carbon dioxide can be reduced to sugar via the Calvin cycle[9].

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DIGITAL COMPUTER SIMULATION OF ECOLOGICAL SYSTEMS

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THE mathematical representation of ecological systems has been investigated for several decades, starting with the pioneer work of Volterra[1] and Lotka[2]. Most of this work has been done with pencil and paper and has necessarily been limited to systems simple enough to permit the analytical solution of the resulting equations. Some work has been done with analogue computers[3], and an application of digital computers to the problem has been made by Watt[4,5]. It is the purpose of this article to describe a general computer method of representing ecological systems of any desired degree of complexity in terms of simultaneous first-order differential equations, with some simple illustrative applications.

The calculations thus far performed are based on the assumption[1,2,6] that the rate at which two species interact is proportional to the product of their numbers. This rate law is identical to the mass action law of chemistry and therefore permits the use of a computer programme[7,8] developed to represent complex chemical systems with the *Univac I* computer. This programme enables the computer, when given as input a set of chemical equations of the form:

$$A + B \rightarrow C + D$$

automatically to write instructions to solve the associated set of differential equations:

$$-\frac{\mathrm{d}A}{\mathrm{d}t} = -\frac{\mathrm{d}B}{\mathrm{d}t} = k\,(A)\,(B)$$

and then perform the solution by numerical methods when given a set of initial conditions. It is possible to write and solve sets of equations having no relationship to chemistry, and it would be possible to substitute some other interaction law for the mass action law, or to make allowance for its limitations[5].

The system examined here (represented by the 'chemical' and differential equations shown in Table 1) consists of a prey, grass (*GRS*), and a predator, rabbits, which may be either adequately fed (*FRB*) or starved (*SRB*). The starved rabbits are assumed to die at a moderately rapid rate if unable to feed; the fed ones, to reproduce. In most of the calculations, it has been assumed that some of the grass is segregated in such a way that the rabbits cannot get at it (that is, roots

Table 1

'Chemical' equations	Rate constants
(1) $GRS \rightarrow GRS$	$k_1 = 24$
(2) $SRB + GRS \rightarrow FRB - SRB - GRS^{a}$	k_2 varied $\begin{cases} 3 \times 10^{-1} \\ 10^{-3} \end{cases}$
(3) $FRB \rightarrow FRB$	$k_3 = 30$
(4) $FRB \rightarrow SRB - FRB$	$k_4 = 300$
(5) $SRB \rightarrow DUM - SRB^{b}$	$k_5 = 33$
(6) $FRB \rightarrow DUM - FRB$	$k_6 = 3$
(7) $FRB + GRS \rightarrow DUM - GRS^{c}$	$k_7 = 0\,012$
(8) $RTS \rightarrow GRS^{d}$	$k_8 = 5 \times 10^{-4}$
(9) $GRS + GRS \rightarrow DUM - GRS^{e}$	$k_9 = 5 \times 10^{-5}$

Corresponding differential equations

$$\frac{\mathrm{d}(GRS)}{\mathrm{d}t} = k_8(RTS) + k_1(GRS) - k_9(GRS)^2 - k_7(FRB)(GRS) - k_2(SRB)(GRS)$$

$$\frac{\mathrm{d}(SRB)}{\mathrm{d}t} = k_4(FRB) - k_5(SRB) - k_2(GRS)(SRB)$$

$$\frac{\mathrm{d}(FRB)}{\mathrm{d}t} = k_3(FRB) + k_2(GRS)(SRB) - k_6(FRB) - k_4(FRB)$$

a, Substances which disappear in a reaction must be listed again on the right side of the equation.

b, This statement is equivalent to '*SRB* disappears'.

c, Fed rabbits continue to eat.

d, *RTS* = roots To abolish the roots, k_8 is set equal to zero.

e, This is to limit the amount of grass that may grow in a fixed land area which they cannot dig up), which is equivalent to a constant influx of grass into the system.

Varying the predative efficiency of the starved rabbits yields results shown in Figs. 1–4. If the rabbits are highly efficient predators with access to all the grass, they will exterminate it and then starve to death (Fig. 1), a situation observed experimentally by Gause[9]. If some of the grass is inaccessible, a steady state results in which the grass is eaten as fast as it appears (Fig. 2). As the predative efficiency of the rabbits is decreased the system goes into an oscillation (Fig. 3) and then a damped oscillation (Fig. 4). An even more inefficient predator effectively starves to death in the midst of plenty.

A given predator may attack more than one prey organism with differing degrees of efficiency[6]. In many cases this may stabilize the system, but it need not do so: when the predators of Fig. 3 were given an alternative prey, which they attacked less efficiently, they first ate approximately all the available preferred prey, and then came to a steady state with the alternative prey.

In an attempt to duplicate a system where a stable predator–prey relationship may be upset with drastic consequences by adding another predator[10], a second predator (foxes) which eats the rabbits was introduced; the foxes are assumed to reproduce (and die of starvation) more slowly than the rabbits. When foxes were introduced into a system (Fig. 4) which would otherwise come to a steady state, it went into wild oscillations (Fig. 5); at the end of the second oscillation the rabbits were approximately extinct.

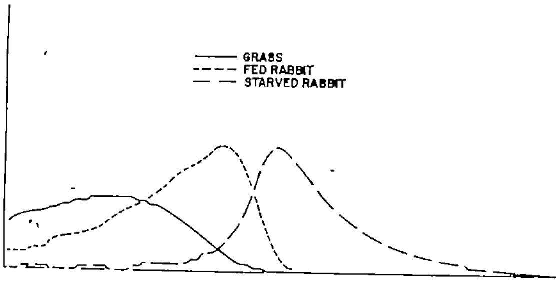

Fig. 1. No. of animals as a function of time with $K_2 = 0·3$ and $K_3 = 0$

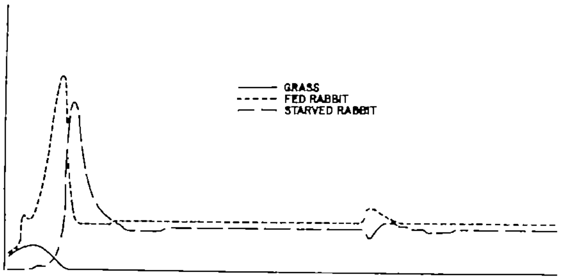

Fig. 2. No. of animals as a function of time with $K_2 = 0·3$ and $K_3 = 0·24$

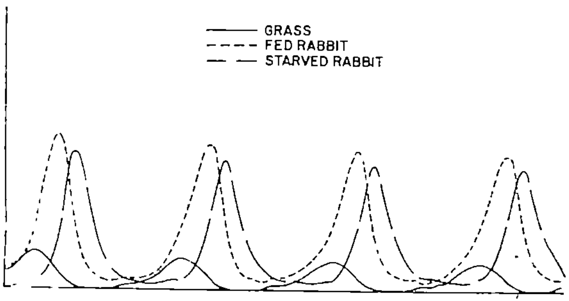

Fig. 3. No. of animals as a function of time with $K_2 = 5 \times 10^{-3}$ and $K_3 = 0·24$

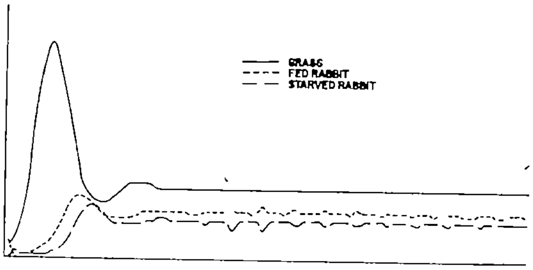

Fig 4 No of animals as a function of time with $K_2 = 10^{-2}$ and $K_3 = 0·24$

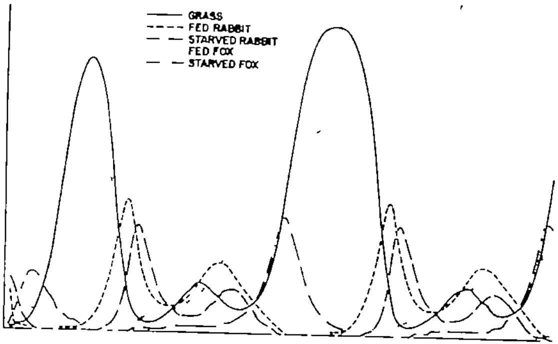

Fig. 5. No. of animals as a function of time with foxes introduced into the system of Fig. 4

Application of this method calculation to ecological problems offers two distinct advantages: (1) The computer can be made to deal with nearly all the mathematics. (2) The limit of complexity is that imposed by the speed and memory size of the computer used (the largest now in existence would be able to handle more than 1,000 species in this manner). By suitably defining new 'species' and taking advantage of other properties of the programme, it is possible to represent environmental variations, disease, different states of nutrition, effect of age, etc.

This method of calculation can be applied both to studies in theoretical ecology and to real situations (such as agricultural pest control). It must, however, be emphasized that the ability to perform calculations in this manner is no substitute for detailed knowledge of the system being studied.

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LETTERS TO THE EDITOR

PHYSICS

Ignition of Gas Mixtures by Electric Discharges between a Heated and a Cold Electrode : Application in Breakflash Apparatus for Intrinsic Safety Testing

THE thermal theory of ignition of gases explains certain phenomena in the ignition of explosive mixtures by electric sparks involving capacitive and break or make discharges. However, a number of important questions for theory as well as practice remain to be answered. In this connexion the author has made some introductory investigations on the ignition of explosive gas mixtures having in mind the simplified assumption that the ignition process is essentially thermal in character, so that some critical temperature is involved in the initiation of the ignition. This temperature may be attained by a single process, such as the heating of a wire, or by more than one process, for example, the heating of a wire plus an electric discharge. In the case of an 8·3 per cent methane–air mixture the initiation of ignition involves a certain critical volume of gas attaining a minimum temperature.

Normally, a considerable proportion of the spark energy is lost in heating the electrodes, though in certain circumstances, for example, capacitance sparks between electrodes at a suitable separation, the loss to the electrodes may be made small. It has been found experimentally that a variety of factors influence the probability of ignition by the break discharge of a given circuit. It appears that these factors affect in various ways the dissipation and utilization of energy in the discharge so that they may increase or decrease the 'incendivity' of the break discharge. These considerations led me to the conclusion that a useful control of the break discharge energy for ignition might be achieved by conducting discharges between a heated and a cold electrode.

The ignition investigations were carried out in a cylindrical chamber of transparent 'Perspex' containing an 8·3 per cent by volume methane–air mixture at atmospheric pressure. The electrode assembly consisted of a stationary heated nichrome wire (80/20 nickel–chromium) of diameter 0·213 mm. (35 S.W.G.), in the shape of a rounded W, and within the middle loop a cold springy wire of tungsten, 0·152 mm. diameter (38 S.W.G.), was arranged to slide and to provide the circuit break. The tungsten wire electrode was driven at a constant speed of 20 r.p.m., so that it passed the heated electrode at a linear speed of about 6 cm./sec.

The preliminary experiments included ignition of the explosive methane–air mixture by (*a*) a heated nichrome electrode alone; (*b*) break discharges from a circuit containing 95 mH. air-cored inductance and a 24-V. d.c. source; (*c*) break discharges from a circuit of very low inductance, also using a 24-V. d.c. source. Further, some observations were made on the behaviour of a cold and hot platinum wire electrode in break discharges.

The experiments completed so far indicate that: (1) The explosive ignition of a methane–air mixture by a heated nichrome electrode alone could be obtained for a few times before the electrode broke. A rapid rise of the electrode temperature gave a high probability of ignition.

(2) The break discharge energy for ignition at a heated electrode decreases as the electrode temperature is increased towards the gas ignition temperature. In Fig. 1 are shown typical ignition probability curves obtained with the inductive circuit at various electrode temperatures (I_h = electrode heating current).

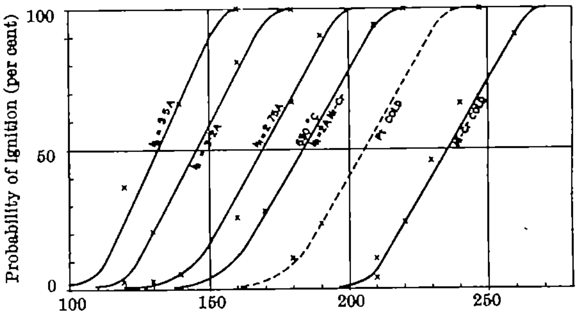

Fig 1

(3) A small number of tests with a circuit of low inductance showed ignitions at break discharges of circuit carrying 1·3 amp. at 24-V. d.c. with electrode heated to about 800° C.

Inductive break discharges between cold platinum wire of 0·274 mm. diameter (32 S.W.G.) and cold moving tungsten wire showed a higher 'incendivity' than that of cold nickel–chromium wire (Fig. 1). This might be attributed to catalytic activity at the surface of the platinum wire. In contrast to this, similar discharges between hot platinum wire showed lower probability of ignition. For a 250 m.amp. circuit current, which produced 100 per cent ignition with cold platinum–tungsten electrodes (Fig. 1), the hot platinum electrode produced only 5 ignitions in 100 tests.

Experiments with electric break (or make) discharges between a heated and cold electrode (or both electrodes might be heated) may yield some information on various aspects of the ignition process, and prove useful in some practical applications. So far, some tentative conclusions can be drawn from the experiments carried out.

(1) The experiments show that electrical break discharges in explosive atmospheres when assisted by appreciable circuit current heating at the point of discharge may create dangerous conditions with currents which are recognized as safe for discharges at cold electrodes.

(2) Operation of a breakflash apparatus with one electrode (or both) heated offers the possibility of an intrinsic safety testing system with high sensitivity (very small igniting currents). The use of a continuously variable heating current in an electrode may enable the safety margins of circuits to be

determined in cases where the application of an over-voltage or an over-current is inconvenient.

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γ-Spectrometry compensated for the Compton Effect

PEIRSON *et al.*[1-3] proposed an efficient technique for obtaining net photopeaks in γ-spectrometry by subtracting the Compton continuum of the NaI(Tl) detector by means of an anthracene scintillator. The photoelectric effect in the latter is indeed negligible and gives rise to the Compton effect only. After equalization for pulse height and counting-rate, net photopeaks are thus obtained.

To minimize counting losses, the detection efficiency of both detectors, in the Compton region, should be approximately the same. This requires equal diameters of the scintillators. The cost of the anthracene detector thus becomes quite high when large NaI(Tl) crystals are used. It appeared, however, that a plastic scintillator (Nuclear Enterprises *NE* 102) is equally suitable for this purpose, resulting in a detector cost reduction by a factor of approximately 50.

The detection efficiency of this plastic is about 60 per cent in comparison with the anthracene, whereas the pulse height is about 67 per cent.

Uncompensated and compensated spectra of cobalt-60, using a 3 in. × 3 in. NaI(Tl) and 3 in. × ½ in. anthracene crystal or a 3 in. × ½ in. *NE* 102 plastic scintillator, are given in Fig. 1. From this it appears that an even better compensation for the Compton edge is obtained with the latter. For the spectrum compensated with the plastic scintillator, the detectors were at an angle of 90° and mounted in lead collimators.

When mounted at an angle of 180°, the back scatter is indeed still quite important and extends up to the 500-KeV. region, whereas at an angle of 90° a reduction in back scatter by a factor 3 is obtained.

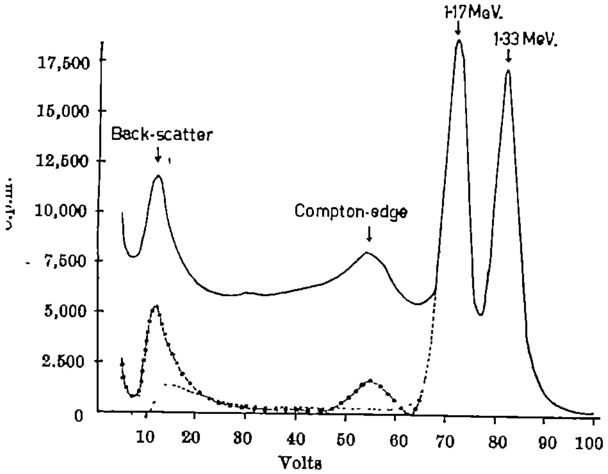

Fig. 1. Spectrum of cobalt-60. ——, Uncompensated (180° angle); –○–, compensated with anthracene (180° angle); - - - -, compensated with plastic scintillator (90° angle and collimator)

The detection efficiency, caused by the lead collimator, is reduced by 20 per cent only.

This technique appeared to be more efficient than the use of a lead absorber before the anthracene crystal as recommended by Peirson *et al.*[4].

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GEOLOGY

Age of Deglaciation in the Ruwenzori Range, Uganda

THE massive fault-block of the Ruwenzori Mountains, which lies on the axis of the Western Rift between Uganda and the Congo, is the wettest of the high mountain areas in East Africa. For that reason it has more glaciers at present and has been more extensively glaciated in the past than any of the other tropical African mountains. Although relative ages have been estimated[1,2], no absolute dates have been published for prehistoric glacial events in this or any other tropical African mountain area.

During July of 1960 Mr. Jonathan Richardson, Mr. Robert Kendall and I collected cores from the sediment that had accumulated in glacial lakes at a variety of altitudes on the Uganda side of the Ruwenzori Range. Much of the sediment was fossiliferous and organic enough to permit radiocarbon dating, and an analysis of one sample by Isotopes, Inc., of Westwood, New Jersey, has provided the first absolute date of a major glacial event in East Africa.

The lowest Ruwenzori moraines are in the Mobuku Valley, where they extend down to 2,100 m. near Ibanda. Lateral moraines at slightly higher altitudes occupy the smaller valleys of the mountains. All these moraines are several orders of magnitude larger than any between them and the present glacier termini. Presumably they have been built up during several glaciations; but good exposures are rare and no evidence of interglacial episodes has come to light.

No lakes are to be found close to the lower ends of the low moraines, but one does lie at the junction of the Mobuku River lateral moraine system with that of its principal tributary, the Bujuku River. This is Mahoma Lake (lat. 0° 21′ N., long. 29° 58′ E.) at an altitude of 3,000 m. Its shape, size, depth and relation to the associated moraines all suggest that it occupies a kettle and there is no evidence of a glacial still-stand between it and the lowest moraines in the valley.

We raised a core almost 6 m. long from the mud under 9·5 m. of water in Mahoma Lake. The drilling operation ceased when the borer entered sandy gravel with pieces of stone more than 4 cm. in diameter and the section appeared to be complete. The lowest 0·4 m. of sediment consisted of coarsely graded grey clay, silt and fine sand. The change to organic sediment began rather sharply at a depth of 5·4 m. and was complete within 0·2 m. The organic lake mud consisted very largely of algal remains and was uniform in gross appearance to the top of the section.

The grey inorganic material looks as if it had been laid down very rapidly while the glacier was still close enough to provide a rich source of fine inorganic sediment, so the carbon date of 14,700 ± 290 years B.P. for sample I–556, which came from the bottom 0·2 m. of organic lake mud, should provide a minimum date that is very close to the true date of deglaciation of the locality. This is the first piece of direct evidence to show that fifteen thousand years ago, while the Wurm glacier in Europe and the Wisconsin glacier in America were going through a halting retreat from their southern limits, the equatorial African mountain glaciers were retreating as well. It strongly supports palynological studies (Martin, P., personal communication, and refs. 3 and 4) indicating that Pleistocene climatic changes in the tropics have been of the same sign as those in the rest of the world and synchronous with them.

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Palygorskite in Leicestershire

CURRENT geological investigations into the ancient rocks of Leicestershire have revealed three occurrences of the clay mineral palygorskite. These are in the lower quarry at Bardon Hill, the Cliffe Hill quarry and the Warren quarry at Enderby. At all three localities the palygorskite is present in joints in dioritic rocks. In every case its identification has been checked by X-ray powder techniques. No sepiolite lines were detected on any of the powder photographs.

At Bardon Hill the palygorskite is found as compact, fibrous masses filling the centre of a joint, the walls of which are lined with coarse calcite crystals. At Cliffe Hill and in a deeply penetrating joint at Enderby, the mineral forms fine specimens of mountain leather and in both cases is intergrown with fine-grained calcite. Elsewhere in the Warren quarry at Enderby palygorskite is almost ubiquitous on the joint surfaces of the upper level, where it is free from carbonate impurities and again takes the form of compact, fibrous masses. At all three localities the palygorskite occurs at levels not far below the overlying Triassic unconformity and in the Warren quarry it can be traced, without a break, from the joints in the diorite into the matrix of the overlying basal Triassic breccia.

Palygorskites found in igneous rocks (usually in joints or crush bands) are generally considered to be of hydrothermal origin[1,2]. When found in sediments the mineral is assumed to be of sedimentary origin[3,4]. So far as the Leicestershire localities are concerned the geological similarities of the three occurrences suggest that the palygorskite in each is of the same age and origin. With regard to the origin, the palygorskite at Enderby cannot have been deposited from a hydrothermal differentiate of the dioritic magma since it is found in the overlying Triassic rocks. We believe that it was deposited from downward-moving waters which were in direct connexion with the lakes and rivers in which the basal Triassic sediments of the area were deposited and not from post-Triassic ascending hydrothermal solutions.

The following observations at Enderby are in agreement with this conclusion. (1) Palygorskite is only found on the second (deeper) level in strong joints which persist upwards to the Triassic unconformity. (2) Its main development is in joints within approximately thirty feet of the unconformity. (3) The mineral is most abundant in the area below wadies. (4) It is poorly developed in, or absent from, adjacent, buried hills which would have acted as anticlinal traps for ascending solutions. It is of interest to note here that in Charnwood Forest a number of other mineral veins with calcite, barytes and clay minerals occur in joints in the Charnian rocks. These can be traced upwards into Triassic sediments where the minerals concerned form parts of the matrices of the basal breccias.

In view of this conclusion, we suggest that occurrences of palygorskite in the joints of igneous rocks should not, without supporting evidence, be assumed to be of hydrothermal origin. Further, having noted that palygorskite is frequently reported from continental deposits and soils (often red)[3,5-7], we propose that occurrences such as those in the Shetland Isles[2] and some of the others reported from various parts of Scotland[8] may point to the former existence of Old Red Sandstone or Triassic unconformities at no great height above the points at which palygorskite is now found.

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Geochemical Characteristics of Carbonatites

THE lava from Fort Portal in western Uganda, recently described by von Knorring and Du Bois[1], was considered to be carbonatitic in view of the high carbonate content and its agreement with the characteristic trace element pattern as observed among true carbonatites, which are in the main calcitic or dolomitic rocks. In pure carbonatite rocks, significant elements like iron, titanium, zirconium and niobium may be accidental or regional to some degree, whereas elements like strontium, barium, rare earths and phosphorus seem to belong to the earlier (primary) constituents of carbonatite magmas.

An examination of another carbonatite lava, collected from the crater of Oldonyo Lengai volcano in Tanganyika by Dr. N. J. Guest, shows a remarkable composition which may correspond more closely to some of the primary carbonatite magmas. Chemically this lava is composed of sodium, calcium and potassium as major constituents, chlorine, fluorine, sulphur, phosphorus, strontium, barium, manganese and iron as minor, whereas other elements are present in amounts less than 1,000 p.p.m. When finely crushed, about 67 per cent of this lava is soluble in hot water and an X-ray examination showed that the soluble part consists mainly of sodium carbonate and the insoluble of calcium carbonate. The powdered lava melts at approximately 650° C.

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Archæocyathine Limestones of Antarctica

ONE of the problems in antarctic geology has been the uncertain stratigraphic position of the Cambrian Archæocyathid limestone found as erratics on the Beardmore Glacier[1,2] and dredged from the Weddell Sea[3]. Probably the Beardmore erratics came from the Mt. Buckley limestone at the head of the glacier; but the field relationships of this limestone are uncertain. In recent syntheses by Fairbridge[4] and Harrington[5] the limestone is left in an uncertain position between the flat-lying Beacon Sandstone, and the underlying steeply dipping Ross System of Harrington. The Beacon Sandstone has yielded upper Palæozoic and Mesozoic fossils, and the Ross System is usually considered pre-Cambrian.

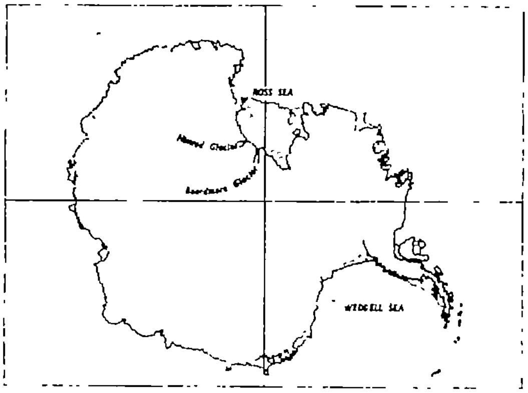

Fig. 1. Locality map of Antarctica

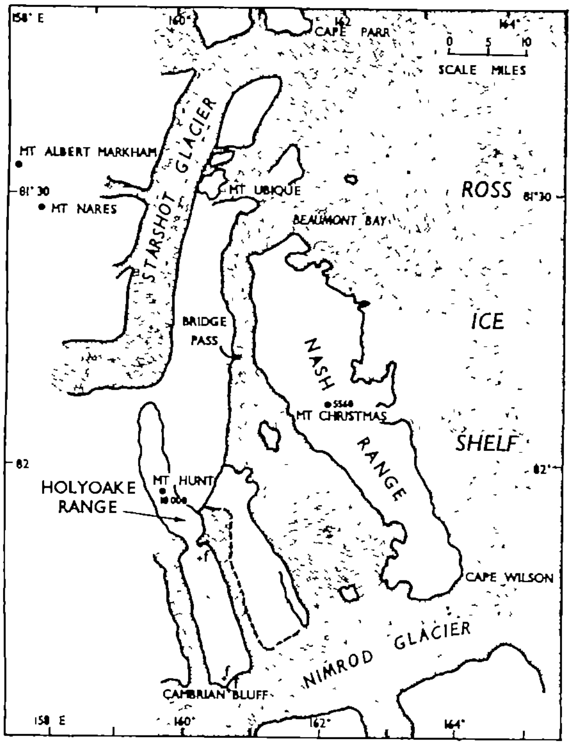

Fig. 2. The Nimrod glacier area, showing fossil localities in Cambrian limestone

Field work during 1960–61 by a New Zealand Geological Survey team suggests that the Archæocyathid limestones belong to the top of the Ross System. Specimens of Archæocyatha were found in place near the Nimrod Glacier in the Holyoake Range (Figs. 1, 2) at three localities, 82° 25′ S., 160° 40′ E.; 82° 23′ 27″ S., 160° 36′ 30″ E.; and 82° 11′ 35″ S., 160° 18′ E. From preliminary examination the fossils belong to several genera of Archaeocyatha, chiefly in the families Coscinocyathidæ and Archaeocyathidæ. They occur in steeply dipping, locally folded, recrystallized and partly recrystallized limestones. The limestones are presumed to overlie greywackes of the Nash Range, which have a similar strike and also dip steeply. These are invaded by plutonic rocks, and clearly belong to the Ross System. An unconformable contact between horizontal Beacon sediments and almost vertical limestone was seen on Mt. Hunt from a distance of six miles. Thus the Cambrian limestones agree with the Ross System in their steep attitude, high degree of folding and partial recrystallization and differ from the flat-lying unmetamorphosed Beacon Sandstone. The Cambrian limestones are therefore assigned to the Ross system, which evidently extends into the Lower Palæozoic.

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METEOROLOGY

A Trend Towards a Longer Dry Season in South-western Nigeria

STEBBING[1] has stated that the climate of West Africa was becoming increasingly drier, and that the Sahara was advancing southwards at an alarming rate. The ensuing controversy, which developed in the late 1930's, has been summarized by Jones[2]. During the past 50 or 100 years there has been a distinct degeneration of the vegetation in many parts of West Africa; but most workers considered this to be caused by human activity rather than by a change in climate and pointed out that there was no meteorological evidence for a progressive, rather than a cyclical, climatic change.

The Olokemeji Forest Reserve, in south-western Nigeria, lies across the forest/derived savanna boundary. The general features and vegetation of the reserve have been described[3], and the seasonal changes in the environment are being analysed in order to compare them with the seasonal changes observed in the vegetation. The rainfall data, in particular, are being analysed in some detail. Records are available from 1906 until 1927 and from 1953 to the present day. The mean annual rainfall is 1,232 mm., and no evidence has been found for a progressive change in this value.

The northern climatic limits of the vegetation zones in West Africa depend on the severity of the dry season rather than on the mean annual rainfall. Consequently, the length (in days) of the rainless periods and, in particular, of the maximum dry period during each dry season (November–February) were analysed. This showed that there was a significant positive correlation between the year of the dry season and the length of its maximum dry period ($r = 0{\cdot}43$, $p =$ about 0·02). In calculating the regression, it was found that the maximum dry period was increasing by 0·59 day per year. (For the dry seasons 1953–54 to 1961–62 the mean length of the maximum dry period was 78·1 days.)

Similarly, calculations were carried out on the length of the maximum dry period during the 'little dry season', which occurs about August. Here also there was a significant positive correlation between the year of the 'little dry season' and the length of its maximum dry period ($r = 0{\cdot}53$, $P < 0{\cdot}01$), and the maximum dry period was found to be increasing by 0·23 day per year. (For the 'little dry seasons' 1954–61 the mean length of the maximum dry period was 19·6 days.)

The evidence presented here, although statistically significant, is from only one station, and it is obviously unwise for too far-reaching a conclusion to be drawn from it. Nevertheless, the analysis of records from other stations may show a similar trend towards an increase in the length of the maximum dry period even though, as at Olokemeji, there is no significant change in the annual rainfall. As dry season severity is more important than mean annual rainfall in determining the distribution of the vegetation zones and the consequent land use policy it is important that records from other stations should be analysed for this factor.

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BIOPHYSICS

Adsorption of Proteins on 'Sephadex'

IN the course of studies on the gel filtration of a number of proteins on 'Sephadex *G*–50' (trade name for a cross-linked polysaccharide dextran made by Pharmacia, Uppsala, Sweden, who publish a booklet containing an extensive bibliography), it was found that in the absence of salts some proteins were strongly adsorbed to the gel. For example, ribonuclease, lysozyme, trypsin, bovine serum albumin and a polytyrosyl derivative of trypsin[1] were all adsorbed and could not be removed by extensive washing with distilled water. However, these proteins were recovered quantitatively by elution with dilute sodium chloride solutions. Since 'Sephadex' has been used extensively for the desalting of protein solutions and for the separation of proteins from materials of low molecular weight, it is important to note that in attempts to remove salts completely by using distilled water as eluant, this phenomenon may lead to complications.

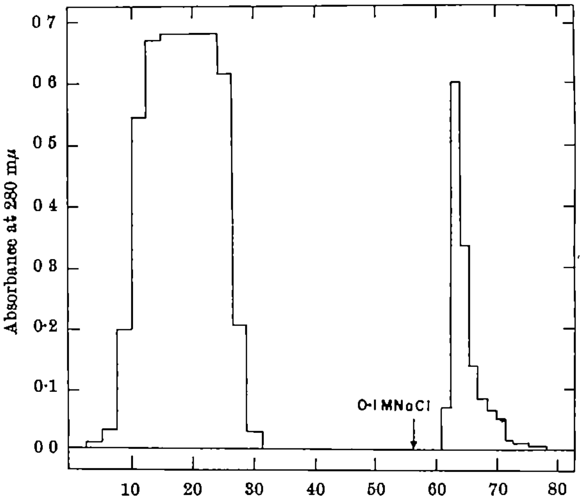

Fig. 1. Adsorption of ribonuclease on 'Sephadex *G*-50' and elution with 0·1 *M* sodium chloride. 20 ml. of ribonuclease solution (1 mgm./ml.) was passed through the column (8·5 cm. × 1·2 cm.), followed by water and 0·1 *M* sodium chloride (arrow)

Two batches of 'Sephadex *G*–50' were used in this work. (The manufacturer's specifications for the 'Sephadex *G*–50' were as follows: "Lot No. To 6809, Dry screen analysis: On U.S. No. 50 0·0 per cent, Through U.S. No. 270 3·3 per cent, Water regain, 4·7 gm. water per gm. dry gel; Lot No. To 6387, Dry screen analysis: On U.S. No. 50 0·1 per cent, Through U.S. No. 270 0·6 per cent, Water regain, 5·0 gm. water per gm. dry gel.") No differences were observed in their behaviour. The dry gel was suspended in a large volume of 0·05 *M* sodium chloride, allowed to settle for about 15 min., and the supernatant decanted. This was repeated twice to remove fines, and the gel was then packed into the column. The gel was washed with distilled water until no chloride was detectable in the effluent. Some of the columns were used repeatedly and were washed with 0·1 *M* sodium chloride and distilled water before each run. The binding capacity of 'Sephadex *G*–50' at 25° was determined for several proteins as follows: a solution containing an amount of protein in excess of the capacity of the gel was passed through the column and the difference between the amount of protein applied and the amount eluted with water was measured. This value agreed with the quantity obtained by subsequent elution with 0·1 *M* sodium chloride. A representative experiment is illustrated in Fig. 1. The binding capacity of an 8·5 cm. × 1·2 cm. bed of 'Sephadex *G*–50' for lysozyme (egg white), ribonuclease (bovine pancreas), and bovine serum albumin was found to be 7·2, 2·8, and 2·4 mgm. respectively. Protein concentrations were determined by ultra-violet spectrophotometry. All the proteins tested were eluted quantitatively with 0·1 *M* sodium chloride. In an experiment with lysozyme, it was found that 10^{-2} *M* sodium chloride also eluted the protein quantitatively, whereas 10^{-3} *M* sodium chloride did not elute any of the adsorbed protein. Trypsin adsorbed to the gel was readily eluted with 10^{-3} *M* hydrochloric acid, but not with 10^{-4} *M* hydrochloric acid.

These findings suggested that use could be made of the strong binding of some proteins to 'Sephadex' to concentrate such proteins from dilute salt-free solutions. The following experiment illustrates this application and gives some indication of the binding strength involved. Four hundred ml. of a dilute ribonuclease solution (0·025 mgm./ml.) was passed through a column of 'Sephadex *G*–50', 11 cm. × 2·2 cm. It was found by determination of enzymatic activity[1] that the concentration of ribonuclease in the eluate was less than 0·1 per cent of that of the applied solution. The column was washed with an additional 300 ml. of water, and the enzyme was then eluted with 0·1 *M* sodium chloride. Fractions of 1·0 ml. were collected. All the enzyme was recovered, 93 per cent being present in 10 ml. Thus, a 40-fold concentration was achieved. The protein concentration in the peak fraction was 1·6 mgm./ml. The activity of the ribonuclease was not affected by adsorption to the gel. Not all proteins tested have been found to behave similarly to those mentioned here. Thus, neither yeast alcohol dehydrogenase nor pig pepsin was found to be adsorbed. The latter two enzymes were tested at 4° in order to minimize autolysis and denaturation.

The fact that the adsorbed proteins are eluted by dilute salt suggests that electrostatic interactions may play a part in the adsorption. Furthermore, the fact that dilute hydrochloric acid was an effective eluent and that pepsin, a highly acidic protein, was not adsorbed points to the possible presence of negatively charged groups in the gel. This view is also supported by the work of Gelotte[3], in which he found that the hydrochlorides of the basic amino-acids lysine, arginine and histidine are strongly adsorbed by 'Sephadex *G*–25' in water, whereas they are eluted by 0·05 *M* sodium chloride. Gelotte ascribed this behaviour to the presence of carboxyl groups in the gel. Other factors such as the aromatic group content[3,4] or the molecular weight of the protein may also influence the degree of binding. Although the adsorptive capacity of 'Sephadex *G*–50' is very small compared with that of ion-exchange celluloses[5], this property of the gel may acquire considerable significance when small quantities of protein are used.

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CHEMISTRY

Estimation of Metal Content in Nickel Soaps by Polarography

HEAVY metal soaps with their many-sided technical and industrial applications present some unsolved problems to the chemists. One of these is the estimation of the amount of metal present in a particular soap. Until now the chemical analysis is generally used for this purpose. This method, besides being tedious, is apt to give less-accurate results, especially when the metal content is low. In the present communication some of the results of the polarography of nickel palmitate and myristate which have found a direct application in estimating the metal concentration are briefly described.

Boner[1] in 1937 showed the existence of complex ion formation between pyridine and metal soaps of Ni^{++}, Co^{++} and Cu^{++}. Polarography of the metal complexes of pyridine in the aqueous medium using potassium chloride as the supporting electrolyte was undertaken by Lingane and Kerlinger[2] in 1941. Since the soaps under investigation became hydrolysed with even traces of water, their polarography in non-aqueous media, using (1) lithium chloride in 1 : 1 benzene/methanol mixture[3]; (2) methyl hydrogen sulphate also in 1 : 1 benzene/methanol mixture[3]; (3) potassium chloride in glycol as supporting electrolyte was investigated. Measurements were carried out with a dropping mercury electrode with Lange's polarometer (model III, No. 46) in conjunction with the Multiflex galvanometer type *MGF* 2 at 25 ± 0·1° C. Inert atmosphere was maintained in the polarographic cell (*H*-type[4]) by passing purified nitrogen through it. The capillary constant $m^{2/3} t^{1/6}$ in the different supporting electrolytes were LiCl = 1·618; $MeHSO_4$ = 1·532; KCl = 1·610 mgm. sec.$^{-1/2}$. All the reagents used were either A.R. samples or product purified by standard methods in the laboratory. Nickel palmitate and myristate were prepared by precipitation method[5]; they were analysed for nickel content.

The polarograms in three different supporting electrolytes showed that the pyridine complex of both the soaps are reducible at the dropping mercury electrode and reversible waves (determined by the normal slope) with the $E_{1/2}$ values shown in Table 1 were obtained.

Table 1

Supporting electrolyte	$E_{1/2}$ (for Ni myristate) vs saturated calomel electrode	$E_{1/2}$ (for Ni palmitate) vs. saturated calomel electrode
0·166 *M* LiCl	– 0·74 volt	– 0 785 volt
0·166 *M* $MeHSO_4$	– 0·74 volt	– 0 700 volt
0·166 *M* KCl	– 0 802 volt	– 0 800 volt

Polarograms obtained with varying amounts of soap in pyridine gave waves having increasing diffusion current values with increasing concentration of the soap. Moreover, the value of diffusion current/concentration of soap was found to be constant for the different mixtures as can be seen from Table 2. From these results it was concluded that the polarographic method can be usefully utilized for the estimation of nickel in its metallic soaps.

Table 2. EFFECT OF SOAP CONCENTRATION ON THE POLAROGRAPHIC WAVES

	Conc. of Ni ions in nickel myristate in m.moles/l.	Diffusion current *id* in 20 × 10^{-2} µamp. 0 83 *M* LiCl		Diffusion current *id* in 20 × 10^{-2} µamp. 0 333 *M* $MeHSO_4$		Diffusion current *id* in 4 × 10^{-2} µamp. 0 166 *M* KCl	
	C	*id*	*id*/*C*–	*id*	*id*/*C*	**id*	*id*/*C*
1	0 636	8 0	13·83	10·0	15 73	19 0	29 61
2	1 009	14 0	13 87	15·5	15 35	30·0	29·83
3	1 261	17 0	13 48	20 0	15 86	36·5	28 94
4	1 513	20 5	13 54	24 0	15 86	44·0	29 08
5	1 765	24 5	13 88	26 5	15·14	51·0	28 89
6	1 892	25 0	13 74	28 5	15 12	56·0	29 06

	Conc. of Ni ions in nickel palmitate in m.moles/l.	Diffusion current *id* in 20 × 10^{-2} µamp. 0 166 *M* LiCl		Diffusion current *id* in 20 × 10^{-2} µamp. 0 1 *M* $MeHSO_4$		Diffusion current *id* in 4 × 10^{-2} µamp. 0 166 *M* KCl	
	C	*id*	*id*/*C*	*id*	*id*/*C*	*id**	*id*/*C*
1	0 517	8 5	16 44	9 5	18 39	21 5	41 58
2	0 828	13·5	16 30	15 0	18·11	34·5	41·61
3	1 035	16·5	15 54	19 0	18 35	43 0	41 54
4	1 242	20·0	16 10	23 0	18 92	52 0	41 86
5	1·449	24·0	16 56	27·5	18·97	59·0	41 06
6	1 552	26 0	16·75	29 0	18 68	63·5	40·91

* The value of *id* (diffusion current) in glycol medium is smaller than those of *id* in benzene–methanol mixture. This is due to high viscosity of glycol medium. *C* = concentration of soap.

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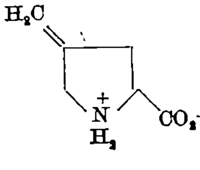
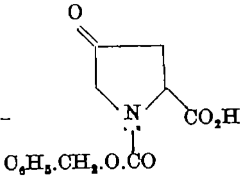
4-Methyleneproline : Synthetic Studies

RECENTLY, Gray and Fowden[1] have reported the isolation of a new amino-acid, 4-methylene proline (I), from seeds of loquat (*Eriobotrya japonica*). Structure (I) was firmly established by both chemical and spectroscopic evidence; but during the course of another investigation we have confirmed it by synthesis. This synthesis serves also to clarify some ambiguity concerning the optical configuration of the new amino-acid.



I



II

$(C_6H_5)_3P{=}CH_2$

III

Wittig reaction[2] between benzyloxycarbonyl-4-oxo-L-proline (II)[3] and methylenetriphenylphosphorane (III) yielded the oily benzyloxycarbonyl derivative of 4-methylene-L-proline. Its crystalline di*cyclo*hexylamine salt had m.p. 160–161° $[\alpha]^{19^\circ}_D - 5.5^\circ$ (*c*. 2 in chloroform) (found: C, 70·89; H, 8·63; N, 6·53. $C_{26}H_{38}O_4N_2$ requires C, 70·55; H, 8·65; N, 6·33 per cent). The natural amino-acid, generously provided by Dr. L. Fowden, was converted into its benzyloxycarbonyl derivative by treatment with benzylchloroformate at *p*H 10·3 (autotitrator) in aqueous acetone; the di*cyclo*hexylamine salt had m.p. 139° and no detectable optical rotation in chloroform solution (found: C, 70·21; H, 8·48; N, 6·63 per cent). In the solid state (nujol mulls) the two salts had distinctly different infra-red spectra, although the absorption bands corresponding to the principal functional groups were similar. In chloroform solution, however, the spectra were identical. Evidently the natural amino-acid is racemic, and this was confirmed by hydrogenation to a mixture of the *cis*- and *trans*-4-methylprolines (mainly the *cis* isomer[1]). We have shown previously that the optical rotations of the *cis* and *trans* isomers of 4-methyl-L-proline are both large and negative (−85° and −57° respectively)[4]. However, the hydrogenation product of the natural 4-methyleneproline had, within the limits of experimental error, no detectable rotation, and it can be concluded that less than 1 per cent of optically active material can be present.

4-Methyleneproline isolated from loquat seeds is therefore racemic, as suspected by Gray and Fowden[5]. This result is surprising, both in view of the rare occurrence of racemic amino-acids in Nature, and since the most plausible biogenetic precursor, 4-methylene-glutamic acid, has already been isolated in an optically active form[6], although from a different plant source.

Benzyloxycarbonyl-4-methylene-L-proline is a possible precursor for stereospecific synthesis of 4-hydroxymethylproline, the configuration of which is at present a matter of discussion[7].

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Use of Amines in Inorganic Hydrolysis

FOLLOWING the work of Smith and Page[1], amines have been widely used for the extraction of acids from aqueous solutions. We have applied this process to the hydrolysis of inorganic salts in aqueous solution without the introduction of any reagents into the aqueous phase. An amine which is immiscible with the aqueous phase is used to extract the acid formed on the hydrolysis of the salt and under suitable conditions insoluble complexes can be precipitated. Thus complexes of UO_2^{++}, Th^{4+}, Fe^{3+} and $RuNO^{3+}$ have been precipitated by us, and although the anion has usually been nitrate we have also carried out precipitations from sulphate media. Other anions could also be used since amines extract many acids efficiently (see, for example, Moore[2]).

The hydrolysis of uranyl nitrate can be made to proceed until a uranium complex is precipitated; for example, in a typical experiment when a 0·89 *M* solution of uranyl nitrate in water was stirred with 0·5 *M* trinonylamine (tri-3 : 5 : 5-trimethylhexylamine) in xylene for 5 min. at room temperature a yellow precipitate was formed. After washing with water and drying at 60° C. the composition of the solid corresponded to $UO_3.2H_2O$ (found: U = 73·2 per cent, H_2O = 12·7 per cent; calculated for $UO_3.2H_2O$, U = 73·8 per cent, H_2O = 11·2 per cent). The product was contaminated by xylene as shown by a carbon content of 0·76 per cent; but nitrogen could not be detected showing that no amine was present. The identity of the product was confirmed by X-ray examination and by differential thermal analysis (which indicated that it was the α-form of $UO_3.2H_2O$).

The precipitated complexes are probably of the type $UO_3.2H_2O.x$ amine and their stability varies with the type of amine, for example, with a tertiary amine, such as trinonylamine, the amine can be removed by thorough washing followed by drying at 60° C., but this is not the case if a primary amine such as 'Primene *JM-T*' (a proprietary mixture of alkyl primary amines with carbon chains ranging from C_{16} to C_{22}) is used. Dioctylamine (DOA, a mixture of the di-iso-octylamine isomers) gives a uranium–amine complex corresponding approximately to $UO_3.2H_2O.$ (DOA) and in stability to washing this is intermediate between the complexes formed by the primary and the tertiary amines examined. The efficiency of precipitation depends on the basicity of the amine and on the relative amounts of amine and acid. We have found precipitation efficiencies of about 99 per cent for the precipitation of uranium with 'Primene *JM-T*' dissolved in kerosene or with DOA dissolved in xylene using amine : uranyl nitrate mole ratios of approximately 3 : 1.

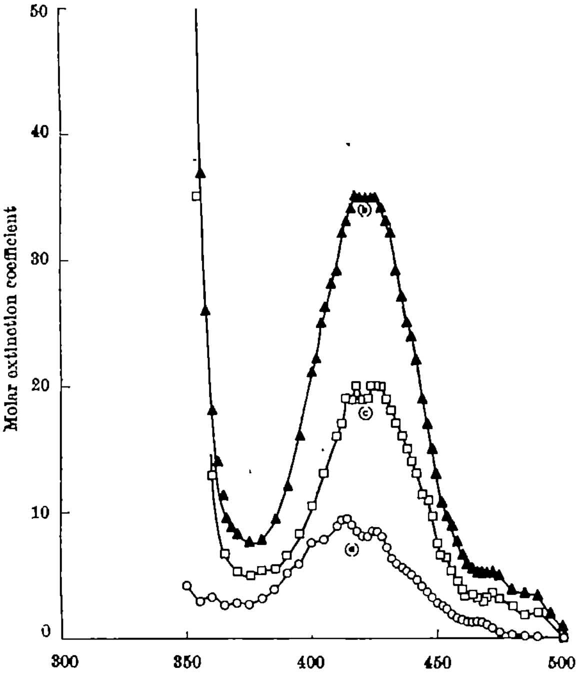

Fig. 1. Absorption spectra: *a*, aqueous uranyl nitrate; *b*, the same solution after extracting 52·9 per cent of the nitrate (no uranium precipitated); *c*, the supernatant liquor after extracting 93 per cent of the nitrate (82 per cent of the uranium precipitated)

The method described here can also be used for the examination of hydrolytic reactions taking place in aqueous solutions, again without introducing reagents into the aqueous phase (the solubility of trinonylamine in water is less than 5 parts per million). This is illustrated in Fig. 1, which gives the absorption spectra of (*a*) aqueous uranyl nitrate solution, (*b*) the same solution after 52·9 per cent of the nitrate has been extracted into trinonylamine (but no uranium has been precipitated) and (*c*) the supernatant liquor after 93 per cent of the nitrate has been extracted into trinonylamine from a 0·24 *M* solution of uranyl nitrate in water, and 82 per cent of the uranium has been precipitated. The spectra of the hydrolytic species resemble those found in the titration of uranyl perchlorate with sodium hydroxide[3], and the extent of hydrolysis may be controlled by choice of amine, concentration of amine and by variation in the relative phase volumes of amine and aqueous phases.

We thank Dr. E. Wait for the X-ray examination and Dr. R. M. Dell for the differential thermal analysis. We are grateful to Imperial Chemical Industries, Ltd., for supplies of trinonylamine and dioctylamine and to C. Lennig and Co., Ltd., for 'Primene *JM-T*'.

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BIOCHEMISTRY

Xanthophyll Pigmentation in Sheep Fat

CASTLE[1] concluded from the observations of Zophoniasson that the yellow fat appearing in some Icelandic sheep is due to a simple recessive character in inheritance. I have recently examined the chemical nature of the yellow pigments in the adipose tissue of some Irish sheep. The yellow fat was rendered from the adipose tissue by heating in an oven at 60° C. One hundred and fifty grams were saponified by heating with 300 ml. of 20 per cent alcoholic potassium hydroxide. After dilution with 900 ml. of water the pigments were extracted using 1,200 ml. of diethyl ether. Complete extraction was accomplished by using 600-ml. portions of ether in two or three subsequent extractions. After washing with water and drying over anhydrous sodium sulphate the ether was distilled off under nitrogen on a water bath. The residue of crude pigments and cholesterol was dissolved in 5 ml. of hexane and chilled. After filtration, from unsaponified material deposited during chilling, the pigment extract was made up to 50 ml. using hexane.

The yellow colour of a petroleum ether 40°–60° C. solution of the pigments was completely extracted by 90 per cent v/v aqueous methanol, thus proving the presence of xanthophylls and the absence of carotene. When the hexane solution was chromatographed on a magnesium oxide column and developed with a mixture containing 82·5 per cent hexane, 15 per cent acetone and 2·5 per cent ethanol, the pigment mixture was resolved into three yellow bands designated *A*, *B* and *C* in order of increasing adsorption. Fractions *A* and *B* were eluted by the developer and collected separately. Fraction *C* was eluted by 8 per cent ethanol in hexane. After washing with water and drying over anhydrous sodium sulphate each of the three fractions was re-chromatographed on a column of de-activated aluminium oxide, and solutions of ethanol in hexane (2 and 4 per cent) were used as developer. Minor bands obtained by this treatment were separated from the main fractions *A*, *B* and *C*. These three fractions after elution from the de-activated aluminium oxide were incapable of further resolution when washed, dried and re-chromatographed on a basic zinc carbonate column

Table 1. SPECTROPHOTOMETRIC CONSTANTS OF LUTEIN, LUTEIN-5 : 6-EPOXIDE, FLAVOXANTHIN AND OF FRACTIONS *A*, *B* AND *C*. MAXIMA ARE EXPRESSED IN MILLIMICRONS

	Hexane	Carbon disulphide	Chloroform	Benzene
Lutein	420,447,477	—	428,456,487	—
Fraction *A*	419,445,475	—	426,450,480	—
Lutein-5 : 6-epoxide	442,471	472,502	—	453,482
Fraction *B*	443,472	473,501	—	453,482
Flavoxanthin	421,450	—	430,459	432,456 (ref. 3)
Fraction *C*	421,448	—	430,455	432,455

and developed with 20 per cent ether in benzene. Fraction *A* and lutein prepared from grass were found to be chromatographically homogeneous.

Table 1 shows the position of the absorption spectra maxima of the three purified fractions *A*, *B* and *C* in various solvents and also gives the spectrophotometric constants of lutein, lutein-5 : 6-epoxide and flavoxanthin as given by Goodwin[2]. It is concluded from the chromatographic homogeneity of fraction *A* and lutein, the positions of the pigments on the magnesium oxide column[1], the spectrophotometric constants of the fractions in the various solvents and the shape of the absorption curves, three of which are shown in Figs. 1, 2 and 3, that fraction *A* is lutein, fraction *B* is very probably lutein-5 : 6-epoxide and fraction *C* is most likely flavoxanthin. All these three xanthophylls are present in green tissues[3]. The lutein-5 : 6-epoxide appeared to be present in greatest concentration, though it is possible that some lutein was lost during the saponification.

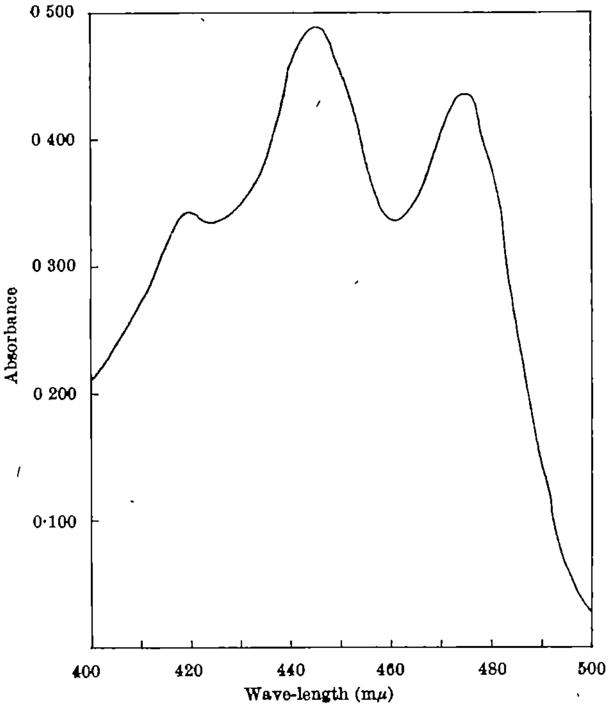

Fig. 1. Visible absorption spectrum in hexane of lutein extracted from yellow sheep fat

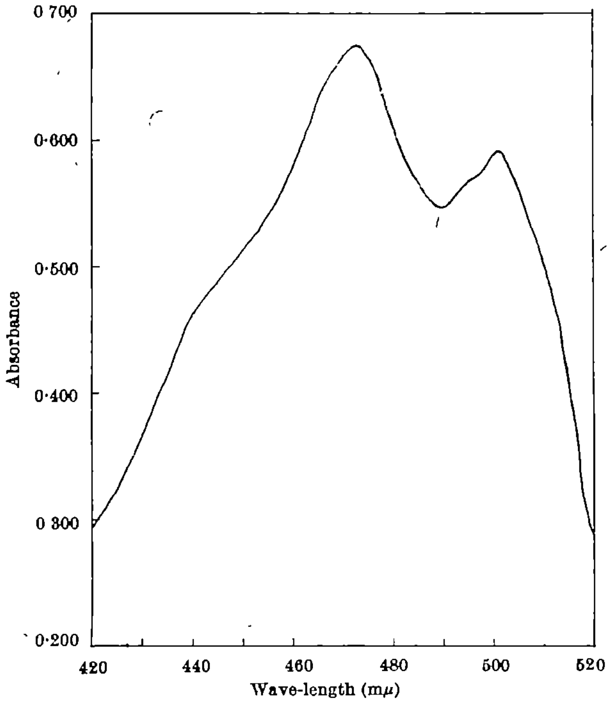

Fig. 2. Visible absorption spectrum in carbon disulphide of lutein-5 : 6-epoxide extracted from yellow sheep fat

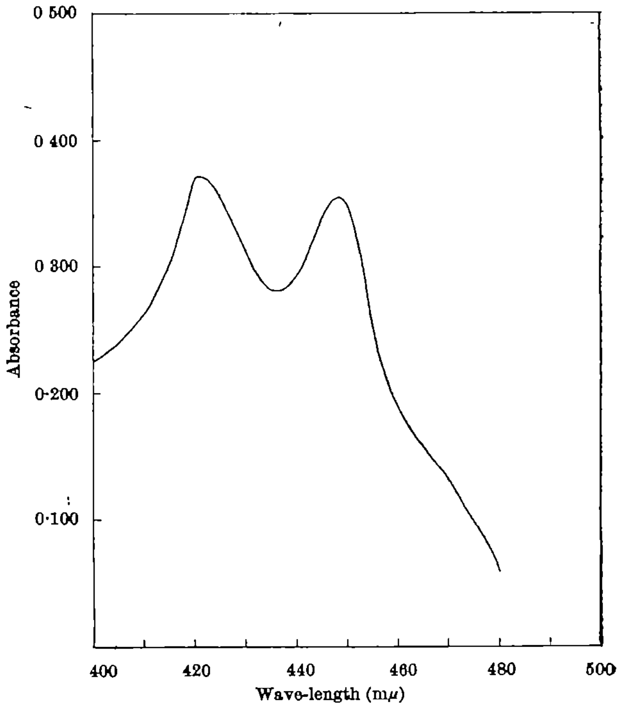

Fig. 3. Visible absorption spectrum in hexane of flavoxanthin extracted from yellow sheep fat

It would seem that the yellow fatted sheep has inherited the inability to oxidize the xanthophylls. The primitive sheep may have been deficient in a 'xanthophyll oxidase' system and the yellow-fatted sheep of to-day may be atavistic in possessing yellow fat. The only other animal which has been reported to store xanthophylls exclusively in its adipose tissue is the yellow-fatted rabbit[4]. A yellow-fatted sheep may be detected before slaughter by the following rapid test. The proteins of a sample of the sheep's blood serum are precipitated by ethyl alcohol. Extraction with diethyl ether brings the xanthophylls into the ether layer. A yellow colour in the ether layer indicates a yellow-fatted sheep. A full account of this work will be published elsewhere[5].

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A Mammalian Peptidase specific for the Hydrolysis of *N*-Terminal α-L-Glutamyl and Aspartyl Residues

THE histochemical visualization of a calcium activated *N*(α-L-glutamyl)β-naphthylamide-cleaving activity has been described in an earlier publication[1]. Hydrolysis of *N*(α-L-aspartyl)β-naphthylamide has now been observed histochemically in sites identical to those described for *N*(α-L-glutamyl)β-naphthylamide (for example, guinea pig pancreatic duct epithelium and islets of Langerhans and human and rat glomeruli) in the presence of the optimal concentration of the activating ion, calcium. The above tissue section localizations of the enzymatically catalysed hydrolysis of the α-L-glutamyl and α-L-aspartyl substrates are distinct from those seen with other β-naphthylamide substrates in the presence of calcium ions.

An enzyme having the characteristics noted here, aminopeptidase *A*, has been partially purified from rat kidney by the following method. The microsomal fraction derived from sucrose or potassium chloride homogenates of rat kidneys was resuspended in 0·001 *M tris*(hydroxyamino)methane buffer at *p*H 7·5. The particulate suspension was subjected to digestion by a mixture of trypsin and chymotrypsin. Centrifugation of the digest at 100,000*g* gave a particulate fraction containing essentially all the aminopeptidase *A* activity and was devoid of significant activity toward glycyl, L-leucyl, α-L-glutaminyl, DL-alanyl, or DL-phenylalanyl-β-naphthylamide.

Parallel calcium ion activation of *N*(α-L-glutamyl)-β-naphthylamide and *N*(α-L-aspartyl)β-naphthylamide is apparent from the results given in Table 1.

Table 1. LIBERATION OF β-NAPHTHYLAMINE (mμMOLES/MIN./MGM ENZYME)

	Additions to incubation solution*	Substrate	
		α-L-Glutamyl-β-naphthylamide	α-L-Aspartyl-β-naphthylamide
1	None	27	3·3
2	0·001 *M* $CaCl_2$	55	9·2
3	0·01 *M* $CaCl_2$	65	11
4	0·02 *M* $CaCl_2$	62	11
5	0·01 *M* Ethylenediamine tetraacetate	3·3	0·3
6	0·01 *M* $CaCl_2$ and 0·002 *M* α-L-Glutamyl-L-phenylalanine	20	2·2

* Incubation solution. Enzyme, 0·06 mgm. dry weight, substrate, 0·41 mgm. α-L-glutamyl-β-naphthylamide or 0·39 mgm. α-L-aspartyl-β-naphthylamide; fast garnet GBC, 0·5 mgm.; in 3 ml. 0·1 *M tris*-(hydroxyamino)methane-maleate buffer, *p*H 7·0, 37° C. The reaction was stopped after 10 min. by the addition of 1 ml. 10 per cent 'Tween 20' in 1 *M* acetate buffer, *p*H 4·2. After 30 min. absorbance at 525 mμ was determined.

Disodium ethylenediamine tetraacetate and α-L-glutamyl-L-phenylalanine produced an inhibitory effect on the hydrolysis of the aspartyl substrate equivalent to that produced on the glutamyl substrate. The inhibition by α-L-glutamyl-L-phenylalanine was found to be of a competitive nature. No effect on hydrolysis was afforded by L-leucinamide, L-leucylglycine or glycyl-glycine (0·002 *M*). The dipeptides α-L-glutamyl-L-phenylalanine and α-L-aspartyl-L-arginine were defined as substrates for aminopeptidase *A* by chromatographic identification of constituent amino-acids of each dipeptide following incubation with the enzyme. No hydrolysis of *N*(α-D-glutamyl)β-naphthylamide, *N*(N^{α}-benzoyl-α-L-glutamyl)β-naphthylamide or *N*-carbobenzoxy-α-L-glutamyl-L-phenylalanine was observed in the presence of high concentrations of aminopeptidase *A*.

Strong evidence that the hydrolysis of both the *N*(α-L-aspartyl)- and *N*(α-L-glutamyl)β-naphthylamide substrates is catalysed by the same enzyme is afforded by the identical effects of activator and inhibitors on the hydrolysis of these substrates and the identical histochemical localization obtained with both. Moreover, the maximum velocity determined by the method of Lineweaver and Burk using an equimolar mixture of naphthylamide substrates was found to lie between the maximum velocities of the individual substrates, again attesting to the fact that only one enzyme is involved in the hydrolytic reactions.

The absence of hydrolysis of *N*(α-D-glutamyl)β-naphthylamide and *N*(N^{α}-benzoyl-α-L-glutamyl)β-naphthylamide in both the histochemical and biochemical systems and of *N*-carbobenzoxy-α-L-glutamyl-L-phenylalanine in the biochemical system indicates that aminopeptidase *A* is stereospecific and exhibits primarily exopeptidase activity.

The biochemical data confirm the existence of the histochemically defined peptidase having a relative specificity for *N*-terminal L-dicarboxylic amino-acids. For convenience and clarity of nomenclature this enzyme is referred to as aminopeptidase *A* (acid α-amino peptidase) to distinguish it from classic leucine amino peptidase[2] and enzymes hydrolysing acidic omega amino[3] and non-acidic α-amino-acid β-naphthylamides[4].

Pig kidney and duodenum are good sources of aminopeptidase *A* and further investigations to obtain a more purified preparation from these sources are in progress. A report of these studies as well as a detailed account of the kinetics of aminopeptidase *A* activity, its application as an end group reagent, the steric and chemical limits of specificity of the enzyme toward synthetic substrates and procedures for the syntheses of these substrates will be the subject of future communications.

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Adrenal Glucose-6-Phosphatase: Alterations in Intracellular Distribution induced by Treatment with Adrenocorticotrophic Hormone

HAYNES *et al.*[1] have proposed a mechanism of the action of adrenocorticotrophic hormone (ACTH) on the adrenal gland in which glucose-6-phosphate (G-6-P) plays a most important part. The increased rate of production of G-6-P, arising from the activation of phosphorylase by ACTH to produce increased quantities of glucose-1-phosphate and the subsequent conversion of the glucose-1-phosphate to G-6-P by phosphoglucomutase, results in an increased metabolism of G-6-P by oxidation to 6-phosphogluconic acid. This oxidation produces reduced triphosphopyridin nucleotide (TPNH) and the TPNH serves as a source of energy for various steps in the production of steroids[2]. However, G-6-P may also be metabolized by dephosphorylation, affected by the enzyme glucose-6-phosphatase (G-6-Ptase).

Since we have been examining the effect of treatment with ACTH on certain adrenal enzymes[3], we felt that an investigation of the effects of ACTH on G-6-Ptase might provide further insight into the metabolism of G-6-P. In this communication results are presented indicating that ACTH treatment caused a change in activity and intracellular distribution of G-6-Ptase.

For preparation of adrenal particulate and soluble cell fractions, approximately 500–700 mgm. of adrenals were collected (100–125 mice) and pooled prior to homogenization. Details of the procedure were given earlier[3]. In order to study the effect of ACTH on enzyme activity in cell fractions, another preparation was made from animals killed 30–45 min. following the subcutaneous injection of 500 milliunits of ACTH. Prior to enzyme assay, each cell fraction was resuspended by briefly homogenizing in a sufficient volume of 0·25 molar sucrose. G-6-Ptase was assayed by Swanson's method[4]. Nitrogen determinations were performed using a modification of the Pregl micro-Kjeldahl method[5]. Enzyme activity (mean of quadruplicate determinations) was expressed in terms of nitrogen.

Table 1 illustrates the intracellular distribution of G-6-Ptase in normal adrenal glands. The results indicate that this enzyme appears to be localized in the microsomal fraction, a result similar to that reported for liver G-6-Ptase[6]. However, following the *in vivo* treatment with ACTH, several differences were observed as recorded in Table 1. The G-6-Ptase activity was found also in the nuclear and mitochondrial fractions, and in small amounts in the soluble cytoplasm as well (less than 2 per cent of the total activity). The most striking difference was the microsomal G-6-Ptase activity, which decreased to less than 50 per cent of the value found in normal glands. Total activity was decreased by approximately 20 per cent.

Table 1. LOCALIZATION OF GLUCOSE-6-PHOSPHATASE ACTIVITY IN ADRENAL CELL FRACTIONS BEFORE AND AFTER ACTH TREATMENT

Cell fraction	Nitrogen (mgm./gm. tissue)	Specific activity*	Total activity†	Total activity (per cent)
	Normal			
Nuclei	5 0	—	—	—
Mitochondria	4·2	—	—	—
Microsomes	2 6	0·402	33·2	100
Soluble cytoplasm	5·7	—	—	—
Total	17 5		33 2	100
	500 milliunits ACTH			
Nuclei	3·6	0·034	3 6	13
Mitochondria	4·5	0·063	6·4	24
Microsomes	3·1	0 162	16·7	61
Soluble cytoplasm	6 8	0 027	0 4	2
Total	18 0		27·1	100

* Specific activity expressed as μgm. inorganic phosphate released/μgm. nitrogen.

† Total activity expressed as mgm inorganic phosphate released/gm. protein

If one assumes that a change in G-6-Ptase activity is a reflexion of some alteration in the metabolism of G-6-P, then the data presented here suggest that treatment with ACTH causes a rapid change in the metabolism of this compound. However, the exact relationship of these changes of enzyme to the production of adrenocortical steroids is not known. One possible explanation is that the decrease in G-6-Ptase activity might shunt the metabolism of G-6-P partly towards oxidation, since it has been shown that an increase in G-6-Ptase activity favours the formation of the oxidized form of TPN[7]. If the metabolism of G-6-P via oxidation (hexose monophosphate shunt) in the microsomes is enhanced, then it would follow that the production of TPNH would be increased. There are reports indicating that, following the administration of ACTH. adrenal TPNH rises[1]. On the other hand, the appearance of extra-microsomal G-6-Ptase activity following ACTH treatment suggests an increased G-6-P metabolism by dephosphorylation, favouring the formation of TPN. Further work is necessary to elucidate these intracellular relationships.

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Effect of Dietary Linolenate on the Proportion of Linoleate and Arachidonite in Liver Fat

PRELIMINARY experiments have suggested that feeding of linseed oil fatty acids depressed the proportion of arachidonic acid in liver and cerebella of chickens[1]. The following experiment was conducted to determine the validity of the original observation.

Day-old Nichols 108 cockerels were fed a purified low-fat diet for seven days. They were then divided into groups of 8 birds each and fed diets containing either ethyl linoleate or linseed fatty acids added at the expense of glucose. The chickens were fed the experimental diets until they were 35 days old. They were then fasted overnight, killed, and the liver analysed for fatty acids using gas chromatography. The results are given in Table 1.

Table 1. EFFECT OF DIETARY FAT ON THE FATTY ACID COMPOSITION OF LIVERS FROM 35-DAY-OLD CHICKENS

	Dietary fat supplement			
Fatty acid	None† (per cent)	2 per cent ethyl linoleate* (per cent)‡	4 per cent ethyl linoleate (per cent)	10 per cent linseed fatty acids§ (per cent)
10 0	0 2	0 2	0 1	0 0
12 0	0 1	0·2±0 1	0 2±0 1	0 3±0 1
14·0	0 4±0 4	0 4±0·4	0 4±0 2	1 5±1 0
16:0	16·1±1·7	33 3±2 6	30 3±4·7	26 6±2 7
16:1	5 9±0 9	4 6±1 2	2 6±3 2	0 4±0 3
18·0	21 7±1 8	27 4±6·7	26 1±5 9	31·5±3 6
18·1	48 0±2 1	16 6±1 6	10 9±2 3	10·4±1 3
18 2	5 4±0 4	11 5±3 1	21·7±1 8	22 0±2 0
18.3	0 0	0 0	0 0	3 9±0 9
20·3	5 9±1 4	0 0	0 0	0 0
20.4	1 2±0 2	5 9±1 7	8 0±1 3	3 4±1 2

* 75 per cent ethyl linoleate, Nutritional Biochemicals, Cleveland, Ohio. This diet contains 1 5 per cent 18:2.

† Diet *C*-21 (casein, amino-acid, glucose, vitamins and minerals[2]) modified by deletion of methyl myristate and increasing arginine to 0 9 per cent

‡ Per cent by weight of total fatty acids in liver. Average of 3 replicates ±*S.D.*

§ Veg. Acid 520, Archer Daniels Midland, Minneapolis, Minn. This contains 47 per cent 18 3 and 17 per cent 18:2. The diet contains 1 7 per cent 18 2.

The linoleic acid content of the diets containing 10 per cent linseed fatty acids or 2 per cent ethyl linoleate is about the same (1·7 per cent and 1·5 per cent respectively). Nevertheless, the percentage of linoleic in the liver of chickens fed the linseed fatty acids was approximately twice the level observed in the livers of the birds fed 2 per cent linoleate. The arachidonic acid was significantly ($P < 0\cdot05$ by the t test) lower in the birds consuming linseed oil fatty acids. Since arachidonic acid is synthesized from linoleic acid[3] our observations suggest that the linolenic acid in the linseed fatty acids is inhibiting the conversion of linoleic acid to arachidonic acid.

This hypothesis, however, should be confirmed by the feeding of pure linolenic acid and preferably by the use of carbon-14 labelled linoleic acid.

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Elastin a Sialoprotein

THE aorta consists of elastin, collagen and a number of other proteins; it also contains about 2 per cent of carbohydrate[1]. Elastin is usually isolated from aorta or ligamentum nuchæ by treatment with acids and alkalis at elevated temperatures; however, a small amount of ill-defined carbohydrate appears to be held strongly and cannot be easily removed[2]. It is possible that this sugar moiety is an integral part of elastin.

The object of the work reported here was to isolate a fragment containing the elastin-sugar linkage using very mild conditions, and to investigate its nature.

A bovine aorta was cleaned, minced in the cold and treated in turn with pepsin at *p*H 2·5, trypsin at *p*H 7·5, and collagenase at *p*H 7·0. The residue was washed well after each treatment, which lasted 30–40 hr. at 37°. The tryptin digest contained most of the acid mucopolysaccharide, as judged by the content of hexosamine and hexuronic acid. The final residue, which accounted for about 50 per cent of dry aorta, was then treated exhaustively with pure elastase at *p*H 8·5 (preparation *E*,I, Hall and Czerkawski[3]), which resulted in more than 90 per cent solubilization. Dialysis of the solubilized material gave approximately equal amounts of diffusate and retentate.

Although both fractions of elastolysate had similar amino-acid composition, the diffusate was devoid of carbohydrate. The retentate, which is practically resistant to further action by elastase, contains about 0·5 per cent of a substance giving the usual reactions for sugars. It contains no glucosamine or galactosamine, but approximately equal amounts of sialic and hexuronic acids. The presence of the former was first indicated by considerable humin formation during hydrolysis in strong mineral acid, and finally confirmed by the positive Bial and direct Ehrlich reactions The actual amount of sialic acid was estimated after a mild acid hydrolysis (1 hr. at 80° in 0·1 *N* sulphuric acid) by the thiobarbiturate and alkali-Ehrlich methods of Aminhoff[4]. Such hydrolysis did no damage to the protein and certainly did not remove the hexuronic acid (Table 1, col. 3), and although it made the sialic acid reactive to thiobarbiturate, probably due to hydrolysis of the glycosidic bond on C2, it did not produce a complete cleavage, because the sugar could not be separated from the residual protein by dialysis. When the products of mild hydrolysis were subjected to dialysis, no sialic acid could be detected in either the diffusate or retentate. The reactivity of sialic acid with the thiobarbiturate reagent could be restored when the retentate was again hydrolysed for 1 hr. at 80° in 0·1 *N* acid. This can be explained by supposing that the mild acid hydrolysis breaks only one of two linkages joining sialic acid to elastin, and that subsequent dialysis results in the reformation of this bond (Table 1, col. 2).

Table 1 ANALYSIS OF SUGAR COMPONENTS AND REACTIVITY TO ELASTASE OF THE RETENTATE FRACTION

Treatment	Apparent content of sialic acid (per cent)	Content of hexuronic acid (per cent)	Elastolytic activity (arbitrary units)
None	0·02	0·23	7·0
Mild acid hydrolysis	0·29	0·23	19·8
Dialysis (retentate)	0·02	0·19	12·0
Another hydrolysis (retentate)	0·23	—	—

The sialic acid was determined by Aminoff's method (ref. 4), and the hexuronic acid was determined directly by the method of Bitter and Ewins (ref. 5). The elastolytic activity was determined by the trichloroacetic acid precipitation method as described by Hall and Czerkawski (ref. 6).

The ability to react with thiobarbiturate is paralleled by the susceptibility of the retentate fraction to further attack by elastase. The mild acid hydrolysis makes the protein susceptible to further proteolytic attack, but this newly acquired susceptibility is greatly reduced after dialysis (Table 1, col. 4).

It is too early to venture any firm conclusions at this stage; but there is every indication that sialic acid is doubly linked to elastin and so is a cross-linking agent, probably in association with the hexuronic acid. It is possible, that it occupies a strategic position, because, although present in such small amount (about 1 mole/10⁵ gm.), it plays an important part in the reaction with elastase. This last fact also makes it unlikely that the sialic and hexuronic acids are part of a large polysaccharide polymer; on the contrary, they are probably uniformly distributed throughout the solid elastin.

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m-Hydroxyphenylpropionic Acid, a Major Urinary Metabolite of (+)-Catechin in the Rat

ALTHOUGH substances of the flavonoid type are present in many foodstuffs of plant origin the metabolic fate of these compounds in mammalian metabolism was until recently largely unknown. The compounds on which recent investigation has been centred, however, are of either the flavonol[1-5], the flavanone or the flavone type[6]. It appeared to be of interest. therefore, to study the fate of a compound of the related catechin type, in which the keto group at C4 is absent.

A group of male albino rats were placed on a standardized, low flavonoid diet based on milk powder and white flour with salt and vitamin supplements. After a period of two weeks, the rats were divided into control and experimental groups, the control group being maintained on the foregoing diet while each rat of the experimental group received 50 mgm. (+)-catechin in admixture with the same food powder. The urine was collected over a period of 3 days following the administration of catechin and ethereal extracts prepared for chromatography by the method of Booth *et al.*[1]. The amount of ethereal extract applied to each chromatogram was equivalent to 2 ml. of fresh urine.

Examination of the extracts in the two-dimensional system, chloroform/acetic acid/water, 2 : 1 : 1 (lower phase) and 20 per cent potassium chloride[1], revealed the presence of a compound (compound *X*, Table 1) in the urine of the catechin-fed rats which was shown to be chromatographically identical with an authentic specimen of *m*-hydroxyphenylpropionic acid. Although the compound was present in relatively large amounts in the urine of catechin-fed rats, it was completely absent from the control chromatograms. The identity of the compound was afterwards confirmed (Table 1) using a second two-dimensional system, employing isopropyl alcohol/aqueous ammonia/water, 8 : 1 : 1 (ref. 7), and benzene/acetic acid/water, 60 : 70 : 30 (ref. 8).

Table 1. CHROMATOGRAPHIC BEHAVIOUR OF *m*-HYDROXYPHENYLPROPIONIC ACID AND COMPOUND *X*

	m-OH-phenyl-propionic acid R_F	Compound *X* R_F
Chloroform, acetic acid, water	0·75	0·75
20 per cent KCl	0·71	0·71
Isopropyl alcohol aq. NH_3, water	0·64	0·64
Benzene, acetic acid, water	0·66	0·66
Diazotized *p*-nitraniline	Red-purple	Red-purple
Diazotized sulphanilic acid	Orange-yellow	Orange-yellow

Both the catechin metabolite described and *m*-hydroxyphenylpropionic acid gave identical colour reactions with the spray reagents diazotized *p*-nitraniline and diazotized sulphanilic acid (purple-red and orange-yellow respectively).

The formation of a phenyl-propionyl derivative from (+)-catechin indicates that ring fission occurs between C4 and the adjacent C atom of ring *A* without any break between C3 and C4. Since ring fission of this type is given by flavan-4-ones and flavones[6] but not by flavonols, which yield only phenylacetyl derivatives[1], it is thought likely that dehydroxylation at C3 accompanied by oxidation of the C4 atom to a carbonyl group occurs before ring fission. Conversion of (+)-catechin to a flavanone structure could be effected in two steps: (1) Oxidation of (+)-catechin to the corresponding flavan-3 : 4-diol. (2) Removal of the elements of water from the 3 : 4-diol grouping to give the corresponding flavan-4-one.

The dehydroxylation observed with respect to the C4′ of the benzenoid ring of the catechin molecule appears to parallel that already reported to occur during metabolism of both 3′,4′-dihydroxy flavonols and 3′ : 4′-dihydroxy-flavanones in the rat[1,6]. *p*-Dehydroxylation of a 3 : 4-dihydroxyphenylalkyl acid also was reported recently by Scheline, Williams and Witt[9].

Of three types of urine sample collected (namely, 0–24 hr., 24–48 hr., 48–72 hr. after administration of catechin), the maximal level of *m*-hydroxyphenylpropionic acid was usually found in the 24–48 hr. sample; the acid not infrequently being wholly absent from the first sample (0–24 hr.) This may be due either to catechin being slowly absorbed from the gut or to formation of *m*-hydroxyphenylpropionic acid from catechin being dependent on the action of the intestinal microflora. Experiments are at present being carried out to clarify this point.

The demonstration of the formation of *m*-hydroxyphenylpropionic acid from (+)-catechin in the rat suggests a possible origin for the *m*-hydroxyphenylpropionic acid, detected by Armstrong *et al.*[7], in human urine, since catechins and their derivatives are known to be present in tea[10] and cocoa[11,12].

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Alkali Metal Requirements in Oxidative Phosphorylation

THE selective binding of potassium ions by mitochondria and their digitonin sub-fragments, coupled with the finding that active electron transport is required for maximal binding[1], indicates the importance of determining the effect of the alkali metal ions on oxidative phosphorylation processes in these organelles.

A loss of K^+ occurs from uncoupled mitochondria using a variety of metabolic inhibitors[1–3], and this effect can be produced even at 0° (refs. 4 and 5). Tapley[6] has suggested that the loss of K^+ from mitochondria may be the primary defect, and in fact be responsible for such uncoupling action.

Boyer, Lardy and Phillips[7] have shown that K^+ were required by liver minces to produce esterification of phosphate during the oxidation of pyruvate. Pressman and Lardy[8] have claimed a stimulation of mitochondrial respiration by K^+, with no effect on phosphorylation, although phosphorylation was not measured directly. Few direct studies have been made since, and most investigations have replaced one metal with another, changing sucrose to maintain tonicity. As sodium may substitute for potassium it was felt that the replacement of one ion by the other, either in part or in full, did not produce conclusive evidence. In addition sucrose alone is inhibitory to mitochondrial function[9].

In this work a medium which was both free of potassium and with a very low concentration of sodium was used. The source of inorganic phosphorus was obtained by neutralizing dilute phosphoric acid to *p*H 7·0 with *tris*hydroxymethylmethane buffer (*tris*). The substrates (α-oxo-glutaric and succinic acids) were used as the free acids also neutralized to pH 7·0 with *tris*. Adenosine triphosphate (Sigma) was included either as the disodium salt for low Na^+ concentrations or prepared as the free acid from a 'Dowex 50 × H' column and then neutralized to pH 7·0 with *tris* to give an Na^+ free medium. Human serum albumin, prepared by exhaustive dialysis of albumin concentrate (Commonwealth Serum Laboratories) against electrolyte free water, was used to provide tonicity.

Rat liver mitochondria were prepared essentially by the method of Schneider[10] and were examined in a conventional Warburg apparatus at 30° C. The mitochondria equivalent to 0·75 gm. of fresh liver tissue were added last, in 0·5 ml. of ice cold 0·25 M sucrose, to each vessel. The final concentration of reactants in each Warburg vessel was as follows: magnesium chloride 5 mM; adenosine triphosphate 2 mM; substrate 10 mM; glucose 16 mM; albumin 0·75 per cent. 30 units of Sigma (III) hexokinase in 1 per cent glucose When required, sodium and potassium ions were provided by the addition of the chloride salts.

The concentration of either Na^+ or K^+ in the alkali ion free medium was shown by flame spectrophotometry to be less than 10^{-4} M.

In this medium, liver mitochondria carried active oxidation and phosphorylation in the presence of both α-oxo-glutarate and succinate. The phosphorus/oxygen ratios obtained under these conditions were 1·9 and 1·0 respectively. The addition of 5 mM Na^+ had little effect but a marked inhibition of both oxidation and phosphorylation occurred when the concentration of Na^+ was increased to 75 mM. An increase in metabolic activity was not produced by the addition of 75 mM K^+ to the medium. A small but constant inhibition of both oxidation and phosphorylation occurred under these conditions. These results are shown in Table 1.

Table 1. EFFECT OF Na^+ AND K^+ ON THE EFFICIENCY OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

Substrate	Alkali ion	concentration (mM)	No of exp.	Mean Pi* uptake	Mean oxygen* utilization	P/O
α-oxo-glutarate	Na^+	0	4	20·9 (19·2–21·9)	10·7 (10·4–11·2)	1·9
	K^+	0				
	Na^+	5	4	19·7 (18·4–22·0)	10·4 (10·0–11·6)	1·9
	K^+	0				
	Na^+	5	2	15·1 (14·6–15·6)	9·3 (9·0–9·6)	1·6
	K^+	75				
	Na^+	75	2	3·6 (1·7–5·9)	4·5 (4·2–4·7)	0·8
	K^+	0				
Succinate	Na^+	0	4	15·1 (12·6–18·7)	15·0 (13·6–18·4)	1·0
	K	0				
	Na^+	5	4	10·9 (9·7–12·6)	9·4 (8·0–10·9)	1·2
	K	0				
	Na^+	5	4	9·2 (8·1–10·1)	8·6 (8·1–9·5)	1·1
	K	75				
	Na^+	75	4	5·0 (3·1–6·8)	6·7 (6·1–7·6)	0·7
	K	0				

*μatoms/12·5 mgm protein/15 min.

Table 2. EFFECT OF SUSPENDING MITOCHONDRIA FOR 10 MIN AT 0° C. IN A SUCROSE MEDIUM CONTAINING 5 mM SODIUM SALICYLATE, ON OXIDATIVE PHOSPHORYLATION

Substrate	Alkali ion	concentration (mM)	No. of exp.	Mean Pi* uptake	Mean oxygen* utilization	P/O
α-oxo-glutarate	Na^+	5	2	24·6 (24·5–24·8)	14·5 (14·5–14·6)	1·7
	K^+	0				
	Na^+	5	2	12·9 (11·5–14·3)	9·0 (8·0–10·0)	1·4
	K^+	75				
Succinate	Na^+	5	2	30·2 (29·9–30·6)	25·3 (24·6–26·0)	1·2
	K^+	0				
	Na^+	5	2	1·2 (0·9–1·54)	15·8 (15·8–15·8)	0·1
	K^+	75				

* μatoms/12·5 mgm. protein/15 min.

In some experiments the measurement of oxidative phosphorylation was made after suspending the mitochondria for 10 min. at 0° C. in a 0·44 M sucrose medium containing 5 mM sodium salicylate prior to final separation. This was done because previous work in these laboratories had shown that this procedure results in a marked increase in the loss of bound K^+ from mitochondria (> 60 per cent) under these conditions, and that the loss of K^+ is not compensated for by replacement with Na^+ (ref. 5).

Results of these experiments are given in Table 2, and demonstrate that even a considerable loss of bound mitochondrial K^+ prior to incubation had little effect on the efficiency of oxidative phosphorylation when the mitochondria were examined in a K^+ free medium, although an increase in respiratory activity was apparent in these mitochondria. The addition of 75 mM K^+ to the medium under these conditions produced considerable inhibition of respiration with either substrate. However, phosphorylation was only slightly reduced when α-oxo-glutarate was the substrate, but was markedly decreased when succinate was the substrate.

These results suggest that K^+ binding and K^+ exchange are not required for efficient mitochondrial respiration and phosphorylation, and that the loss of bound K^+ from mitochondria *per se* will not uncouple oxidative phosphorylation.

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PHYSIOLOGY

Effect of DL-Methionine Sulphoximine on Incorporation of ^{35}S-Methionine into Proteins of Brain Cortex Slices

IT has been shown by a number of authors that the administration of methionine sulphoximine (MSI) to experimental animals produces convulsions similar to epileptic seizures. Many metabolic disturbances occurred in the tissue of the brain under these conditions[1,2], one of these being the inhibition of ^{35}S-methionine incorporation into brain proteins *in vivo*[3-5]. However, the exact mode of action of MSI remains, so far, unknown.

To obtain more information about the connexion between MSI and protein metabolism of the brain, I have examined the effect of this substance on incorporation of ^{35}S-methionine into proteins of brain cortex slices *in vitro*.

The brain was rapidly excised following decapitation of the animal and sliced on an ice-cold block. The slices were incubated in a Warburg apparatus in Krebs–Ringer phosphate-saline, pH 7·4, containing 0·01 M glucose and 6 μc. ^{35}S-methionine (per vessel) of 279 mc./gm. specific activity. DL-Methionine

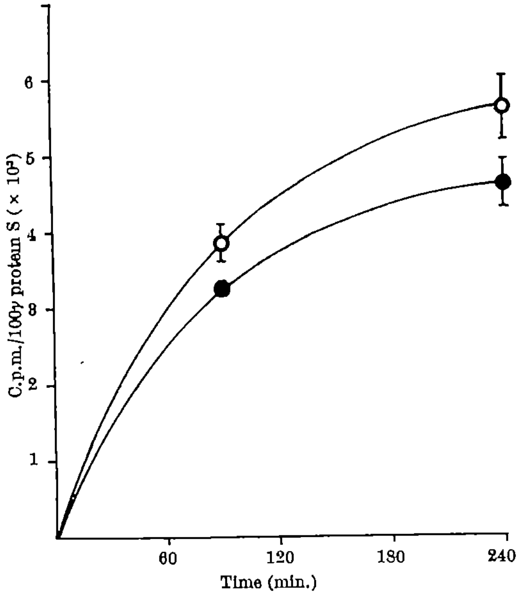

Fig. 1. Time course of ^{35}S-methionine incorporation into proteins of brain cortex slices in the presence (●) or absence (○) of MSI in the incubation medium. Slices were incubated in Krebs–Ringer phosphate-saline pH 7·4, containing glucose and ^{35}S-methionine, with or without MSI (10^{-2} *M*) at 37° C., gaseous phase oxygen. Curves are drawn through points corresponding to mean values of four independent experiments ± *S.E.*

sulphoximine (MSI) was obtained from the California Corporation for Biochemical Research, Los Angeles.

Following incubation, the protein fraction of brain cortex slices was obtained by the procedure described elsewhere[6]. Protein-bound sulphur was oxidized to sulphate, and then precipitated as benzidine sulphate, the radioactivity of which was measured in a Frieseke–Hoepfner scaler. The preparation of samples for the measurement of radioactivity and their further treatment has also been described in a previous report[6]. Results were expressed as c.p.m./100γ protein sulphur. Further details of experimental procedure are given in Table 1 and Figs. 1–3.

As can be seen from Table 1, the concentrations of MSI which are known to inhibit markedly proteosynthesis in other tissues *in vitro* (Ehrlich ascites carcinoma cells, liver, pancreas[7–10]) had no effect on ^{35}S-methionine incorporation into proteins of guinea pig brain cortex slices. Similar results were obtained with brain cortex slices of the rat and rabbit (Table 1). An increase in concentration of MSI to 10^{-2} *M* resulted in a slight inhibition of incorporation only. In the absence of substrate in the incubation medium, MSI failed to have any effect on the process investigated (Table 1).

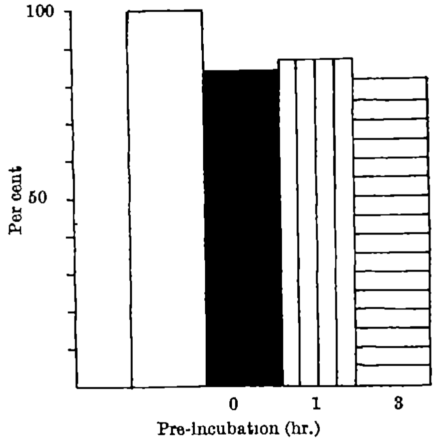

Fig. 2. Effect of pre-incubation of brain cortex slices with MSI on ^{35}S-methionine incorporation into brain proteins. Slices were pre-incubated in Krebs–Ringer phosphate-saline pH 7·4, containing glucose (10^{-2} *M*), with or without MSI (10^{-2} *M*) for 0, 1 or 3 hr and then incubated in the presence of ^{35}S-methionine for 90 min. at 37° C., gaseous phase oxygen. (The values of ^{35}S-methionine incorporation after the pre-incubation without MSI are expressed as 100 per cent; each column represents mean value of four independent experiments)

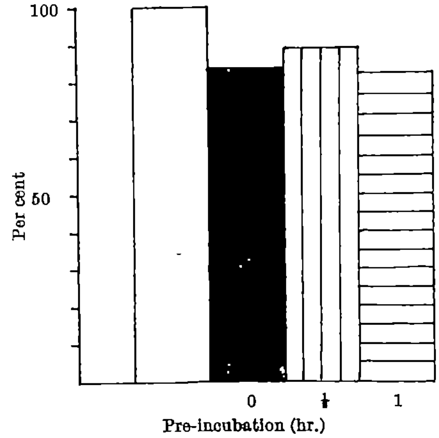

Fig. 3. Effect of pre-incubation of brain cortex slices in the absence of substrate upon MSI inhibition of ^{35}S-methionine incorporation into proteins. Slices were pre-incubated in Krebs–Ringer phosphate-saline pH 7·4, without glucose for 0, ½ or 1 hr. at 37° C. and then incubated in the presence of glucose, ^{35}S-methionine, with or without MSI (10^{-2} *M*) for 90 min. at 37° C., gaseous phase oxygen. (The values of ^{35}S-methionine incorporation in the absence of MSI are expressed as 100 per cent; each column represents mean value of four independent experiments)

Prolongation of the incubation period up to 4 hr. did not cause a further increase in the low degree of inhibition of incorporation (Fig. 1). Similarly, the pre-incubation of brain cortex slices with MSI did not influence further the inhibitory effect (Fig. 2).

Table 1. Effect of dl-Methionine Sulphoximine (MSI) on Uptake of Oxygen and ^{35}S-Methionine Incorporation into Proteins of Brain Cortex Slices

Animal	Concentration of MSI (*M*)	No. of exps.	c.p.m./100γ protein S	*P*	No. of exps.	μ*M*O$_2$/hr./gm. fresh tissue
Guinea pig	0	7	4,158±179		3	83·9±1·8
	2×10^{-3}	7	4,034±161		3	83·5±2·1
Guinea pig	0	8	3,269±210	<0 5	5	77·8±1·6
	4×10^{-3}	8	2,854±188		5	80 4±1·9
Rat	0	3	3,506±130			
	4×10^{-3}	3	3,680±183			
Rabbit	0	4	2,790±488		3	64 4±3 0
	4×10^{-3}	4	2,888±199		3	64·1±3·7
Guinea pig	0	5	3,800±209	<0·05	5	77·5±2 3
	10^{-2}	5	3,194±88		6	76·1±2·4
Guinea pig*	0	4	1,636±67			
	10^{-2}	4	1,594±145			

Slices were incubated in Krebs–Ringer phosphate-saline pH 7·4, containing glucose (without glucose*), ^{35}S-methionine, with or without MSI for 90 min. at 37° C., gaseous phase oxygen. Mean values ± *S.E.*, the number of experiments and statistical evaluation by *t*-test (*P*) are given.

When guinea pig brain cortex slices were pre-incubated in Krebs–Ringer phosphate-saline without substrate and then transferred to normal medium for incubation (Fig. 3), only a slight inhibition was also observed.

Preliminary experiments with homogenates of brain cortex tissue have shown that under these conditions MSI (4×10^{-3} *M*) failed to have any inhibitory effect on ^{35}S-methionine incorporation into proteins.

It follows from Table 1 that the uptake of oxygen by brain cortex slices in the presence of MSI concentrations used remained unaffected.

It is difficult to explain why MSI has little effect *in vitro* in spite of its inhibitory effect on ^{35}S-methionine incorporation into brain proteins *in vivo*. It seems that permeability phenomena are probably not the main factors involved. However, the possibility that, for example, some metabolic products of MSI are being formed in other tissues than the brain tissue cannot be ignored or that there is a connexion with the level of intracellular brain glutamine. These and other possibilities are under investigation.

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Effect of Dimethylbenzanthracene on the Cellular Proliferation Cycle

THE carcinogenic hydrocarbon 7,12-dimethylbenz[α]anthracene (DMBA) inhibits the incorporation of tritiated thymidine into the deoxyribonucleic acid (DNA) of the cells of the growing hair follicle of the mouse to the extent of approximately 50 per cent in 24 hr.[1]. We have now examined the effect of this hydrocarbon on the timing of the phases of the proliferative cycle of these cells.

The animals used were females, 8–12 weeks old, of an inbred strain derived from Walter Reed Hospital Swiss mice. Hair was plucked from the back in order to stimulate cell division in the hair follicle[2]. Three days later, 0·15 ml. of 0·05 per cent DMBA in acetone was applied to 2 cm.^{2} of the plucked skin[3,4]. After 6 hr., when an effect on incorporation of the label could be expected[1], tritiated thymidine (50–100 μc., specific activity 3·9 c./m*M*) was injected intraperitoneally, and 1 hr.–24 hr. thereafter pairs of animals were killed. Small samples of the treated and adjacent untreated skin were fixed in ethanol/acetic acid (3 : 1). The skin was stained by the Feulgen procedure. The bulbs of individual hair follicles were then dissected and squashed. The slides were dipped in melted Kodak *NTB* emulsion, stored until suitably exposed, and developed. Mitotic figures (late prophase to early telophase) were scored for presence or absence of label. The results are recorded in Fig. 1.

The mean duration of the phase of DNA-synthesis (*S*-phase) and pre-mitotic G_2-phase[5] were estimated according to Quastler and Sherman[6]. G_2-phase occupied 1·5 hr. in both untreated and DMBA-treated follicles.

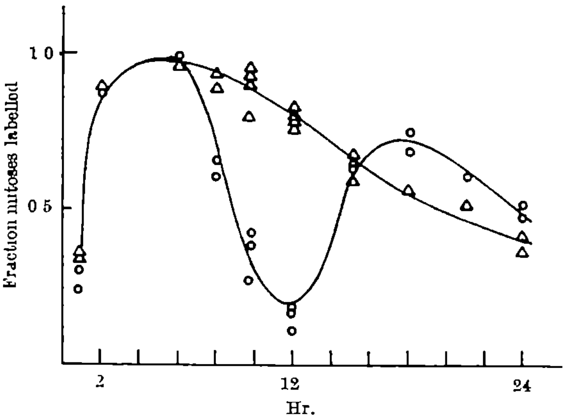

Fig. 1. Fraction of mitotic figures labelled in DMBA-treated (triangles) and untreated (circles) hair follicles of mice at different times after injection of tritiated thymidine. Each point derived from one mouse

In the untreated follicles, *S*-phase is about 7·5 hr. with a standard deviation of approximately 1 hr. The duration of the cell-cycle estimated from Fig. 1 is about 12 hr. These values agree closely with those obtained by Cattaneo *et al.*[7] in the growing hair follicle of *CF*-1 male mouse.

In contrast, the duration of *S*-phase in the DMBA-treated follicle is about 18·5 hr. with a standard deviation of approximately 9 hr. It is evident that DMBA has a marked but variable effect on cells synthesizing DNA.

The duration of mitosis was not measured, but it was evident that DMBA did not produce a block in mitosis, for the proportions of the various stages were not markedly changed in the treated cells.

In the animals which provided the data of Table 1, the fraction labelled cells/total cells was 57 per cent for control follicles and 65 per cent for treated. These values and those for the corresponding duration of *S*-phase provide a calculated mean duration of the cell-cycle of 13 hr. in the control (12 hr. from Fig. 1) and about 28·5 hr. for treated cells (cannot be estimated from Fig. 1).

Table 1. RELATIVE PROPORTIONS OF STAGES OF MITOSIS IN CONTROL AND DMBA-TREATED* GROWING HAIR FOLLICLES

Mouse		Prophase	Metaphase	Anaphase	Telophase
1	Treated	10	47	24	19
	Control	3	54	11	33
2	Treated	5	50	19	26
	Control	7	46	21	25
3	Treated	12	53	23	13
	Control	7	59	25	12

* 0 15 ml. of 0·05 per cent DMBA in acetone applied to 2 cm.^{2} of plucked skin. Animals killed 24 hr. later when percentage inhibition of incorporation of tritiated thymidine was: mouse 1 = 43, mouse 2 = 45, mouse 3 = 68

The uncertainty in the calculated duration of the generation time makes it impossible to decide if the post-mitotic phase (G_1) is affected by treatment with DMBA. The observation referred to here that the labelling index is somewhat higher (65 per cent) in the treated cells than in the untreated cells (57 per cent) shows that it is unlikely that DMBA produces a *block* in G_1. If cells were prevented from passing through G_1- into *S*-phase, fewer cells should be labelled in the treated than in the control follicles.

We conclude that cells of the growing hair follicle of this strain of mouse, treated with DMBA, are slowed in their passage through *S*-phase. The rate of passage is reduced to about the same degree as the rate of uptake of tracer into DNA[1]. There is no effect on the passage through G_2, and probably none on passage through G_1 and mitosis.

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Response of the Hodgkin–Huxley Model to Temperature and Chemical Impulses

Tasaki *et al.*[1,2] have demonstrated the existence of prolonged action potentials in the squid axon under tetraethylammonium and of a "hyperpolarizing response" of the normal squid axon in enriched potassium solutions. In a series of experiments they have evaluated the properties of the squid axon membrane in these two particular environments and were led to the conclusion that the Hodgkin–Huxley[3] equations did not account for their observations, which had to be explained instead in terms of a "bi-stable state" hypothesis.

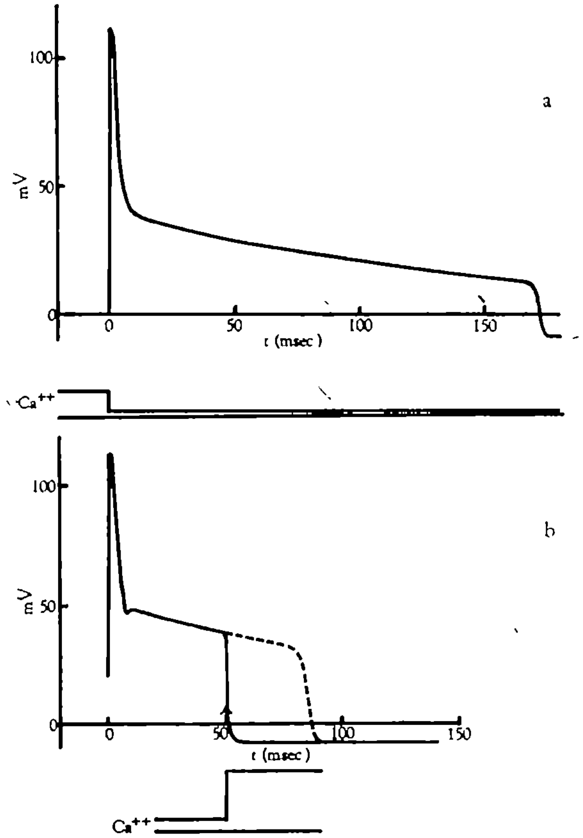

Fig. 1. Response of membrane with prolonged action potential to sudden changes of external calcium *a*, A five-fold reduction in [Ca]$_0$ initiates an action potential which is longer than normal; *b*, a five-fold increase in [Ca]$_0$ is seen to produce abolition

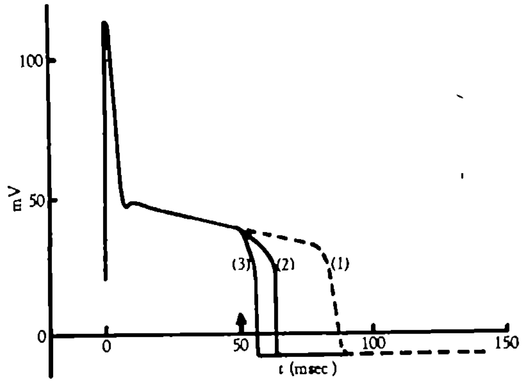

Fig. 2. Response of the Hodgkin–Huxley model to sudden increases of ambient temperatures. (1) Contact temperature; (2) $\Delta T = + 10°$ C. at $t = 50$ msec., (3) $\Delta T = + 20°$ C. at $t = 50$ msec.

In a recent communication[4] we demonstrated the existence of solutions of the Hodgkin–Huxley equations which correspond rather closely with the observations of Tasaki *et al.*, and that while the concept of a "bi-stable state" may form a useful aid to thought in considering action potentials in the squid axon under certain experimental conditions, it was in fact already implied in the Hodgkin–Huxley formalism. A similar conclusion had also been reached by Fitzhugh[5].

Recently, Spyropoulos[6] has published further findings on prolonged action potentials, this time on action potentials in frog node prolonged by the application of Ni^{++}. Spyropoulos has shown that a prolonged action potential may be initiated by a sudden reduction in the external Ca^{++} concentration, and may be abolished by a sudden increase in external Ca^{++}. Abolition may also be produced by sudden pulses of increased temperature.

Using the fast digital computer *Silliac*, we were therefore led to explore whether these responses also were consistent with the Hodgkin–Huxley equations and have found that they are.

Fig. 1, for example, shows the response to chemical pulses of Ca^{++}. The effect of changes in external calcium was simulated in the Hodgkin–Huxley equations by a prescription already used by Huxley[7], namely, to alter the voltages in the Hodgkin–Huxley equations involving the sodium and potassium current carriers by an amount:

$$\Delta V. = k \log[Ca]_0/[Ca]_i$$

where k was so adjusted such that a 5-fold change in [Ca]$_0$ corresponded to $\Delta V. = -15$ mV., as had been shown previously by Frankenhaeuser and Hodgkin[8].

Fig. 1*a* shows that a 5-fold decrease of [Ca]$_0$ will produce a response in a membrane satisfying the Hodgkin–Huxley equations and Fig. 1*b* that a 5-fold increase of [Ca]$_0$ will switch it off.

As in our previous computations[4], the prolonged action potentials were produced by slowing down the potassium current carrying mechanism by a factor of one hundred times.

The effect of a sudden increase in temperature is shown in Fig. 2. It is seen that the prolonged action potential in a Hodgkin–Huxley membrane is abolished by a rise in temperature of 10° C. and even more rapidly by an increase in temperature of 20° C.

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Ageing and Environmental Manifestations in Pyruvate Metabolism of Rabbit Fœtal and Neonatal Liver

Fœtal and perinatal cell differentiation may be considered as a sequence of progressive metabolic alterations determined in part by genetic and in part by environmental influences. Implied in genetically organized differentiation are the biological processes of maturation, growth and senescence which are collectively referred to as the ageing of the cell.

In an effort to distinguish between metabolic activity related to environmental needs as contrasted with the requirement of the ageing process, the following investigation was carried out.

Pregnant rabbits were rendered post-mature by subcutaneous injection of 40 units/kgm. 'Antuitrin-*S*' (kindly supplied by Dr. L. T. Blouin, Parke, Davis Laboratories, Ann Arbor) at 24 days of gestation. The litters were delivered by Cæsarian section 4 days after the calculated day of confinement. In a second group of untreated rabbits, spontaneous delivery occurred at term and in a third group, term-delivered litters were allowed to be breast-fed for four days. Fœtus and new-born were killed by decapitation and the livers placed immediately in ice-cold isotonic sodium chloride. Following a previously described technique[1], liver slices 0·3–0·5 mm. thick were incubated with 30 μmoles of pyruvate-2-^{14}C in a Warburg respirator for 2 hr. in 100 per cent oxygen. Oxygen consumption, glycogen utilization, pyruvate utilization and production, conversion of labelled substrate to $^{14}CO_2$ and production of lipids and lactate were measured[1] and the results expressed per gram tissue dry weight. The data obtained were statistically analysed by Student's *t* (ref. 2).

In the 4-day-old rabbit, the liver utilized 112 per cent more oxygen, 61 per cent more pyruvate and produced 100 per cent more labelled respiratory $^{14}CO_2$, but formed only one-third as much lipid and contained only 32 per cent of the glycogen as compared with new-born liver. Post-mature rabbit liver utilized 75 per cent more oxygen, 63 per cent more pyruvate and converted 122 per cent more labelled substrate to respiratory carbon dioxide than new-born liver. On the other hand, post-mature liver consumed 18 per cent less oxygen, utilized the same amount of pyruvate and converted 10 times more labelled substrate to lipids as compared with 4-day-old liver. Net lactate production was higher in the 4-day old post-mature liver than in the liver of the 4-day-old and of the new-born. Glycogen content of post-mature liver was not statistically different from that in the 4-day-old liver.

Table 1. *In vitro* Incubation of Rabbit Liver Slices at Different Stages of Development with Pyruvate-2-^{14}C

	4-day-old	New-born	4-day post-mature
	μmoles/gm. tissue dry weight ± *S.E.*		
Oxygen utilization	*434 ± 21 (10)	204 ± 16 (10)	†357 ± 19 (15)
Glycogen content	*92 ± 87 (10)	290 ± 97 (6)	*64 ± 20·4 (8)
Utilized glycogen	*99 ± 26 (14)	236 ± 19 (11)	*44 ± 10 (10)
Utilized pyruvate	*295 ± 17 (11)	183 ± 11 (13)	*298 ± 16 (14)
Conversion of labelled pyruvate to CO_2	*22·6 ± 1·7 (9)	11·2 ± 1·6 (9)	*24·8 ± 1·4 (9)
Conversion of labelled pyruvate to lipids	*0·38 ± 0·09 (6)	1·1 ± 0·09 (5)	†3·9 ± 1·4 (6)
Lactate production	86·3 ± 7 (11)	82 ± 12 (11)	*113 ± 5 (11)

No. of determinations given in parentheses.
*$P < 0·05$ when compared to the new-born.
†$P < 0·05$ when compared to the 4-day-old.

Oxidation of pyruvate-2-^{14}C to respiratory carbon dioxide, pyruvate utilization and tissue oxygen consumption permit an estimate of the overall rate of cellular oxidative metabolism.

Our results indicate that pyruvate oxidative metabolism is twice as active in the 4-day-old rabbit liver than in the new-born. Since in the post-mature rabbit liver, the pyruvate oxidative metabolism is comparable to the 4-day-old liver, the characteristic metabolic determinants which support total oxidative metabolism *in utero* are functions of the ageing process of the fœtal liver cell and not of the environment.

In contrast to these findings, conversion of pyruvate to lipids, hepatic glycogen content and the rate of glycolysis are influenced by environmental factors. Thus, the liver of the new-born and of the post-mature has a higher rate of lipid synthesis than that of the 4-day-old. Dawkins[3] has demonstrated in the neonatal liver of the same animal that glucose-6-phosphatase activity increases as a result of environmental factors. Therefore, the liver of the 4-day-old has a lower glycogen content than that of the new-born.

The glycogen content of the post-mature is as low as that of the 4-day-old. As the glucose-6-phosphatase does not increase in the post-mature fœtus[3], the reason for this low glycogen content is that the post-mature fœtus is hypoxic[4] and the resistance to hypoxia has been shown to depend in part on the rate of glycolysis[5] in the fœtal tissue. The higher rate of lactate production (31 per cent) supports this assumption. The post-mature converts pyruvate to lipids at a rate 10 times as high as the 4-day-old and 3 times as much as the new-born. This is another indication of hypoxia as lipid synthesis could represent an adaptive mechanism for the conservation of oxygen.

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Inhibition of Histamine Formation *in vivo*

Quantitative studies of the inhibition of histidine decarboxylase *in vivo* are now feasible with the introduction of two methods of assessing the rate of endogenous histamine formation in the rat. One method, devised by Schayer, measures the amount of ^{14}C-histamine excreted in the urine following the injection of a known amount of ^{14}C-histidine. The other method depends on the demonstration that in the female rat the urinary excretion of free histamine parallels the endogenous formation of the amine[1,2]. Using both these methods, it was found that omitting pyridoxine from the diet for a week causes a reduction in histamine formation to about half normal, and that the rate of formation can be reduced further by superimposing injections of semicarbazide on the pyridoxine-deficient diet[3].

In the present work α-methyl-histidine was found to be a more specific and less toxic inhibitor of histamine formation than semicarbazide, which is known to inhibit a variety of enzymes, among them various amino-acid decarboxylases and diamine oxidases. α-Methyl-histidine was provided by Merck, Rahway, New Jersey, through the kind office of Dr. Schayer. This compound was tested in two types of experiments. One group of rats was fed the standard, adequate, histamine-free diet[1]. In another group pyridoxine was omitted from the diet until a reduced steady level of histamine formation was obtained, and α-methyl-histidine administered while the deficient diet was maintained. This was done to see whether inhibition of histamine formation by this compound was due to interference with the active centre of histidine decarboxylase or merely to an action on the co-enzyme pyridoxal-5-phosphate, the probable mode of action of semicarbazide. In addition, the urinary excretion of 5-hydroxyindolyl acetic acid (5-HIAA), which is believed to reflect the rate of endogenous formation of 5-hydroxytryptamine (5-HT), was followed. This, again, was carried out to see if α-methyl-histidine also inhibits the formation of 5-HT, which is known to result from hydroxylation of tryptophan to 5-hydroxytryptophan and the subsequent decarboxylation of this amino-acid.

Typical results in a rat fed the adequate diet are shown in Fig. 1; α-methyl-histidine injected under the skin in doses of 200 mgm./kgm., 4 times daily during four days, results in a reduction of the over-all formation of histamine, as determined by the urinary excretion, to less than half normal. On discontinuing the injections the original level of histamine formation is promptly restored and even transitory rebounds above this level are seen. The excretion of 5-HIAA, by contrast, remains unaltered.

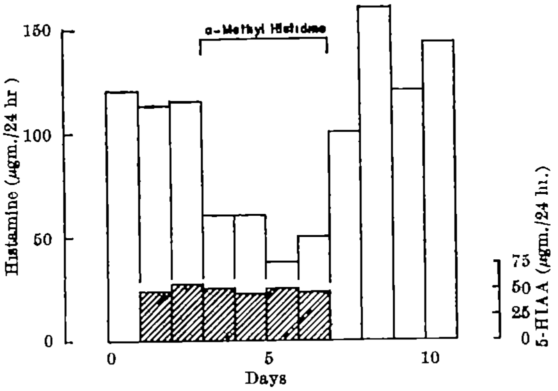

Fig. 1. The white columns represent amounts of daily excretion of free histamine in a female rat fed an adequate histamine-free diet. The hatched columns represent daily urinary excretion of 5-HIAA. α-Methyl-histidine was injected for a period of four days. Aminoguanidine was administered during the entire course of observations to prevent the inactivation of histamine by histaminase

In the group of rats on deficient diet, in a typical example, deficiency in pyridoxine for 24 days reduced the level of urinary histamine to about 40 per cent of normal, whence it remained steady for the period of observation. Superimposing injections of α-methyl-histidine on the maintained deficient diet, 4 × 200 mgm./kgm. daily for 4 days, beginning at the 24th day of deficiency, precipitated a further reduction in the formation of histamine to about half the level prevailing after 24 days of only pyridoxine deficiency. On cessation of the injections the level of urinary histamine promptly rose to the level at which administration of the inhibitor was begun. In the same group of rats ^{14}C-histidine was injected and the urinary ^{14}C-histamine measured, first on adequate diet, then at the reduced steady level of pyridoxine deficiency, and finally under conditions of superimposed injections of α-methyl-histidine on the deficient diet. The results with the radioactive method were the same as with the simpler one used simultaneously. Here again, injections of the inhibitor significantly reduced the ^{14}C-histamine excretion in the period when the deficiency of pyridoxine alone did not cause any further reduction in the formation of histamine. In these experiments α-methyl-histidine appears to act directly on the enzyme centre and not on the co-enzyme pyridoxal-5-phosphate, particularly because it does not inhibit the formation of 5-HT, as indicated by the excretion of 5-HIAA, to the production of which amine pyridoxal-5-phosphate is known to be a co-enzyme.

Injections of α-methyl-histidine in the amounts stated did not cause detectable psychical or physical ill effects, this in contrast to semicarbazide superimposed on the deficient diet which may elicit states of hyperirritability and panic, and in varying degrees a general deterioration of the physical state of the animal.

Finally, the effects of α-methyl-3,4-dihydroxyphenylalanine (α-methyl-DOPA) were similarly examined. In rats on adequate diet this compound in doses of 100–200 mgm./kgm., injected 4 times daily for four days, caused slight (about 25 per cent), or inconsistent, reductions in urinary histamine. In cases where the inhibition was definite, the urinary excretion of 5-HIAA was reduced to about the same extent as urinary histamine. Injections of α-methyl-DOPA caused prostration, diarrhoea, loss of appetite and body-weight.

In conclusion, α-methyl-histidine appears a promising weapon in further investigations of the physiology of histamine.

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HÆMATOLOGY

A Direct Relationship between Adenosine Triphosphate-level and *in vivo* Viability of Erythrocytes

Although Loehr *et al.*[1] described in 1959 that adenosine triphosphate content (ATP) in erythrocytes decreased gradually during their life-span of 120 days on the average in blood vessels and suggested that the drop of the ATP-level caused destruction of erythrocytes *in vivo*, no concrete evidence for this theory has yet been presented.

Recently, some of the authors demonstrated that ATP increased rapidly through incubation with adenine and nucleoside in the erythrocytes which had been preserved for a long period[2,3], even when the addition of inosine alone was no longer effective. With the aid of this method of ATP regeneration, it was found that there was an intimate relationship between ATP-level in the erythrocytes and their shape[4,5]. In this communication, evidence will be presented concerning the relationship between ATP-level and *in vivo* viability of erythrocytes obtained by the same method as already described[4,5,11].

Acid-citrate-dextrose (ACD) blood collected from healthy volunteers was divided into three portions, to one of which one-tenth volume of physiological saline containing 12·5 µmoles/l. of adenine and 25 µmoles/l. of inosine was added (*B*) and nothing to the other two (*A*, *C*). After storage for eight weeks at 4° C. aseptically, the specimen *A*, preserved without any addition, was incubated with 12·5 µmoles/l. of adenine and 25 µmoles/l. of inosine for 2·5 hr. at 37° C. and then ATP content of the erythrocytes was estimated by Cohn and Carter's method[6]. The osmotic fragility was tested according to the median corpuscular fragility method[7]. Hexokinase activity in hæmolysate and utilization of glucose were determined according to the method of Kashket *et al.*[8]. Post-transfusion viability was determined by the method using chromate labelled with chromium-51. The shape of erythrocytes was observed with an ordinal light microscope using hanging-drop preparations.

After incubation with inosine and adenine ATP in the specimen *A* was regenerated to almost normal level, accompanying change of the shape from smooth sphere to discoidal, as reported before[2,3]. But the treatment had no effect on the osmotic fragility and hexokinase activity (Table 1). These results indicate that the damage to the enzyme activity, which occurred during storage, did not recover at all even when a great amount of ATP was regenerated. However, rather unexpected results were obtained on the post-transfusion viability of the erythrocytes as indicated in Fig. 1. The treatment with inosine and adenine showed a great deal of improvement on the post-transfusion viability of the erythrocytes preserved for a long period of time. These data were exactly reproducible through several series of experiments, although results of a single experiment alone are demonstrated in Table 1 and Fig. 1.

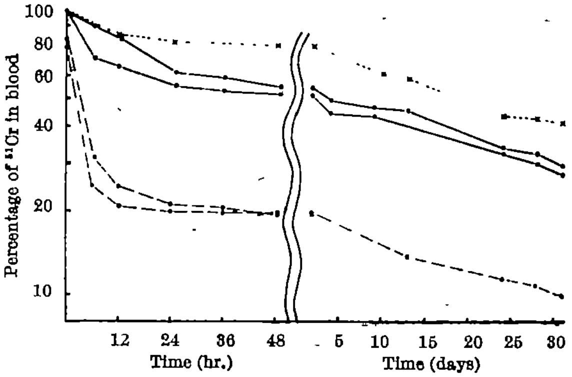

Fig. 1. Decay of radioactivity in circulating blood after transfusion of erythrocytes stored for eight weeks and labelled with radioactive chromate. Radioactivity of the blood 5 min. after the transfusion was represented as 100 per cent. ●—●, Erythrocytes stored in ACD and then incubated with inosine and adenine at 37° C. (specimen *A*); ×---×, erythrocytes stored in ACD containing inosine and adenine (specimen *B*); ●– – ●, erythrocytes stored in ACD alone (specimen *C*)

Table 1. Various Characteristics in the Erythrocytes treated with Inosine and Adenine after Preservation in ACD for Eight Weeks at 4° C.

	A Preserved in ACD without any addit. and then incubated with inosine and adenine	*B* Preserved in ACD with inosine and adenine	*C* Preserved in ACD
ATP µmoles per 100 ml. of cells	77	89	5 9
Osmotic fragility NaCl per cent at 50 per cent hæmolysis	0 66	0·49	0 61
Shape	discoidal	discoidal	smooth sphere
Hexokinase activ. in hæmolysate	127	206	124
Glucose utilizat. in cells, µgm /40 min 10·5 ml. blood	51	61	31

The results obtained emphasize a direct interrelationship between ATP content and a moment of *in vivo* destruction of the erythrocytes according to the following reasons.

(1) Although incubation with inosine and adenine resulted in no improvement either of hexokinase activity or of osmotic fragility, indicating that an irreversible change proceeded during storage, the reversal of post-transfusion viability was observed in association with the marked regeneration of ATP and the reversal of the shape of the erythrocytes. As the shape of erythrocytes depends on the ATP content in the cells as stated before, the great flexibility in shape of erythrocytes was observed even after eight weeks' storage. The viability seems to be essentially based not on the shape but on the ATP-level.

(2) The single addition either of inosine or of adenine to the erythrocytes, which had been stored in ACD solution for a long period of time, did not restore the ATP-level at all as reported before[3]. Furthermore, the preservation of erythrocytes in ACD solution containing either an adequate amount of inosine or adenine could maintain the ATP content and post-transfusion viability far less than that in ACD containing both of them[9-11]. These facts indicated that the effect of the treatment with inosine and adenine was not due to the mere presence of inosine or adenine itself, but to the metabolites derived from inosine and adenine. The metabolites related to inosine and adenine, which are generally accepted to be present in erythrocytes, are adenine nucleotides, hypoxanthine, inosine monophosphate, lactate, pyruvate, and phosphate esters which belong to the Warburg–Dickens and Embden–Meyerhof systems. The last five substances, however, could be demonstrated even in the erythrocytes incubated with inosine alone[12]. Recently, some of the authors presented evidence that the adenine nucleotide, especially ATP in the preserved erythrocytes, definitely regenerates through

incubation with inosine and adenine, and also a presumable pathway, by which the ATP regeneration may proceed during incubation, was advocated[13]. Now, an accumulation of results of our previous and recent experiments lead us reasonably to conclude that the ATP-level is most likely to be responsible for reversal of the post-transfusion viability of the preserved erythrocytes.

Thus, the organism may distinguish deteriorated erythrocytes from younger or vital cells not by their mechanical fragility, but by adenosine triphosphate-level in the erythrocytes. presumably mediated by the changes of still undetermined nature, which probably occur at the surface of the erythrocytes. These results also suggest an existence of an intimate and rather direct relationship between adenosine triphosphate-level and erythrocytes integrity.

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Conditions Influencing the Stability of Human Antihæmophilic Factor

The lability of human antihæmophilic factor in both plasma and concentrates is a major hazard during purification, even when the reaction of solutions is maintained within the rather restricted optimum range of stability, which we find to be *p*H 7·1–7·2, and when preparative procedures are conducted at temperatures close to 0°.

Antihæmophilic factor is carried down with the fibrinogen fraction when this is separated from fresh normal human plasma. In addition the fibrinogen fraction, among other contaminants, contains plasminogen, and the separation of antihæmophilic factor from fibrinogen and plasminogen is a matter of some difficulty.

When solvents such as ethanol[1] or diethyl ether[2] are used to fractionate plasma proteins, small quantities of an actively fibrinogenolytic substance, probably plasmin, tend to be present in partially purified fibrinogen preparations. The presence of this fibrinogenolytic agent is manifested by the eventual loss of the ability of solutions of fibrinogen, after prolonged incubation under standard conditions, to form a clot when samples are added to a solution of thrombin. Concentrates of human antihæmophilic factor, which we have made by a modification of the method of Kekwick and Wolf[3], contain traces of this fibrinogenolytic agent, of the order of 0·001 Loomis fibrinolysin units/ml. Since Wagner, Brinkhous and Penick[4] have shown that canine antihæmophilic factor is destroyed rapidly by bovine plasmin, it was of interest to see whether the stability of human antihæmophilic factor concentrates could be enhanced by adding ε-aminocaproic acid, which is known to inhibit plasmin activity[5].

From the results presented in Table 1 it can be seen that 0·05 and 0·10 *M* ε-aminocaproic acid had no significant influence on the stability of the antihæmophilic factor in a concentrate although the fibrinogenolytic activity was reduced to 20–25 per cent of the initial level. With one preparation the stability of the antihæmophilic factor was slightly improved with 0·1 *M*, but an increase in the amount of ε-aminocaproic acid to 0·4 *M* caused no further improvement. When the concentrate (Table 1) was treated with 0·002 *M* di-isopropyl-phospho-fluoridate (DFP) there was no detectable decrement in antihæmophilic factor activity after 7 hr. and 92 per cent of the activity remained after 24 hr. incubation at 25°. The antihæmophilic factor activity of the test solutions was adjusted to be approximately equivalent to that of fresh normal human plasma, in a citrate–phosphate–saline buffer *p*H 7·2, *I* 0·30.

Table 1. Effect of ε-Aminocaproic Acid and Di-isopropyl phospho-fluoridate on the Stability of Human Antihæmophilic Factor (AHF) in Concentrates

Sample	ε-Amino-caproic acid (*M*)	Percentage AHF remaining after 7 hr. at 25°	Fibrinogen life (hr. at 37°)
Original concentrate	0 00	64	30
,, ,,	0 05	64	168
,, ,,	0 10	60	110
DFP-treated concentrate	—	100 (92 after 24 hr.)	—

The AHF activity was assayed in a thromboplastin generation test based on that described by Pool and Robinson (ref. 6).

The fibrinogen life. Solutions of a fraction containing 0·2 gm. protein/100 ml. in veronal saline *p*H 7·3 *I* 0 15, with other additions as indicated, were incubated at 37°. The fibrinogen life is the period of incubation for which the solution retained the ability to form a clot when 0 20 ml. was added to 0 20 ml. thrombin solution. Clotting time of samples at beginning of incubation about 10 sec. The fibrinogen content of all the fractions was about 70 per cent of the total protein.

When another antihæmophilic factor concentrate obtained as a precipitate was extracted at 0° with a citrate–phosphate buffer *p*H 7·2 *I* 0·15, the total protein was about equally divided between the extract and residue, but 90–95 per cent of the antihæmophilic factor activity remained in the residue. Despite the fact that most of the fibrinogenolytic activity also remained in the residue, the stability of the antihæmophilic factor in it was much higher than in the original concentrate. Thus under the conditions of testing, after 7 hr. incubation at 25°, 97 per cent of the antihæmophilic factor activity was present in solutions of the residue whereas only 74 per cent of the antihæmophilic factor activity remained in solutions of the original concentrate (Table 2). When the residue and the extract were mixed in the original proportions, the stability of the antihæmophilic factor was effectively the same as in solutions of the starting material. However, when the extract was treated with DFP, and the excess of this removed by dialysing before mixing the DFP-treated extract with the residue, the stability of the antihæmophilic factor in this mixture was the same as in solutions of the residue alone (Table 2).

Table 2. EFFECT OF TREATMENT OF CONCENTRATE AND EXTRACT WITH DI-ISOPROPYL-PHOSPHO-FLUORIDATE ON THE STABILITY OF AHF IN THE CONCENTRATE AND MIXTURES OF THE EXTRACT AND RESIDUE

Sample	Percentage AHF remaining after 7 hr. at 25°	Fibrinogen life (hr. at 37°)
Original concentrate	74	130
Residue	97	76
Extract	—	> 130
Residue + extract	70	—
Original concentrate treated with DFP	96	—
Residue + DFP-treated extract	94	—

See footnotes to Table 1.

These results suggest that the instability of human antihæmophilic factor in solutions of concentrates is largely due to the action of a DFP-sensitive component other than plasmin; the nature of this component is under investigation. The enhanced stability of antihæmophilic factor in DFP-treated concentrates should facilitate further purification.

Fuller details of this work, for the support of which we are indebted to the Medical Research Council, will be published elsewhere.

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HISTOCHEMISTRY

Effect of 3-Methylcholanthrene Crystals and Solutions on the Chorioallantoic Membrane of the Developing Chick Embryo

METAPLASTIC changes of the chorioallantoic membrane of the developing chick embryo, such as keratinization and epidermoid pearl formation, have been reported by several authors[1-4].

We have subjected the chorioallantoic membrane of chick embryos of various ages to single topical applications of crystals of 3-methylcholanthrene as well as to various concentrations of the carcinogen dissolved in acetone or polyethylene glycol. In order to determine the effects of carcinogen *per se*, the vehicles alone, as well as particles of egg-shell, have also been tested.

The results so far have shown that the response depends in part on the age of the embryo. After initial necrosis and inflammation, the most impressive reaction of the chorionic ectoderm was metaplasia, such as stratification of epithelium and of prickle cell, keratin and epithelial pearl formation. An additional reaction seen in 10-day chorioallantoic membranes subjected to methylcholanthrene crystals was the presence of cords, columns and clusters of epithelial cells deep in the mesenchyme. Such accelerated response under similar conditions of embryonic age was not noticed with the application of the non-carcinogenic irritant.

The egg-shell powder produced a characteristic foreign-body giant cell response.

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PATHOLOGY

Urinary Phenolic Acids in Infective Hepatitis

AN abnormal urinary excretion of aromatic metabolites of tyrosine (that is, tyrosyluria) is well known to occur in scurvy. Recently, Robinson and Smith[1,2] reported tyrosyluria in severe stress (spinal shock, burns, etc.) and various pathological conditions, in patients receiving an adequate dietary intake of ascorbic acid.

Since tyrosine is metabolized in the liver, it seemed worth while examining the urines of patients suffering from parenchymatous liver damage for tyrosine metabolites.

Urine specimens were collected over a period of 24 hr. from 6 patients suffering from infective hepatitis. All the patients had increased serum glutamic-oxalacetic transaminase activities, reduced serum albumin concentrations and raised thymol turbidities. Their dietary intake of ascorbic acid was adequate.

The urinary phenolic acids were extracted by the method of Hill *et al.*[3]. A quantity of extract equivalent to the volume of urine passed in 1 min. was subjected to two-dimensional paper chromatography using isopropanol–ammonia followed by *n*-butanol–pyridine–water[3]. After drying, the chromatograms were sprayed with 10 per cent sodium carbonate solution, dried again and resprayed with diazotized sulphanilic acid. Hydrolysed urine samples were not examined.

Using this technique, *p*-hydroxyphenylacetic acid is almost invariably found in small quantities in normal urine, but *p*-hydroxyphenyllactic acid is rarely detected.

All six patients suffering from infective hepatitis excreted increased amounts of both *p*-hydroxyphenylacetic and *p*-hydroxyphenyllactic acids. A seventh patient with hepatic cirrhosis also excreted increased amounts of these phenolic acids. None of the patients excreted significantly increased amounts of 4-hydroxy-3-methoxymandelic acid.

It seems probable that parenchymatous liver damage can be the commonest cause of tyrosyluria. The question arises whether the tyrosyluria which Robinson and Smith[1,2] observed in stress and in various pathological conditions was due to some degree of liver dysfunction. This is being investigated. Preliminary results show that in myocardial infarction this is probably true.

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Cell Turnover in Pulmonary Tissues

In recent years considerable interest has been shown in the turnover of epithelial cells, particularly those in the gastro-intestinal tract[1,2]. These investigations have been facilitated by the introduction of autoradiographic techniques and the use of tritium-labelled thymidine. This is incorporated specifically by the nuclei of cells in pre-mitosis, at which time synthesis of deoxyribonucleic acid (DNA) is occurring[3].

Bertalanffy and Leblond[4] reported their findings in an investigation of cell turnover in the lung in the albino rat, in which they used the technique of mitotic counting after colchicine arrest; but extensive search of the literature has not revealed any other studies on the pattern of cell turnover in pulmonary tissue.

This communication records the findings of an investigation into turnover of the cells in mouse lung using tritium-labelled thymidine and autoradiography. It has been assumed that the labelled cells behave in the same way as normal cells although it is possible that the use of tritium-labelled substances may result in modification of the DNA molecule[5].

18-day albino mice were used. 100 μc. of tritium-labelled thymidine (Atomic Energy Research Establishment, Harwell; 100 mc./m*M*) in 0·2 ml. saline were injected intraperitoneally into each animal at 11 a.m. on the first day of the experiment. Afterwards the animals were maintained in free-run cages with full access to chow and water. Paired animals were killed by ether anæsthesia at intervals of 1, 6, 24, 48, 72 and 96 hr. after injection and further pairs after 1 week, 10 days and 3 weeks respectively.

The trachea and lungs were dissected immediately and fixed in 10 per cent formalin solution. Paraffin-embedded sections of whole lungs were cut to include parts of the main bronchi and autoradiographs were prepared using stripping-film technique (Kodak; *AR*10). After exposure for one month, and developing, the sections were stained with neutral red. All the slides were examined by light microscopy.

1 hr. following injection it was found that only scattered epithelial cells in the large bronchi showed nuclear labelling. The labelled cells were situated mainly, but not exclusively, in the basal layer of the bronchial epithelium. The superficial cells showing labelling at 1 hr. were no longer present at 3 days.

Over the three-week period of observation the labelled cells in the basal layer showed migration towards the surface of the epithelium. Very occasional labelled cells, however, were still present at the end of this time. In the small bronchi and respiratory bronchioles only scattered, labelled epithelial cells were seen at 1 hr. Turnover was faster than in the large bronchi and no labelled cells were present at 10 days.

With the techniques used it was clear that the scattered, labelled cells seen in the walls of pulmonary alveoli were macrophages. It was impossible, however, accurately to differentiate histologically between the vacuolated and non-vacuolated forms described by Bertalanffy and Leblond[4].

At 7 days the labelled cells showed an abrupt decrease, but thereafter the appearances remained roughly constant with, at 3 weeks, very occasional cells still showing labelling. There was no evidence of shedding of labelled cells into alveolar spaces in any of the sections.

In the pleural tissues scattered mesothelial cells showed labelling at 1 hr. While occasional cells still showed labelling at 10 days, none was evident at 3 weeks.

It is apparent, therefore, that unlike the widespread uptake of tritium-labelled thymidine shown by gastro-intestinal epithelium, the uptake by respiratory epithelium is limited to scattered cells. This low degree of mitotic activity and slow turnover of cells may be related to the fact that, under normal conditions, respiratory epithelium is not exposed to significant amounts of trauma, nor is it called on to secrete large quantities of enzyme, as is the situation, for example, in the small intestine. It is also of interest to find that, contrary to orthodox opinion, mitosis is not confined to cells in the basal layer of bronchial epithelium but also occurs in the intermediate and superficial layers.

From our observations we have concluded that the turnover time for epithelial cells in the small bronchi and respiratory bronchioles is 7–10 days. In the large bronchi it was apparent that the superficial cells showing labelling at 1 hr. had a shorter life-span (3 days) than the overall figure of 3 weeks while occasional basal cells had a longer life-span, since labelling was still present at 3 weeks. These epithelial cells with greater and lesser life-span did not differ histologically from those epithelial cells with a turnover time of 3 weeks.

In the pulmonary alveoli, despite the failure to make a histological differentiation between vacuolated and non-vacuolated macrophages with the techniques used, the marked decrease in labelled cells at 7 days indicated that the majority of these macrophage cells had a life-span of approximately 7 days. It is considered that these probably corresponded to non-vacuolated macrophage cells which were estimated by Bertalanffy and Leblond[4], using mitotic counting, to have a life-span of 8 days. The alveolar cells showing labelling after 7 days later proved to have a life-span in excess of 3 weeks. These probably represented vacuolated macrophages which Bertalanffy and Leblond[4] estimated to have a life-span of 29 days.

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ANATOMY

Significance of Arachnoidal Cell Clusters in Man

Since the early part of this century arachnoidal cell clusters in man have generally been accepted as a manifestation of advancing age. They were first described by Meyer[1] as a post-mortem finding in the meninges of patients suffering from mental disease. It has since become evident that they occurred not only in man but also in various laboratory animals[2,3]. Their association with advancing age can be attributed largely to the work of Weed[4], who, in a series of cats of

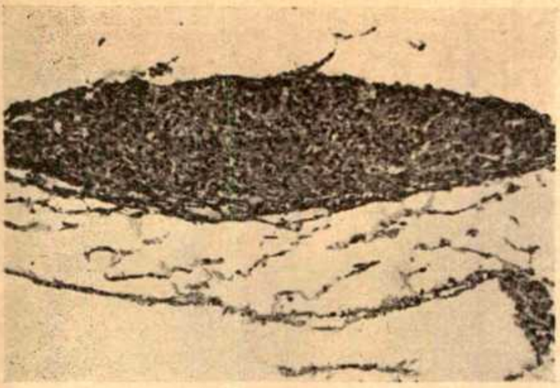

Fig. 1. An arachnoidal cell nest in the human fœtus. (× 107)

different ages, reported that in very old animals the cell clusters were always present, while in very young animals they were never found.

In man, however, arachnoidal cell proliferations can be found even in the unborn human fœtus and hence factors other than those of old age must be considered in their ætiology.

In a series of post-mortems on infants, 'milk-spots' in the meninges of the brain were observed in nearly two-thirds of all stillbirths and in about one-third of all neonatal deaths. They were more frequent in full term infants but were noted even in premature previable fœtuses. The histological features varied from typical arachnoidal cell clusters (Fig. 1) to a more fibrous structure sometimes containing capillary vessels and often showing degenerative changes. The example illustrated is from a stillborn fœtus (body-weight 3,350 gm.) that died of birth trauma.

The significance of these cell clusters in the human fœtus is uncertain. It has been shown experimentally that under conditions of anoxæmia the cells of the arachnoid do proliferate and form cell clusters as well as freely wandering macrophages[5]. Such a mechanism could certainly operate *in utero*, but it is likely that other factors are also involved. In any event, it would seem unnecessary at present to postulate that the human species may be afflicted with old age even from before birth.

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IMMUNOLOGY

In vitro Synthesis of Auto-Antibodies by Rabbit Lymph Node Cells following Cross-Immunization *in vivo*

CROSS-reactions between phylogenically related antigens with an immune serum produced in an animal immunized with a substance similar to these antigens is a familiar concept. The idea that this cross-reaction could extend to self-substances of the immunized animal is not so widely accepted, although there are no essential differences between the cross-reactions involving two foreign substances or the cross-reaction between one foreign and one autologous substance. If auto-antibodies can be synthesized in certain circumstances of immunization (that is, by a mammal immunized with antigens of another species of mammal) then, *in vivo*, in the spectrum of different antibody molecules synthesized in response to the stimulation, those which will bear an antibody specificity complementary to self-substances will immediately disappear.

On the other hand, tissue culture conditions open new opportunities to detect the synthesis of such auto-antibodies since the auto-antigen is not necessarily present in the culture.

In vitro synthesis of anti-erythrocytes antibodies, by lymph-node cells of rabbits, in a stage of secondary response, has been followed by a technique described previously[1]. Sheep erythrocytes were injected in the foot-pad of rabbits and the popliteal lymph nodes were taken three days after a booster injection. All suspensions were made in Parker's medium 1066 supplemented with bovine amniotic fluid, with or without normal rabbit serum, depending on the experiment. All lymph-node cell suspensions were started with 10^7 cells/ml. fluid. In a few experiments small washed fragments (about 1 mm.^{3}) were used instead of cells. A mixture of carbon-14-labelled valine, leucine, lysine and threonine was added to the medium. The tissue culture fluids, obtained after short-term survival experiments (one or two days), were studied for their content in radioactive material precipitable by trichloroacetic acid or in radioactive material susceptible to be fixed on different erythrocytes. Three kinds of erythrocytes have been used: the homologous antigen sheep erythrocytes and cross-reacting antigens; normal rabbit erythrocytes or rabbit erythrocytes taken from the donor of the lymph node cells. It is thus possible to observe three kinds of antibody specificity: heterospecificity (of rabbit antibody towards sheep erythrocytes), isospecificity (of rabbit antibody towards normal rabbit erythrocyte) and auto-specificity (of rabbit antibody against its own erythrocytes; rabbit erythrocytes taken from the donor of the lymph node cells).

Our tracer results show that surviving cells can synthesize antibodies; the evidence that we are dealing with true antibodies being principally of two kinds: agglutination of rabbit red cells by the tissue culture medium and fixation of radioactivity on the red cells.

Agglutination has been performed with a micro-technique[2] using small concentrations of red cells (10^7/ml.) and microscopic examination. This micro-technique markedly increased the sensitivity of the detection. We have observed that, in most cases, antibodies agglutinating sheep erythrocytes, normal rabbit erythrocytes, and rabbit erythrocytes taken from the donor of the lymph node cells could be detected in the tissue culture fluid. Sometimes, this agglutination could be followed up to a final dilution of 1/80 for 10^7 cells, the end point of the reaction being given by the presence of occasional clusters of 2 or 3 cells clumped in the microscope field. Tissue culture fluids from non-immunized rabbits never showed any agglutinating activity.

By incubation with tissue culture fluid, radioactive material is fixed on sheep erythrocytes, normal rabbit erythrocytes and rabbit erythrocytes taken from the donor of the lymph node cells, as can be seen in Table 1, according to a technique described else-

Table 1

					Radioactivity fixable on erythrocytes			
					Sheep erythrocytes		Rabbit own erythrocytes	
Exp. no.	Lymph node weight (gm.)	Type of culture	Duration of exp. (hr.)	TCA precipitate (c.p.m./ml.)	in c.p.m./ml.	Per cent of TCA precipitate	in c.p.m./ml.	Per cent of TCA precipitate
20	2·31	Lymph node cells	24	8,200	1,890	23	1,230	15
34	1·46	Lymph node cells	24	7,000	560	8	308	4·4
38	0·5	Lymph node cells	48	26,500	6,600	25	500	2
38	0·5	Lymph node fragments	48	61,100	8,000	13	550	0·9
45	0·39	Lymph node fragments	48	30,000	990	3·3	300	1

where[1]. This radioactivity increases with the duration of culture, is related to the cell metabolism (inhibited by dinitrophenol) and is related also to the level of immunization (titre of agglutination of sheep erythrocyte in the serum). Normal rabbit lymph node cells kept in the same conditions with radioactive amino-acids did not show any formation of such radioactive substances. 'Cold' controls showed very little radioactivity fixation on red cells. Dialysis of the culture fluid did not decrease the radioactivity fixable on rabbit erythrocytes taken from the donor of the lymph node cells or on sheep erythrocytes, but the radioactive material is precipitated by ammonium sulphate at 40 per cent saturation or by a goat specific anti-rabbit γ-globulin serum. Presence of large amounts of 'cold' anti-sheep erythrocyte serum inhibits the fixation of the radioactive material, presumably by competition. The positive results given by micro-agglutination and tracer techniques with rabbit erythrocytes taken from the donor of the lymph node cells lead us to believe that we have observed the synthesis of auto-antibodies in tissue culture.

However, in most experiments radioactivity fixable on normal rabbit erythrocytes is higher than radioactivity fixable on rabbit erythrocytes taken from the donor of the lymph node cells. This can be interpreted in two ways: either that there are less auto-antibodies than iso-antibodies formed (just as there are less iso-antibodies than hetero-antibodies), or that the autologous red cells are already coated by some sort of auto-antibodies synthesized *in vivo*, when they are collected.

Synthesis of antibodies *in vitro* indicates that the usual absence of circulating auto-antibodies in an animal may not be due to the specific disappearance of all cells competent for the synthesis of this kind of antibodies, such as is postulated in the 'forbidden clone' theory[3].

The possibility that such a synthesis of antibodies occurs also *in vivo* must be considered and is actually under experimental analysis.

These experiments lead us to believe that tissue culture offers an approach to the problem of formation of true auto-antibodies, the synthesis of which has been stimulated by an immunization with an immunogen structurally related to an antigen of the body.

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RADIOBIOLOGY

Cross-linking of Deoxyribonucleic Acid to Protein following Ultra-Violet Irradiation of Different Cells

If deoxyribonucleic acid is exposed to ultra-violet light, especially to the 2537 Å. mercury arc line, a variety of changes occur. In very dilute solution the first physicochemical change is denaturation leading to a fall in viscosity without a change in molecular weight[1]. With higher doses, main-chain scission occurs and this is markedly enhanced by the presence of oxygen. There is chemical evidence for the formation of a dimer when thymine is irradiated in frozen aqueous solution[2] and for cross-linking between molecules when DNA is irradiated in dilute solution[3] or as a thin film[4]. The work reported here has shown that the changes produced in DNA when irradiated as a nucleoprotein inside cells are qualitatively and quantitatively different from those that are produced when pure DNA is treated.

Well-agitated suspensions in physiological saline (equilibrated with either nitrogen or oxygen) of *E. coli B*, murine lymphoma cells or salmon sperm heads were irradiated in physiological saline with a low-pressure 'Vycor' jacketed mercury arc in an apparatus[1] which enabled the dose of radiation absorbed by the DNA in the different cells to be computed, allowance being made for scattered light. To ensure that the alterations produced in the DNA were the direct consequence of the ultra-violet light (and not secondary effects of metabolism), the cells were irradiated at 0° C. and their DNA content extracted immediately afterwards. The test for the detection of cross-links between DNA helices was the formation of an insoluble gel network.

The cells were dispersed in 2 *M* sodium chloride and centrifuged for 1 hr. at 10,000 r.p.m. at 0° C. In the unirradiated cells all the DNA (as determined by the Burton method) was present in the supernatant. After irradiation, the cells appeared to disperse in 2 *M* sodium chloride, but some DNA was removed from solution on centrifugation in the case of sperm heads (Fig. 1) though no such effect was seen with *E. coli* or the lymphoma cells. This readily sedimentable DNA was seen to be in the form of a loose gel which we assume to be covalently cross-linked because it does not disperse in 8 *M* urea[5].

When the DNA was isolated from the cells by standard deproteinization procedures (Marmur's method[6] for *E. coli* and lymphoma cells and modified Dounce's method for sperm heads) the amount recovered decreased markedly on irradiation in all three systems (Fig. 2). We assume that the reason why some of the DNA from the irradiated cells is found in the insoluble protein fraction, instead of in the supernatant, is due to the fact that protein became covalently linked to the DNA and that this

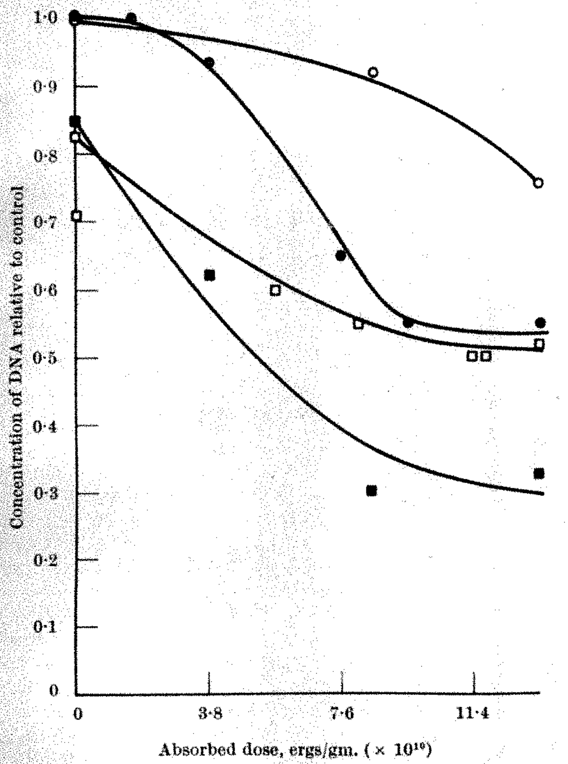

Fig. 1. Effect of ultra-violet light on the DNA in the nuclei of salmon sperm. Top lines, Fraction of DNA soluble in 2 *M* salt; the difference represents DNA in gel form due to DNA–DNA cross-links. ○, Irradiated under oxygen; ●, irradiated under nitrogen. Bottom lines, Fraction of DNA which can be isolated by the standard deproteinization procedure; the difference represents DNA in gel form + DNA linked to protein. ■, Irradiated under oxygen; □, irradiated under nitrogen

complex travels with the protein fraction during the separation procedure. This interpretation is supported by the fact that treatment of the cell suspensions with 0·5 per cent trypsin for 10 min. prior to deproteinization renders all the DNA extractable, presumably because the bound protein has been largely digested away. Trypsinization is without effect on the gel fraction ascribed to DNA–DNA cross-links in sperm heads.

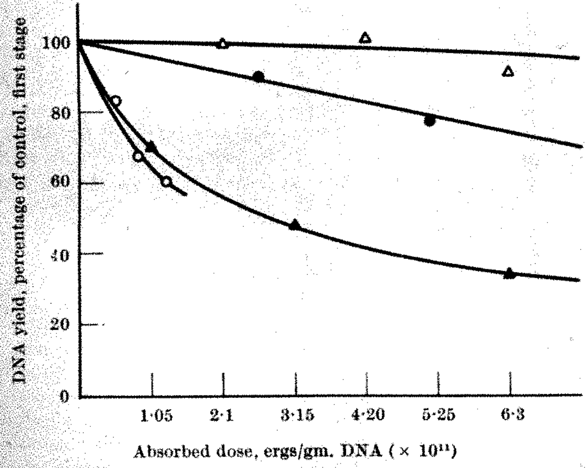

Fig. 2. Effect of ultra-violet light on the fraction of DNA that can be extracted from different cells by standard isolation procedures. ●, Murine lymphoma cells; ▲, *E. coli B*; △, *E. coli B* treated with trypsin before DNA extraction; ○, salmon sperm heads

Fig. 1 shows that while the presence of oxygen markedly reduces the extent of DNA–DNA cross-linking, it is initially without effect on the DNA-protein cross-links and at high radiation doses even enhances this reaction. The physical-chemical properties of the DNA which can be isolated are markedly affected by radiation and main-chain scission occurs much more readily when the DNA is irradiated within cells than in dilute solution. These results will be reported in detail elsewhere.

We suggest that the linking of protein to DNA may contribute significantly to the killing of cells by ultra-violet light, since this reaction is readily detectable by physico-chemical methods with amounts of radiation that are necessary for killing 99·9 per cent of the cells, and smaller doses are therefore likely to alter biologically significant amounts of DNA.

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Alterations in Deoxyribonucleic Acid from Regenerating Rat Liver after X-radiation *in vivo*

While it is thought that X-radiation *in vivo* does not produce any changes in the properties of deoxyribonucleic acid (DNA) which are associated with H bonding[1], alterations in its structure are suggested from differences in fractionation pattern on *ECTEOLA*[2,3] and from the diminished capacity of nuclei isolated after irradiation *in vivo* to act as templates for DNA synthesis[4]. The validity of the chromatographical evidence has been questioned for normal animals by Kondo and Osawa[5] and after irradiation[6,7], due to our ignorance of the means by which fractionation on *ECTEOLA* is attained and because of the possibility that the differences found between DNA from control and irradiated animals could be due to the action of DNases which are known to be activated in thymus after X-radiation. In view of these uncertainties, it was decided to extend our earlier observations on thymus DNA to regenerating rat liver. This tissue is advantageous since it is possible to irradiate at a known period in the mitotic cycle, and because in normal rat liver

Table 1. PROPERTIES OF LIVER DNA ISOLATED 25 HR. AFTER PARTIAL HEPATECTOMY FROM CONTROL RATS AND FROM THOSE WHICH HAD RECEIVED 2,000 R. 30 MIN. EARLIER

	Experiment 1			Experiment 2		
	Control (*C*)	Irradiated (*R*)	*R*/*C* (per cent)	*C*	*R*	*R*/*C* (per cent)
ε P (260)	7,387	6,705	—	7,792	6,989	—
Protein (per cent)	0·54	0·28	—	0·52	0·81	—
Phosphorus-32 counts/min./*E* = 1	79·5	39·8	50	69·4	29·4	42·4
DNA eluted as percentage of total DNA eluted						
1 0·5 *M* sodium chloride, *p*H 7	6·5	6·4	99	4·9	6	122
2 2 *M* sodium chloride/0·1 *M* ammonia	58·9	64·1	109	48·3	53·9	112
3 2 *M* sodium chloride/*M* ammonia	19·3	17·1	89	35·7	30·2	85
4 0·5 *M* sodium hydroxide	15·4	12·4	80	11·1	9·8	89
Specific activities of peaks 1	65·6 ± 4·00	46·2 ± 1·16	70·5	52·2 ± 8·72	40·8 ± 7·18	78·0
2	89·0 ± 3·33	40·3 ± 1·73	45·3	119·6 ± 3·26	40·8 ± 1·58	34·1
3	78·9 ± 1·70	25·4 ± 1·23	32·2	125·4 ± 4·86	33·2 ± 2·02	26·5
4	80·9 ± 2·07	30·6 ± 2·78	37·9	50·8 ± 2·52	24·4 ± 4·06	48·1

activation of DNases post-irradiation is much less marked than in thymus[8]. DNA was isolated by Kirby's method[9], which would reduce to a minimum DNase action during the isolation.

Male animals (200 ± 20 gm.) of the laboratory stock of Wistar rats were used. 24 hr. after hepatectomy, that is, in the first peak of DNA synthesis, they were given 2,000 r. total-body irradiation (77 r./min.). Immediately following exposure they received 100 μc. phosphorus-32/100 gm. body-weight and were killed 30 min. later. Control animals were treated similarly except for the absence of irradiation. DNA from control and irradiated rats was prepared simultaneously. 24 animals were used per group, and 20 or 30 mgm. material was applied to *ECTEOLA* columns[10] and the fractionation carried out in parallel with a common reservoir and mixing flasks. Preliminary experiments established that identical samples of DNA on the two columns showed less than 3 per cent difference in ultra-violet and phosphorus-32 elution patterns in peaks 2 and 3 (Table 1) and less than 6 per cent difference in peak 4. Peak 1, which represented a minor component, was thought to contain denatured material and was more variable (13 per cent).

X-radiation (Table 1) produced no change in the ultra-violet absorption characteristics of the unfractionated material, although incorporation of phosphorus-32 was reduced to 40–50 per cent of the control-level. The material eluted by 2 *M* sodium chloride/0·1 *M* ammonia (peak 2) was increased after exposure at the expense of peaks 3 and 4. In both experiments the inhibition of the uptake of phosphorus-32 was most marked in peak 3. Measurement of the melting point temperatures[12] of peak 2 and the base ratios of peaks 2 and 3 failed to show any differences in these respects between the control and irradiated fractions.

The difference in behaviour of the different peaks after exposure and the lack of change in H bonding suggest that the altered fractionation pattern is not due to DNase action. Although the effects are small this is to be expected since the DNA was isolated by procedures which might eliminate grossly damaged molecules. The changes observed provide additional evidence for alterations in DNA structure after X-radiation *in vivo*.

We thank Dr. G. E. Harrison of the Medical Research Council Radiobiological Research Unit, Harwell, for providing some of the facilities for these experiments, and the Medical Research Council for a grant to one of us (R. F.). Financial assistance for this work has been provided by the Department of Scientific and Industrial Research and the International Atomic Energy Agency.

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Ray Cataract in the Lens of the Eye caused by Wounding after X-Irradiation

SOME years ago I found that, on being turned about in the anterio-posterior direction, the inverted rounded lens of an amphibian larva underwent a reverse turn and in a few days came to occupy its previous position[1,2].

A single roentgen-ray action (500 r.) on a tadpole reduces the electric potential in the retina and the lens and prevents a reverse turning of the inverted lens, and a 1,500-r. dose results in destroying the lens. This unexpected result may be explained only by an intra-ocular surgical interference made after a relatively strong ionizing irradiation, because eyes irradiated with the same dose but left unoperated, or unirradiated but operated eyes, remained quite normal without showing any phenomenon of 'surgical after-action'[3].

I have now carried out similar experiments on adult frogs (*Rana temporaria*) and on white stock mice. The heads of these animals were irradiated in a diagnostic roentgen apparatus (*RUD* 100 kV./20 m.amp.) and after 2 days the X-rayed lenses were cut to a depth of half their diameter.

Up to the present time such operations have been made on 49 frogs and 30 mice. The right eye of the

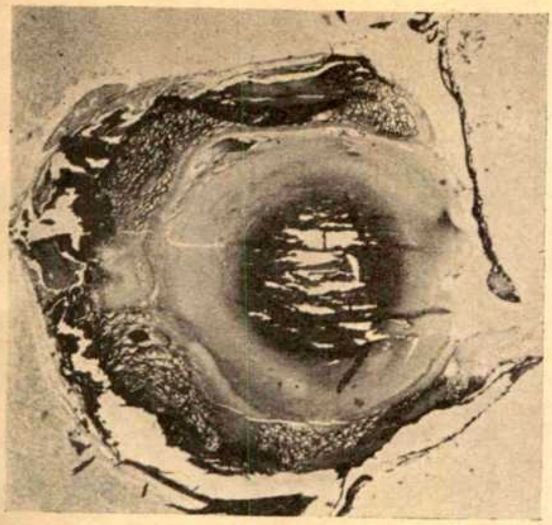

Fig. 1. Lens of a frog irradiated with 1,000 r. showing traces of incision 3–14 days after operation. A homogenized fibrous mass and numerous spheric cells can be seen. (× 20)

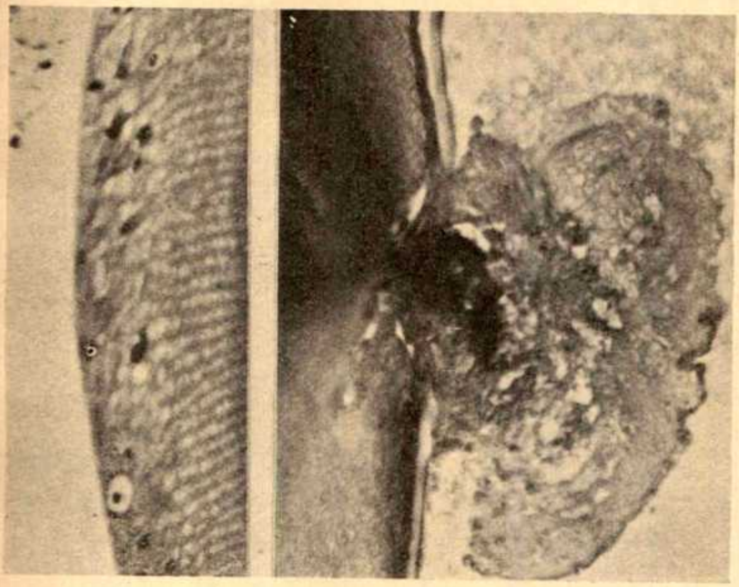

Fig. 2. Beginning of cataract-changes in the lens of a mouse irradiated with 800 r. on the second day after operation. The formation of epithelium on the posterior (left) and traces of incision on the anterior (right) poles. (× 230)

irradiated animal was operated on, while the left one served as control. Moreover, some control experiments took the form of incision of the lens of unirradiated animals.

Twenty frogs received 1,000 r., 18–2,000 r. and 11–4,000 r.

Even on the third or fourth day following the operation, either a slight or a most pronounced, but always a more or less steady, opacity of lens became evident. 8–9 days after operation 90 per cent of lenses were very opaque, and after about a fortnight the milk-white contents of a rather shrivelled lens began to emerge in the form of a loose mass into the aqueous humour of the anterior chamber of the eye. Similar changes took place after an irradiation with 2,000 r.; but in this case a very strong total opacity of lens appeared as a rule even on the first–second day after operation, reaching its limit in 7–9 days. Finally, after a 4,000-r. irradiation almost the entire lens became densely opaque on the day following treatment.

Nineteen mice received 350 r. and 11–800 r. There were ten control experiments.

Irradiation with 350 r. caused a change in the lens 10 days after operation in living mice. In this case, therefore, unlike frogs, the lens became opaque later and opacification was rather unsteady. Approximately by the eighteenth day it reached its greatest density. Irradiation with 800 r. resulted in accelerating the process, and as early as on the fifth day there began to form a rather dense cataract which reached its highest development by the eighth–tenth day.

It is worth noting that the left unoperated eyes of test frogs and mice remained clinically quite normal during the whole experiment, that is, for 20 days.

In the control experiments, the unirradiated lenses became slightly opaque in the region of incision; but this soon disappeared. In individual cases only such a local opacity, increasing somewhat in dimensions, remained until the end of the experiment. But even then no complete traumatic cataract of the lens appeared.

Histological examination of my material at different dates after operation has shown that opacity occurs very rapidly after the first irradiation, and there is a connexion between wounding and the formation of cataract. It seems that a ray cataract is caused by the act of wounding by incision.

The development of the cataract proceeded so violently, in frogs particularly, that it was impossible to follow the usual stages of its formation. In frogs the lens began to swell at once, which did not allow it to become separated from that of a ripe and often even an over-ripe cataract. The most characteristic stages in changes in the lens of frogs and mice are shown in Figs. 1 and 2.

The experiments render it necessary to take into consideration a possible risk during an intra-ocular operation on a patient, if such operation is preceded by a relatively strong ionizing irradiation. Furthermore, the question arises as to the terms of an intra-ocular surgical intervention in connexion with a probable increase and disappearance of a 'surgical after-action'. Moreover, it would be pertinent to investigate the probable importance of an accumulation of small, but multiple, doses of ionizing radiation for the manifestation of a negative effect detected in my experiments. Finally, it seems that the rapidly reacting eyes described here could be successfully used in more extensive work on chemical protection of the eye from the harmful action of ionizing radiations.

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A Pool Size Problem associated with the Use of Tritiated Thymidine

HELL *et al.*[1] have directed the attention of research workers to the fact that errors may result from the use of high specific activity radioactive compounds when the intracellular concentration of the corresponding metabolites is very low. In particular they mentioned tritiated thymidine and showed that the apparent effects of irradiation on the incorporation of this compound by Ehrlich ascites cells depended on whether tritiated thymidine of high or low specific activity was used. They ascribed this effect to differences in pool size in the irradiated cells.

In a set of experiments designed to investigate variations in the amount of deoxyribonucleic acid (DNA) per HeLa cell during the course of infection with herpes virus, which have been described in detail elsewhere[2], we have obtained similar results which can be attributed to a marked variation in size of nucleotide pool during the course of the experiments.

Four of these experiments are relevant: (1) Measurements were made of the total amount of DNA in single HeLa cells in Feulgen-stained cultures fixed at various times after infection with herpes virus. These measurements were made using a microspectrophotometer and the two wave-length method[3-5]. Measurements on about 30 cells per culture gave an estimate, in arbitrary units, of the mean amount of DNA per cell and this was found to vary with time (Fig. 1). These results confirmed those obtained by Newton and Stoker[6], who measured the increase in DNA content of herpes-infected HeLa cells by a chemical method.

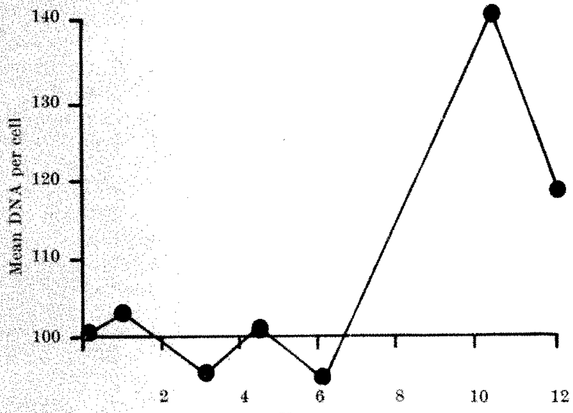

Fig. 1. Ratio of mean DNA per cell in the infected cells to that in controls averaged over three experiments

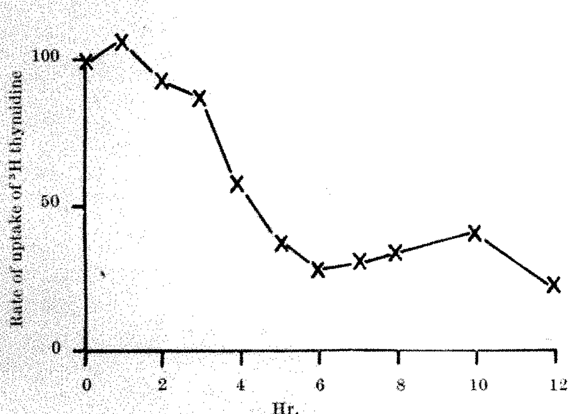

Fig. 2. Average of two experiments in which the rate of uptake of tritiated thymidine by infected cells in grains/nucleus/0·5 min. is expressed as a percentage of that in uninfected control cells

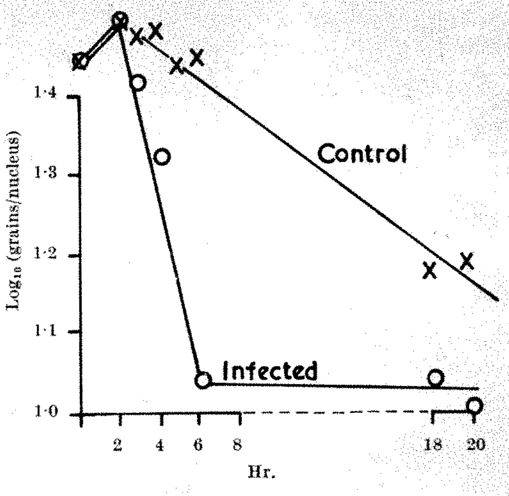

Fig. 3. Curve showing the loss of label from cells following infection. The cells had been incubated with 0·19 c./m.mole tritiated thymidine for 24 hr. prior to infection

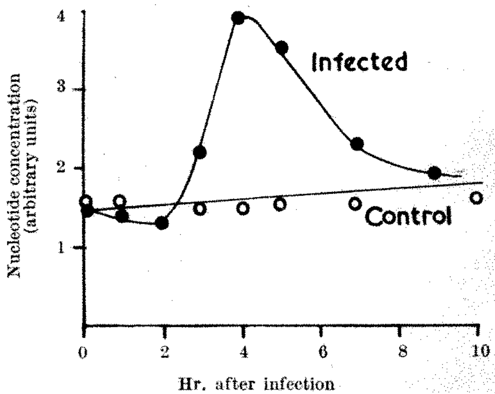

Fig. 4. Nucleotide content of HeLa cells infected with herpes virus

(2) The rate of uptake of tritiated thymidine into HeLa cells at various times after infection was measured by placing the cultures in tritiated thymidine for 0·5 hr. at varying times after infection, fixing immediately, making autoradiographs, and measuring the mean number of developed grains over the nuclei in the autoradiographs. This mean grain count reflects the rate of uptake of tritiated thymidine and might be thought to give an estimate of the rate of DNA synthesis. The results obtained are shown in Fig. 2.

(3) HeLa cells were labelled with tritiated thymidine before infection. Immediately before infection the tritiated thymidine was replaced by an equal concentration of unlabelled thymidine and the amount of label remaining in the cells was measured autoradiographically at different times after infection. The amount of label in infected cells fell rapidly to a low value and then remained constant (Fig. 3).

The second experiment indicates only a continuously decreasing rate of uptake of tritiated thymidine with time, whereas the microspectrophotometric results show that DNA is being rapidly synthesized between 6 and 10 hr. after infection. The explanation which we favour is that during the period

2–6 hr. after infection, cell nucleic acid is being rapidly broken down by the virus to give a large intracellular pool of nucleotides, polynucleotides and nucleosides. The results of the third experiment are consistent with these ideas.

(4) The fourth experiment was designed to investigate this point. The amount of acid soluble nucleotide extractable from the cells was estimated at varying times after infection (Fig. 4). These measurements indicate that there is indeed an increase in the size of pool in infected cells.

In consequence, even though DNA synthesis (probably induced by the virus) is rapid during the 6–10-hr. period, the tritiated thymidine is now only a small fraction of the nucleotide pool. Hence the rate of incorporation of label decreases continuously and in no way reflects the rate of DNA synthesis. This seems to be an extreme example of phenomena in which the effects of size of pool must be taken into account and in this case the difficulty would arise even with low specific activity precursor.

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BIOLOGY

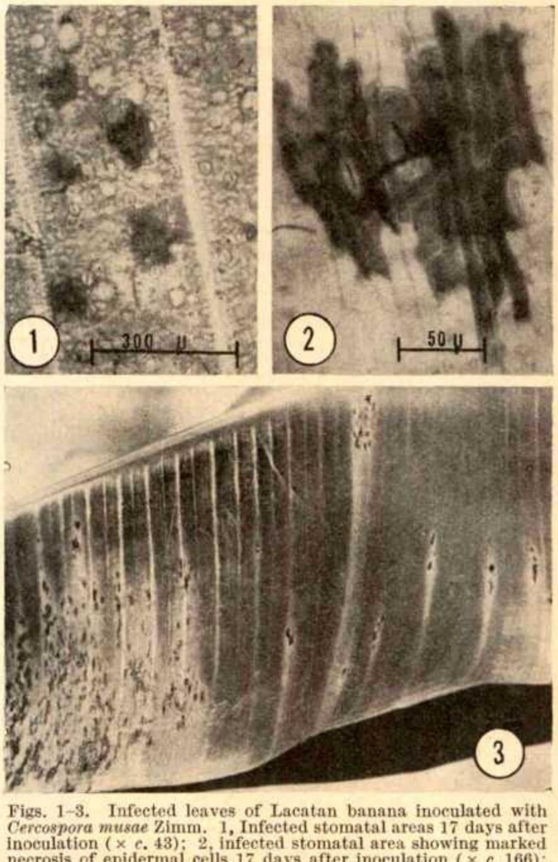
Establishment of Sigatoka Disease of Bananas in the Greenhouse

ALTHOUGH Sigatoka disease of banana has been known for a half-century and is a serious problem in most banana-growing areas, many fundamental aspects of the disease and of the life-history of the causal organism, *Mycosphaerella musicola* Leach (= *Cercospora musae* Zimm.), remain poorly understood. Present knowledge has been derived largely from field observation of the disease and pathogen and from field inoculation studies[1–4]. Successful greenhouse inoculations have not been reported, although many investigators have been interested in studying the disease under controlled conditions. In the field, Sigatoka outbreaks are coincident with periods of wet, humid weather[1,2], and laboratory investigations have shown that conidia of *C. musae* must be immersed in a water film for germination to occur[2]. Failure to obtain adequate humidity and/or moisture films on the leaf surface may account for the lack of reported greenhouse studies of this disease.

This communication is a preliminary report on greenhouse inoculation studies with *C. musae*, and describes a method by which successful infections were obtained.

A sporulating culture of *C. musae*[5], originally obtained from Dr. L. Calpouzos, U.S. Department of Agriculture Experimental Station, Mayaguez, Puerto Rico, and maintained in our laboratory for three or more years, was used for this work. Inoculum was obtained from week-old plate cultures grown on Difco mycological agar. Plates were inoculated by spreading a heavy conidial suspension evenly over the agar surface, and were incubated at 25° C. Conidia were collected by flooding the plates with sterile distilled water and gently massaging the culture surface with a rubber 'policeman'. Inoculations were made on susceptible varieties by spraying conidia on to the lower surface of the youngest unfurled leaf with an atomizer and compressed air. Microscopic observations of spore germination and stomatal penetration were made periodically by examining sections of inoculated leaves stained with aqueous crystal violet.

In preliminary experiments, inoculated leaves were enclosed individually in plastic bags immediately after inoculation to maintain a high relative humidity near the leaf surface. Uncovered inoculated leaves were maintained as controls. On covered leaves, germination began with 24 hr. of inoculation, and 80–95 per cent germination was attained within 48 hr. No germination occurred on uncovered leaves, even after several weeks. Stomatal penetration was generally evident on covered leaves 4–6 days after



Figs. 1–3. Infected leaves of Lacatan banana inoculated with *Cercospora musae* Zimm. 1, Infected stomatal areas 17 days after inoculation (× *c*. 43); 2, infected stomatal area showing marked necrosis of epidermal cells 17 days after inoculation (× *c*. 166); 3, streak and spot symptoms 7 weeks after inoculation

inoculation, and nine to sixteen days after inoculation infected areas were barely visible macroscopically as small necrotic spots (Figs. 1, 2). Symptom development ceased at approximately this stage, even on leaves kept continuously covered for as long as two months. Adjustment of environmental conditions by daily removal and replacement of bags was also without effect in inducing streak and spot development. The fungus was recovered from infected leaves 12 weeks after inoculation, however, indicating that failure to obtain advanced symptoms was not due to death of the fungus.

Results of these experiments suggested that suitable environmental conditions for pathogenesis had not been achieved by bagging the leaves, or that an avirulent strain of the fungus had been used. Subsequent experiments were attempted, therefore, in which inoculated plants were placed in a plastic tent equipped with an overhead misting system controlled by a humidistat. With this system, relative humidity was maintained near 100 per cent, and the plants were subjected to a simulated mist rain. Plants were inoculated as in the previous experiments with two strains of *C. musae*: (1) the strain used in the preliminary experiments; (2) an isolate newly recovered from infected leaf material in Puerto Rico and sent to us by Dr. L. Calpouzos. Inoculated leaves covered with plastic bags, or left uncovered in the open greenhouse, were also included in the experiment. Following inoculation, plants in the plastic tent were maintained under constant high humidity for a period of nine days to induce spore germination. For the next three weeks, these plants were subjected to periods of alternate wetting and drying by daily opening of the tent for 6-hr. periods. It has been suggested that alternate wetting and drying may be a factor favouring development of this disease[6]. Four weeks after inoculation, the plants were removed from the tent to normal greenhouse conditions. At this time, small necrotic areas, similar to those observed on bagged leaves in the preliminary experiments, were the only visible evidence of infection.

Seven weeks after inoculation, typical streak and spot symptoms began to develop on those leaves inoculated with the newly isolated strain of *C. musae*, but only on those plants which had been held in the plastic tent (Fig. 3). Streaks and spots failed to develop on leaves inoculated with the strain used in the preliminary studies, indicating that this strain was probably avirulent. Neither strain induced symptoms beyond the small necrotic spots on leaves enclosed individually in plastic bags even after eleven weeks, although a few spots did develop on a single leaf inoculated with the virulent strain after thirteen weeks. Conidia failed to germinate on uncovered leaves. The fungus was readily recovered from infected leaves by placing leaf spots in moist chambers to induce sporulation, and transferring conidia to agar slants.

Work is now in progress to determine more precisely the conditions necessary for stomatal penetration and development of disease. Greenhouse investigations of this disease may be applicable to a variety of unsolved problems, including examination of the use of fungicides and oils in disease control, natural resistance mechanisms, selection of resistant varieties from hybrid progeny, determination of the factors influencing development of the ascigerous stage, and the role of the ascospore in the establishment of new infections.

We thank Dr. L. Calpouzos for providing cultures and for his suggestions.

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Control of Penicillium Decay of Citrus Fruits with 2-Aminobutane

IN evaluating a number of simple aliphatic amines as volatile post-harvest fungicides for use on citrus fruits, we found that 2-aminobutane (*sec*-butylamine) was remarkably effective in reducing decay incited by *Penicillium digitatum* Sacc.[1]. Subsequent experiments[2] revealed that effectiveness of this amine is mainly due to the fungistatic action of butylammonium salt residues persisting on the fruit after fumigation. *In vitro* tests showed that germination of *Penicillium* spores was almost completely inhibited by 10^{-3} *M* 2-aminobutane, and, furthermore, that this inhibition could be completely reversed by washing the spores in neutral phosphate buffer, followed by transfer of the spores to amine-free medium. A number of other lower ($< C_6$) primary monoamines were tested by this procedure and found to be ineffective in preventing spore germination. These results prompted an evaluation of 2-aminobutane in fruit washes and wax emulsions for control of *Penicillium* decay of citrus fruits.

Ice-cold aqueous solutions of 2-aminobutane were neutralized with an equivalent quantity of dilute hydrochloric acid and the resulting salt solution diluted with either water or wax emulsion to the desired amine concentration. For evaluation as a wash treatment, the diluted amine solution was adjusted to *p*H 10·5 (*pKa* 2-aminobutane = 10·56) or to *p*H 6·5. Since the paraffin-carnuba wax used was emulsified with triethanolamine oleate, the *p*H of the concentrated amine hydrochloride solution was adjusted to that of the emulsion, *p*H 8, before the two were combined. Valencia oranges or Eureka lemons were inoculated by scratching the fruit with a rotary saw blade[3] passing through a suspension of *Penicillium* spores containing 5×10^6 spores per c.c. The inoculated fruit was then incubated at 24° C. for 16 hr. before application of the amine treatments. The inoculated oranges were submerged in the amine solution for 4 min. and either dried directly or rinsed in a tank of water for 5 sec. and then dried. Three replicate lots of 30 oranges each were treated with each of the solutions listed in Table 1 and packed in 6 × 9 × 11 in. fibreboard cartons. Lemons were inoculated in the manner described for oranges, immersed in the appropriate wax formulation (Table 2) for 5 sec. and, after the excess wax drained from the fruit, were placed in open wood boxes. All fruit were stored at 20° C. to permit development of decay. Sodium *o*-phenylphenate, an extensively used citrus decay control agent, was included for comparison in both wash and wax tests.

Table 1. CONTROL OF *Penicillium digitatum* ON ORANGES BY AQUEOUS SOLUTIONS OF 2-AMINOBUTANE

	Treating solution	*p*H	Water rinse	Per cent decay in	
				7 days	14 days
A	Water only—control	10·5	yes	98	100
B	0·5 per cent 2-aminobutane	10·5	yes	1	22
C	0·5 per cent 2-aminobutane	10·5	no	0	2
D	0·5 per cent 2-aminobutane	6·5	yes	12	49
E	0·5 per cent 2-aminobutane	6·5	no	1	14
F	0·5 per cent sodium *o*-phenylphenate	12·0	yes	1	14

Table 2. COMPARISON OF WATER WAX EMULSIONS CONTAINING 2-AMINOBUTANE AND SODIUM *o*-PHENYLPHENATE IN RETARDING *Penicillium digitatum* ON LEMONS

Fruit treatment	Per cent decay in 14 days
Wax emulsion only	58
Wax emulsion with 1 per cent 2-aminobutane	7
Wax emulsion with 2 per cent 2-aminobutane	0
Wax emulsion with 1 per cent sodium *o*-phenylphenate	2

Table 1 shows that washing oranges in aqueous 0·5 per cent 2-aminobutane solutions greatly retarded the development of *Penicillium* decay and, at *p*H 10·5, the fungistatic activity of the rinsed amine treatment was only slightly less than that of sodium *o*-phenylphenate. The solutions at *p*H 10·5, which contained appreciable quantities of non-ionized amine, were more effective than those adjusted to *p*H 6·5, and efficiency at both *p*H values was enhanced by allowing 2-aminobutane residues to remain on the fruit. Although non-ionized 2-aminobutane undoubtedly penetrates lypophilic barriers of the fruit and fungus more readily than the butylammonium ion, persistence of amine salts on the fruit can satisfactorily account for the effectiveness of the wash treatment (cf. treatments *B*, *C*, and *E*, Table 1). The greater penetrating power of the non-ionized amine probably facilitates movement of the amine to the potential infection site. In other experiments, treatment of fruit with 1 per cent 2-aminobutane solution at any *p*H, followed by rinsing, has virtually eliminated decay.

Table 2 shows that a water wax emulsion containing 2-aminobutane provides decay control equivalent to one containing sodium *o*-phenylphenate. Although the triethanolamine oleate emulsifier necessitated treatment at *p*H 8 in this instance, we have obtained excellent decay control at *p*H 6 with 2-aminobutane water waxes containing non-ionic emulsifiers.

A number of other simple monoamines have been tested as citrus fruit washes at the molar equivalent of 0·5 per cent 2-aminobutane and at their respective *pKa* values. All other amines evaluated were completely ineffective. In screening trials carried out in Florida[4-6], a number of amines have been evaluated in wash solutions for control of citrus fruit decay. With the exception of several complex quaternary ammonium compounds, the amines tested were not considered effective for this purpose. Furthermore, certain amines were reported[5,6] to cause fruit rind injury. In our tests with 2-aminobutane we have never observed fruit injury attributable to the amine treatment, even when residues from 2 per cent 2-aminobutane solutions were permitted to dry on the fruit.

Unlike many commonly used citrus fruit-wash chemicals, such as sodium tetraborate and sodium carbonate, 2-aminobutane is an efficient decay-control agent when applied at room temperatures (about 25° C.). Sodium *o*-phenylphenate is widely used as a low-temperature fruit-wash; but the potential hazard of fruit injury from this chemical is well known[7]. Furthermore, there is considerable evidence[8] that continuous use of sodium *o*-phenylphenate washes in lemon packing houses may lead to the build-up of *P. digitatum* strains which possess resistance not only to *o*-phenylphenol, but also to biphenyl, a fungistat commonly used in citrus shipments. We have tested 2-aminobutane against both biphenyl-sensitive and biphenyl-resistant strains of *P. digitatum* and found both groups equally inhibited at concentration of 10^{-3} *M* amine. The dissimilarities in chemical structure, and probably mechanism of inhibition, makes it highly unlikely that *P. digitatum* could develop cross-resistance to biphenyl and 2-aminobutane.

From results obtained in the small-scale tests reported here, 2-aminobutane appears to offer considerable advantage over existing commercial fruit-wash and wax fungicides. The combined virtues of effective control of *P. digitatum* at room temperatures and over a wide *p*H-range, with negligible hazard of fruit injury, give 2-aminobutane considerable potential as a fruit decay control agent for use in California. However, in other citrus producing areas where major decay problems are due to organisms other than *Penicillium*, the utility of this fungistat may be limited.

Preliminary tests have shown that 0·5 per cent 2-aminobutane solutions effectively control *Penicillium expansum* Thom on apples, even when the solution temperature is held at 2° C.

An analytical method has been developed for 2-aminobutane residues on treated fruit, and commercial citrus fruit treatment trials are in progress.

We thank Mr. R. L. Slusher, of this laboratory, for assistance in conducting the fruit decay control tests and Mr. D. Cothron, Brogdex Co., for assistance in the development of commercial water wax formulations containing 2-aminobutane. Generous samples of 2-aminobutane were supplied by the Pennsalt Chemicals Corp.

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A Peculiar Ericalean Pollen Grain

IN the genus *Styphelia* Sm., the Epacridaceae include a pollen form previously not known in this or related families. I have examined the patterns of ektexine ornamentation in pollen from all genera of the Epacridaceae. The grains are mostly similar to those of allied families, in that they are either smooth or have an ornamentation which is inconspicuous except under oil immersion. Those of *Styphelia*, however, are strikingly different. Of the eleven species recognized by Bentham[1], I have been able to investigate ten, and in these the grains are always provided with warts (Fig. 1), the largest of

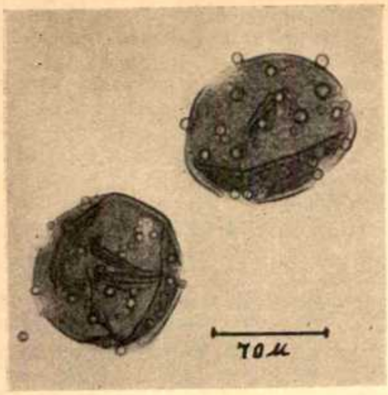

Fig. 1. Acetolysed pollen grains of *Styphelia triflora* Andr.

which are usually conspicuous at quite low magnifications and may be more than 10μ in diameter. As can be seen in the photograph, the warts are rather easily detached, and at first sight they could be mistaken for oil droplets. The grains of *S. tenuiflora* and *S. melaleucoides* have smaller warts than do the other species, but even here they are easily distinguished from those of related groups. Similar forms occur in distant families (for example, Nymphaeaceae), but the pollen of *Styphelia* appears to be without parallel in the Ericales.

Styphelia is now confined to Australia, with the disjunct distribution of a relict genus. Its near relatives are almost exclusively Australasian or Malaysian, but from the occurrence of *Leucopogon* fruits in the London clay[2], it is evident that the group must formerly have had a far wider range. Therefore it is possible that *Styphelia* pollen has been observed in fossil samples, and that palynologists failed to identify it.

Several taxonomists, including a few modern ones, have suggested that a number of allied genera should be regarded as sections of *Styphelia*. Australian botanists, however, who are more intimately concerned with these plants than anyone else, would not welcome the addition of several hundred synonyms to a confused nomenclature. Furthermore, the evidence from pollen structure indicates that the amalgamation might be as taxonomically unsound as it is unpractical.

I thank Dr. J. W. Franks, of the Manchester Museum, for his advice; the Keeper of the Herbarium at the Royal Botanic Gardens, Kew, for the loan of specimens; and the Department of Scientific and Industrial Research, for financial support.

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Role of the Corpus Luteum in Marsupial Reproduction

IN marsupials the histological changes which occur in the ovaries and uteri of unmated females after ovulation appear to be very similar both in degree and duration to those occurring during pregnancy[1]. The gestation period is shorter than the length of an œstrous cycle in all the polyœstrous marsupial species so far investigated, and some workers consider that this can be accounted for by a similarity between the duration of the functional life of the corpus luteum in pregnant and non-pregnant females, and of the gestation period[2]. Thus, Sharman[3] and Amoroso[4] postulate that the hormonal control both of the œstrous cycle and of pregnancy may be identical and that the simple yolk-sac placenta characteristic of most marsupials probably has little or no endocrine function comparable to that of the true allantoic placenta of eutherian mammals. One of the implications of this hypothesis is that pregnancy in marsupials would be largely maintained by the progesterone produced by the corpus luteum. However, a few marsupials, including the long-nosed bandicoot *Perameles nasuta* Geoffroy, have true allantoic placentæ[5,6] and therefore the reproductive physiology of such species is of considerable interest.

Observations were made on 57 *P. nasuta* females. Vaginal smears were taken from 31 animals. The interval separating successive peaks of vaginal cornification in 8 œstrous cycles averaged 26 days (range 17–34 days). Ovulation occurred irrespective of the presence of males. Examination of the reproductive tracts of 21 females on capture indicated that both ovaries were functional. The total number of ova shed in one cycle was most frequently either two or three. In all but two of the females the number of corpora lutea corresponded exactly with either the number of uterine embryos or of pouch-young. In the two exceptions the number of pouch-young was less than the number of corpora lutea. The gestation period (the interval separating copulation and parturition) was greater than 8 days in one instance, and 12 ± 1 days in another.

In the examination of reproductive tracts, uteri from four pregnant females were included (the blood vascular system of the trophoblast was either in the process of development or recently developed). Seventeen females were carrying pouch-young and represented a graded series during pouch life; seven were killed between parturition and 11 days of pouch life. There was no macroscopic evidence of degenerative changes in the corpora lutea or of the imminence of œstrus until the litter was more than 40 days old (the ages of pouch-young were estimated from unpublished growth-curves of Dr. A. G. Lyne). Vaginal smears taken during pregnancy and the first 40 days of lactation likewise gave no evidence of œstrus.

The fully formed corpora lutea of pregnancy had a diameter of about 2·8 mm. During the first week of pouch life the diameter decreased to about 2·2 mm. Microscopic examination of the ovaries showed that the large spherical nuclei of the luteal cells also decreased in diameter at this time. No further reduction occurred in the size of the corpora lutea until the litter was more than 40 days old. Rapid regression then followed, the luteal cells degenerating and being replaced by connective tissue which also eventually disappeared.

It appears that the corpora lutea of pregnancy do not normally involute until the offspring have suckled for about 45 days (approximately two-thirds of the total period of lactation). The degeneration of the corpora lutea at this stage permits females to return to heat and become pregnant before the completion of suckling (6 records). Parturition occurs after the suckling litter was weaned. If suckling is terminated by removal of the litter from the pouch the corpora lutea disappear within 10–14 days. Five females carrying litters between 27 and 40 days of age returned to œstrus 8–9 days after removal of the litter. Twelve other females carrying litters younger than 27 days or older than 40 days failed to return to œstrus after the removal of their litters. Œstrus was taken as the degree of vaginal cornification or uterine proliferation and vascularity that had previously been found to be associated with mating and ovulation.

The persistence of the corpus luteum of pregnancy as the corpus luteum of lactation has also been reported in another Australian marsupial, *Dasyurus quoll* (= *D. viverrinus*). Sandes[7] found that the corpus luteum in this species formed within 3 days after ovulation and persisted virtually unchanged throughout pregnancy and the greater part of lactation, ultimately disappearing when the litter was capable of leading an independent life. Although the allantois in *D. quoll* does not enter into the formation of the placenta in the same way as it does in *P. nasuta*, it nevertheless shows a greater development than that found in the majority of marsupial species that have only a yolk-sac placenta.

P. nasuta is similar to all other polyœstrous marsupials investigated in that the gestation period is less than the length of an œstrous cycle. However, the occurrence of parturition in the presence of an apparently functional corpus luteum raises some doubts as to the validity of the hypothesis that the hormonal control of pregnancy is identical with that of the normal œstrous cycle. This hypothesis rests largely on the occurrence of parturition after the degeneration of the corpora lutea of pregnancy in *Setonix*, *Trichosurus* and *Didelphys*[2].

In *Setonix brachyurus* the corpus luteum begins to degenerate quite some time (about 7 days) prior to parturition[1], so that it appears parturition is not as closely related to the functional life of the corpus luteum, at least in *Setonix*, *Perameles* and *Dasyurus*, as was originally thought. It would be premature at this juncture to speculate whether or not the marsupial placenta produces hormones.

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Effect of Cortisone on the Developing Mouse Fœtus

THE discovery that cortisone acts as a teratogenic agent was made first in Fraser's laboratory at McGill[1]. In the intervening years, Fraser and his students have published a number of papers reporting investigations which have extended the original findings (for a general review see Fraser[2]). Within the framework of these experiments it appears that the effect of the cortisone on the mouse fœtus is specific. The only gross defect which has been reported so far is cleft palate (although some minor morphological effects have been recorded by Kalter[3]). The purpose of this communication is to report a new effect of cortisone on the developing mouse fœtus.

During the course of an investigation[4] in which cortisone and fasting[5] were used as teratogenic agents in various inbred mouse strains, areas of yellow-brown pigment were noted in the birth membranes of the fœtuses of a *C57BL/6* Jax strain female autopsied on the nineteenth day of pregnancy. This female had received cortisone injections (2·5 mgm. of cortisone acetate intramuscularly in the flank) on the twelfth, thirteenth and fourteenth day of pregnancy. All fœtuses removed from uteri during the remainder of the experiment were examined for this feature. The results of these observations are recorded in Table 1. There was an inter- and intra-litter variability in the amount of pigment in the birth membranes and since a quantitative scoring method was not feasible, Table 1 is based simply on the presence or absence of pigment.

Table 1. FREQUENCY OF OCCURRENCE OF PIGMENTED AREAS ON FOETAL MEMBRANES AND ITS RELATION TO CLEFT PALATE IN THE *C57BL/6* JAX FOETUSES

			Relation of cleft palate and occurrence of pigment areas in birth membranes					
			Fœtuses with cleft palate			Fœtuses without cleft palate		
Treatment	No. of litters	No. of fœtuses	No.	No. with pigment	Per cent with pigment	No.	No. with pigment	Per cent with pigment
Controls	10	61	1	1	100	60	2	3
Cortisone	28	154	49	47	96	105	97	92
Fast 9th day + cortisone	10	43	21	21	100	22	20	91
Fast*	19	99	0	0	0	99	2†	2

* Combining data from groups fasted on 8th or 9th day

† Both animals with exencephaly and bilateral eye defects.

The most significant feature of the observations is the high frequency of pigment patches in the fœtal membranes of fœtuses the dams of which had received cortisone. However, the presence of the pigment appears to be independent of the occurrence of cleft palate, since 96 per cent of the fœtuses with cleft palate and 92 per cent of the fœtuses without cleft palate in the cortisone-treated group had pigmented membranes. It will be noted that five animals with pigmented membranes (three in the control series and two in the fasted group) were born to dams which had not received cortisone. The three animals in the control series were all much lighter than their litter mates when they were removed from the uterus. Two of these, including the one with cleft palate, occurred in one litter. The two animals in the fasting-treatment series were the only fœtuses in a litter of a female who was fasted during the eighth day of pregnancy. Both fœtuses had exencephaly and bilateral eye defects, and were much lighter in weight than the average new-born of this strain.

In addition to these observations on the *C57BL/6* Jax strain, pigmented birth membranes were noted in the fœtuses of some strain *LG/Rr* females which had received cortisone. The pigment was not easy to detect in this strain, and may well have been overlooked during some of the earlier autopsies. As a result, the numbers are so few and unreliable that they are not included in this communication. Pigmented membranes were also observed in a fœtus with exencephaly but a normal plate the dam (*LG/Rr* strain) of which had been subjected to the fasting plus cortisone treatment.

To my knowledge, there have been two previous instances in which similar pigmented fœtal membranes have been observed after the administration of teratogenic agents. In Runner's laboratory at the R. B. Jackson Memorial Laboratory, similar patches of yellowish-brown pigment were observed in the birth membranes of fœtuses the dams of which (prepuberal) had received insulin injections (Runner, M. N., personal communication). Haskins[6], using nitrogen mustard as a teratogenic agent in the rat, describes areas of "brown, necrotic tissue . . . around the periphery of the placentæ of the more grossly affected fœtuses".

On the basis of the present observations, it would seem reasonable to conclude that the abnormal features of placental development, manifested by the pigment areas, represent expressions of the cortisone treatment which are not related causally to the production of cleft palate. Confirmation of this conclusion must await the results of further studies. However, the findings presented in this communication do direct attention to the fact that the placenta is an integral part of the maternal-fœtal complex and, as such, cannot be neglected in the study of mammalian development.

This work was carried out while I was holding a fellowship from the Roscoe B. Jackson Memorial Laboratory.

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Auto-inhibition of Reproduction in *Cura formanii* (Turbellaria)

EVIDENCE for the widespread phenomenon of organismic interaction through chemicals released into the environment is summarized by Lucas[1]. One type of such interaction occurs between organisms of the same species. In tadpoles[2], for example, the effect of such substances is to inhibit the growth of individuals exposed to sufficient concentrations. This mechanism tends to limit the number of individuals of a given species within an area and thereby also limits depletion of environmental resources.

The eggs of the triclad turbellarian, *Cura* (= *Curtesia*) *formanii*, are normally fertilized by sperm from the same animal[3]. Copulation has never been observed, and individuals isolated from birth produce cocoons from which normal young emerge[4]. A clone of this flatworm was produced from a single individual by cycles of bisection and subsequent regeneration.

When the clone contained 160 adults, the worms were randomly distributed among 64 straight-sided bottles so that groups of 16 bottles contained 1, 2, 3 and 4 worms per bottle, respectively. One half of the worms, equally divided with respect to number of worms per bottle, were cultured in 40 ml of pond-water; the remainder were cultured in 80 ml. All animals were fed as much fresh mouse liver as they would consume three times a week, and following each feeding the water in the bottle was replaced with fresh pond-water.

Fig. 1 gives the number of cocoons produced per eight worms as a function of time.

Chi-squares for the differences between the various populations illustrated in Fig. 1 are given in Table 1.

Table 1.

	No. of worms per bottle 1 vs. 2	2 vs. 3	3 vs. 4	
40 ml. H_2O	7·5*	3·2	2·5	
80 ml. H_2O	8 7*	0·5	6 1†	
	No. of worms per bottle 1	2	3	4
Between 40 and 80 ml. per bottle	1 5	1 0	0 0	0 9

* Significant at the 99 per cent level.
† Significant at the 95 per cent level.

In a similar experiment (except that the design was factorial) 63 cocoons were produced within 64 bottles, each containing one worm, and 50 cocoons were produced in an equal number of bottles containing two worms each. The χ^2 for the difference is 25·5, which is very significant. In addition, 63 cocoons were produced by worms living in aerated water while 50 were produced by an equal number of worms which were not aerated but otherwise were living under similar conditions. The χ^2 here is 1·5.

The results of these experiments are consistent with the hypothesis that *Cura formanii* releases materials into the environment which inhibit reproduction by individuals of the species. The very small effect, if any, of aeration, indicates that the inhibitory material is not highly volatile and that the major inhibitory effect is not the result of change in concentration of dissolved oxygen or carbon dioxide. The lack of a demonstrable difference between the number of cocoons produced in 40 and 80 ml. of water indicates that cocoon production was not limited by scarcity of some substance present in the water and required for reproduction. The inhibitory effect is not decreased by the presence of snails (*Physa*) as is the case with the anti-growth factor produced by tadpoles.

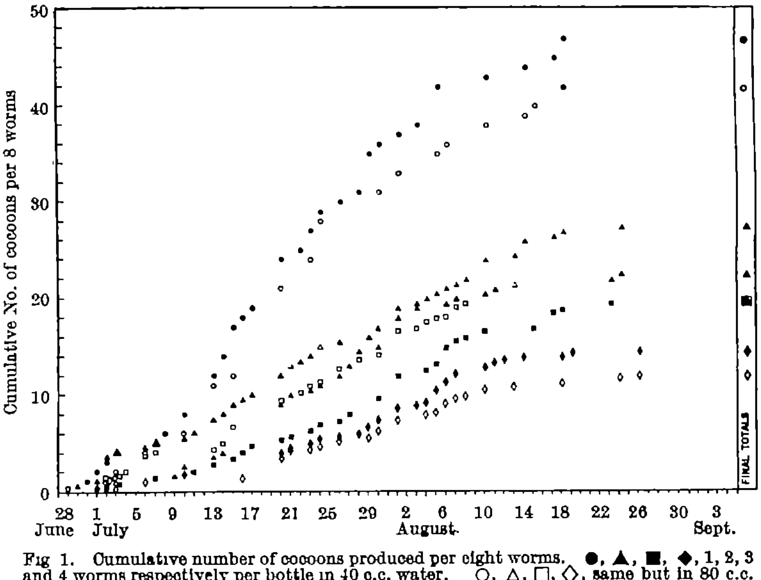

Fig 1. Cumulative number of cocoons produced per eight worms. ●, ▲, ■, ◆, 1, 2, 3 and 4 worms respectively per bottle in 40 c.c. water. ○, △, □, ◇, same but in 80 c.c. water

The growth inhibition mechanism of tadpoles does not prevent the production of many young individuals in an already crowded environment. The population regulation mechanism in *Cura formanii* is more efficient. Here, when the population density reaches a certain level, reproduction is inhibited and no further increase of drain on environmental resources occurs.

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VIROLOGY

Properties of Bovine Virus Diarrhœa Virus

RECENTLY it has been shown[1] that a virus isolated from cattle with signs of 'mucosal disease'[2] is antigenically related to the virus of bovine virus diarrhœa[3]. In northern Sweden, a type of mucosal disease ('Umeå disease') was found to be accompanied or preceded by a respiratory disease. In paired serum samples from diseased animals increasing titres of antibodies were demonstrated against both bovine virus diarrhœa virus and para-influenza 3 virus[4], but only para-influenza 3 virus was isolated[5]. The incidence of infections with bovine virus diarrhœa virus among Swedish cattle seems to be about 20 per cent as judged from the results of a serological survey in 1959–60[4]. In the present work the properties on which Cooper[6] bases his classification of animal viruses have been investigated for bovine virus diarrhœa virus.

The bovine virus diarrhœa virus was represented by the strain Oregon *C24V*, which exerts a cytopathic effect on embryonic calf kidney cultures[7]. Since embryonic cells have a tendency to early spontaneous degeneration, new-born calf kidney cultures were used for propagation and titration of the virus. The maintenance medium consisted of Hanks's salt solution with 0·5 per cent lactalbumin hydrolysate.

The sedimentation coefficient of bovine virus diarrhœa virus was estimated by centrifugation in a Spinco model *L* centrifuge and compared with that of poliovirus type 1 and reovirus type 3. The viruses in infective tissue culture fluids were centrifuged in a *SW*39 rotor at different speeds and for different lengths of time and the supernatants titrated in roller tubes. In order to remove aggregates of virus the virus suspensions were pre-centrifuged for 1 hr. at 25,000*g* for poliovirus and bovine virus diarrhœa virus and for 1 hr. at 6,000*g* for reovirus. Poliovirus and reovirus were titrated in monkey kidney cultures and bovine virus diarrhœa virus in calf kidney cultures. The results for one experiment are recorded in Table 1. The bovine virus diarrhœa virus appears to sediment slower than reovirus and faster than poliovirus. The sedimentation coefficient of bovine virus diarrhœa virus was estimated to be approximately 80–90 Svedberg units (*S*). Since the calculated sedimentation coefficient of poliovirus is lower than the values reported previously[8], the value of bovine virus diarrhœa virus may actually be higher.

Table 1. ESTIMATION OF SIZE BY CENTRIFUGATION

Speed (r.p.m.)	Poliovirus* 1 hr.	Poliovirus* 2 hr.	Reovirus* 1 hr.	Reovirus* 2 hr.	Bovine virus diarrhœa virus* 1 hr.	Bovine virus diarrhœa virus* 2 hr
19,000	5 3	ND	4 7	ND	5 3	ND
27,000	4 9	4 9	3 7	ND	5·3	4 9
32,000	4·7	3 5	3 5	3·1	5 1	3 5
40,000	4 9	3 5	2 9	3·1	4·1	2·9
Sedimentation coefficient	60–80*S*		>180*S*		80–90*S*	

* Titre in neg. ^{10}log TCD_{50}/ml. of the supernatant.
ND, not done

The virus suspensions were filtrated through Millipore filters (Bedford, Mass.) of different pore size. Poliovirus and bovine virus diarrhœa virus passed through 50 mμ filters while reovirus was retained by 100 mμ filters (Table 2).

Table 2. ESTIMATION OF SIZE BY FILTRATION

Pore size (mμ)	Poliovirus*	Reovirus*	Bovine virus diarrhœa virus*
50	6 1	<0·5	1 1
100	7·5	<0·5	3 3
Not filtered	7·9	5 5	4 3

* Titre in neg. ^{10}log TCD_{50}/ml.

Electron micrographs of ultra-thin sections of virus pellets revealed a few spherical particles with a diameter of about 40 mμ. Specimens for electron microscopy were prepared from infective tissue culture fluid. The virus was concentrated in aqueous two-phase systems[9] and sedimented by centrifugation at 105,000*g* for 1 hr. Since the titre of the tissue culture fluid and disrupted cells never exceeded $10^{5\cdot7}$ TCD_{50}/ml. the concentration of virus in the pellet was too low to permit an identification of the spherical particles as the virus particles of bovine virus diarrhœa virus.

The sensitivity of the Oregon strain to ether and chloroform was tested according to methods described previously[10,11]. The Oregon virus was readily inactivated by these lipid-solvents and appears therefore to be a lipo-virus.

The type of nucleic acid of this virus was investigated by the use of 5-iodo-deoxyuridine, which is known as an inhibitor of synthesis of deoxyribonucleic acid (DNA)[12]. Calf kidney cultures containing 50 μgm. of the inhibitor per ml. medium were inoculated with bovine virus diarrhœa virus, and as a control, with bovine enterovirus and pseudorabies virus. The multiplication of pseudorabies virus, a DNA virus, was clearly inhibited by 5-iodo-deoxy-

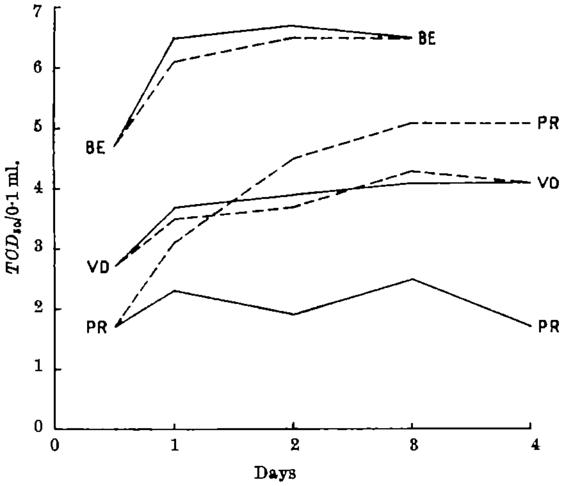

Fig. 1. Growth of different viruses with or without 5-iodo-deoxyuridine (IUDR) added to the medium. BE, bovine enterovirus; PR, pseudorabies virus; VD, virus diarrhœa virus. ——, with IUDR; – – –, without IUDR

uridine whereas bovine virus diarrhœa virus and bovine enterovirus were not inhibited at all (Fig. 1). Bovine virus diarrhœa virus can therefore be tentatively considered as an RNA virus.

Other compounds known as inhibitors of DNA synthesis[12–14] were also tested. 5-bromo-deoxyuridine[12] was found to be less inhibitory for the multiplication of pseudorabies virus than 5-iodo-deoxyuridine while 5-fluoro-deoxyuridine[13] and aminopterine[14] had no effect on the growth of this virus in calf kidney cultures. The results presented here confirm the observation[12] that 5-iodo- and 5-bromo-deoxyuridine are superior to other inhibitors of DNA synthesis in some species of cells.

The results of cross-neutralization tests showed no antigenical relationship between the Oregon strain and para-influenza-3 virus, infectious bovine rhinotracheitis virus, or bovine enteroviruses. On the other hand, hyperimmune swine fever serums had a neutralizing effect on the Oregon strain[15], and bovine virus diarrhœa virus will protect pigs against swine fever[16]. Darbyshire[17] has shown earlier that antigens in organ suspensions derived from pigs with swine fever and from cattle with a British mucosal disease[18] cross-precipitate in agar-gel.

To summarize, the bovine virus diarrhœa virus as represented by the strain Oregon *C24V* appears to be an RNA-containing lipo-virus with a size of approximately 40 mμ and antigenically related to the swine fever virus.

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Release of Deoxyribonucleic Acid from Vaccinia Virus by 2-Mercaptoethanol and Pronase

IN an earlier report the isolation of a single-stranded DNA from highly purified vaccinia virus was described[1]. The DNA was extracted under mild conditions with water-saturated phenol and deoxycholate, according to the standard procedure used for obtaining purified and undegraded nucleic acids from many other viruses and micro-organisms[2]. It was considered unlikely that this single-stranded DNA was derived by denaturation of originally double-stranded DNA as a result of phenol treatment; first, because there are no reports in the extensive literature that phenol extraction at 4° C. can cause such degradation, and secondly because salmon-sperm DNA treated in our laboratory with the identical procedure used for vaccinia showed no evidence of denaturation. Despite repeated attempts to improve the yield of phenol-extracted DNA from vaccinia, the method was quantitatively unsatisfactory as not more than 10–20 per cent of the total viral DNA was recovered. Alternative methods of isolating the DNA from the virus in higher yield and in as pure a form as possible have therefore been investigated.

Earlier workers disrupted vaccinia by treatment with 1 per cent sodium carbonate[3] or 5 per cent sodium hydroxide[4]. In our hands these classic methods, which were originally applied to virus cultivated on rabbit skin, released less than 1 per cent of the total DNA from the egg-adapted Levaditi strain purified with fluorocarbon. About the same efficiency of extraction (1 per cent) has been obtained on treatment with the following reagents: 2 per cent sodium lauryl sulphate[5], saturated guanidinium chloride[6], 8 *M* urea[7], 1 per cent sodium deoxycholate[8], and hot (50° C.) phenol[9]. Furthermore, we have found that treatment with *N* sodium hydroxide at 20° C., as used by Wyatt and Cohen[10] to disrupt vaccinia for base-ratio determinations, gave about the same yield as the above methods. Sonication (Raytheon-10*KC*) of fluorocarbon-purified vaccinia for 15 min. at 4° C. in the presence of 80 per cent phenol released about 50 per cent of the total DNA. Although this DNA was highly purified (260/280 mμ ratio of 2 0), the method was not pursued further since the DNA was undoubtedly being degraded[11]. Various combinations of enzymatic treatment (lysozyme, trypsin, chymotrypsin) also failed to release nucleic acid.

Strikingly similar difficulties have been encountered in attempting to liberate DNA from human and bull sperm[12]. Successful release of DNA from sperm was recently reported by Borenfreund, Fitt and Bendich by degrading the keratin-like outer membrane on incubation with 2-mercaptoethanol and trypsin[13]. This technique, with certain essential modifications, has now been applied to vaccinia with the result that we have routinely been able to extract 90–95 per cent of the DNA from the virus.

Purified virus[1] was washed once with absolute ethanol, deposited by centrifugation at 20,000*g* for 1 hr., and resuspended in a 1 : 1 mixture of absolute ethanol and ether (about 6 ml./5–10 mgm. dry weight of virus). The vaccinia was sedimented again and dried overnight in a 40° C. oven. The dried virus was resuspended in 5 ml. of 0·15 *M* sodium chloride–0·015 *M* sodium citrate, and sufficient 2-mercaptoethanol was added to bring the final concentration to 2 per cent. This suspension was then stirred slowly for 18–24 hr. at 4° C. During the first few hours of stirring it was important to break up clumped material with a glass rod. Slow stirring was then continued for 12 hr. at 37° C. in the presence of 0·5 mgm. 'Pronase'[14], a broad spectrum protease from *Streptomyces griseus* (Calif. Corp. Biochem. Res Lot. No. 109080). This was followed by centrifugation at 20,000*g* for 1 hr. After the supernatant was set aside, the pellet was resuspended in saline–citrate and 'Pronase', as above. This was stirred for 8 hr. at 37° C. and then treated exactly as before. A third cycle involved stirring for only 4 hr. The 3 supernatants were then combined and the DNA was sedimented in a Spinco *SW* 39 rotor at 35,000 r.p.m. for 5 hr. Thus, in contrast to the original method[12], the periods of incubation were generally longer, 'Pronase' was used instead of trypsin, and enzymatic digestion was carried out at 37° C. (at 4° C. only about 20 per cent of the total amount of DNA was released).

The sedimented DNA, its supernatant, and the final pellet containing disrupted vaccinia (the latter hydrolysed with 5 per cent TCA for 15 min. at 90° C. (ref. 14)) were analysed by the method of Keck[15]. Typical DNA content of these fractions was 96, 1, and 3 per cent respectively of the total contained in the virus. That vaccinia contains 5·6 per cent DNA[4] seems to be substantiated from our calculations based on the amount of DNA extracted in the present experiments from weighed samples of dried vaccinia. A typical nucleic acid absorption spectrum (260/280 mμ ratio of 2·0) was obtained from the mercaptoethanol-released DNA. The purity of this DNA was also established by following its thermal transition (temperature versus absorbance at 260 mμ)[1,16]. There was no detectable increase in absorbance up to 75° C., but thereafter a sharp rise in absorbance occurred with the midpoint of this rise, or T_m, at 84° C. This indicated that the bulk of the DNA was double-stranded. The average hyperchromic effect was about 25 per cent. Further treatment of the DNA with dupanol[11] or phenol[1] altered neither the absorption spectrum nor the T_m.

Base ratio analysis of the DNA was carried out by hydrolysis with 90 per cent formic acid (175° C. for 30 min.)[10], followed by chromatography using Whatman No. 1 filter paper and the standard isopropanol–water–hydrochloric acid solvent system[10]. A short wave-length 'Mineralight' was used to locate the ultra-violet absorbing spots, which were then eluted with 0·1 *N* hydrochloric acid. The absorbance of each base was determined in the Beckman *DU* spectrophotometer, and their concentration was calculated from the values of Bendich[17]. The mole per cent (based on two determinations) of adenine, thymine, guanine, and cytosine was 30, 31, 20, and 19, respectively. The guanine–cytosine content of 39 is consistent with that value predicted[16] by the T_m, and with that previously reported for vaccinia[10].

It would be of interest to determine whether the nucleic acid released by 2-mercaptoethanol contains a small fraction of single-stranded DNA, and, if so, whether this fraction is similar to that extracted from the virus by phenol. Assuming that the single-stranded DNA extracted in phenol is not produced by denaturation, as seems likely from past experience[18] and from our finding that the double-stranded DNA extracted with 2-mercaptoethanol has the same T_m after phenol treatment, then it seems possible that phenol preferentially extracts only one type of DNA from vaccinia (that is, single-stranded). Thus each virus particle may contain both types of DNA, or, alternatively, there may be two classes of virus particles, the majority containing double-stranded DNA and the remainder, possibly incomplete, containing single-stranded DNA. Since the presence or absence of a small single-stranded fraction in the DNA isolated by mercaptoethanol cannot be established by the thermal denaturation curves obtained, or from the base ratios, further experiments designed to clarify this point are in progress.

The failure of repeated attempts in our laboratory[19] and elsewhere (Fenner, F., personal communication) to isolate infectious DNA from vaccinia by the standard phenol procedures may well be due to the fact that phenol removes a relatively small atypical sample of the total DNA from the virus. Thus phenol may not be suitable for the preparation of infectious nucleic acids from certain large viruses, although it has been the standard reagent used for this purpose with many small DNA and RNA animal viruses[2]. Following our experience with vaccinia it seems that 2-mercaptoethanol might be worth investigating as a means of releasing nucleic acids, and possibly infectious nucleic acids, from other viruses. Preliminary attempts to demonstrate infectious DNA from vaccinia by this method have so far been unsuccessful.

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Effect of Irradiation on Poliovirus Yield of Cultured Kidney Cells of the Rhesus Monkey

ALTHOUGH X-irradiation of established lines was followed by the formation of giant cells[1], irradiation of primary cells was not[2]. Irradiation of cells also consistently increased the poliovirus yield per cell in the established lines, but not in primary monkey kidney, which suggested a relationship between the formation of giant cells and the yield of virus[3]. This communication demonstrates that irradiated monkey kidney cells which consistently form giants also yield more poliovirus per cell.

Giant cells were obtained consistently when cell suspensions were prepared from monolayers of primary monkey kidney and were irradiated at the time of plating. For these experiments, monolayers of primary rhesus monkey kidney were treated with 0·05 per cent trypsin (Difco, 1 : 250) containing 0·15 per cent disodium dihydrogen 'Versinate' (Dow Chemical Co. Trade name for disodium salt of ethylenediamine tetraacetic acid dihydrate). The cells were distributed into 50-mm. Petri dishes (plastic) in a 20 per cent calf-serum lactal medium[3], at a level of 1×10^5 or 3×10^5 cells per dish, and were subjected to 1,500–2,000 r. of X-ray from a Westinghouse Industrial X-ray machine operating at 140 kV. and 9 m.amp., filtered with 0·25 mm. copper and 1 mm. aluminium. Control monolayers prepared at the same time were seeded with 5×10^4 or 1×10^5 non-irradiated cells. All plates were incubated at 37° C. in an atmosphere of 4 per cent carbon dioxide in air. The medium was renewed every 2–3 days until the plates were infected. Then the growth medium was removed, and the monolayers were washed once with phosphate-buffered saline[4], infected with 0·5 ml. of Leon strain type 3 poliovirus (titre: $1 \cdot 2 \times 10^8$ per ml.) in 0·5 ml. of phosphate-buffered saline, incubated for 1–1½ hr. at 37° to permit virus attachment, washed 3 times with phosphate-buffered saline, overlaid with Eagle's medium[5] enriched with 2 per cent calf-serum, and returned to the carbon dioxide incubator. When the cells had been destroyed, monolayers were collected.

For the counting of virus yields, cell suspensions were prepared at the time of infection from representative irradiated and non-irradiated monolayers, as described. Table 1 summarizes the results of several experiments in which monolayers were infected 6 or 7 days after plating. In all cases the irradiated cells yielded more virus.

Table 1. POLIOVIRUS YIELDS OF RHESUS MONKEY KIDNEY CELLS NON-IRRADIATED AND IRRADIATED ON THE DAY OF PLATING

Time of infection (No. days after plating)	Exp. No	Virus yield (plaque-forming units per cell) Non-irrad.	Irradiated
6	1	195	1,200
	2	160	425
	3	380	1,000
7	1	120	540
	2	208	1,150
	3	150	640

Since microscopic examination of the plates revealed that almost all cells were enlarged, an experiment was designed to determine the size of the irradiated and non-irradiated cells 6 and 7 days after plating. For calculation of cell volume, the monolayers were trypsinized and the diameters of the first 10 cells in each set were measured with a calibrated ocular micrometer. Both the major and the minor axes of ellipsoidal cells were measured. Since in the series studied the axial ratio never exceeded 1 : 4, the volumes were calculated as though the ellipsoidal cells were spheres with diameters equal to the mean of their major and minor axes. This resulted in an over-estimation of volume of no more than 25 per cent. Table 2 gives the mean volumes obtained, with their standard deviations. Irradiation obviously resulted in an increase in cell volume, as well as an increased poliovirus yield approximately equal to this increase in volume. A similar correlation for X-irradiated HeLa cells, which formed giants and yielded more Newcastle disease virus, was reported by Marcus and Tolmach[6].

Apparently the correlation between the development of large cells and an increase in virus yield, which was observed in non-irradiated cells by Dunnebacke and Reaume[7], applies also to irradiated cells.

Table 2. MEAN VOLUMES (WITH STANDARD DEVIATIONS) OF RHESUS MONKEY KIDNEY CELLS, NON-IRRADIATED AND IRRADIATED ON THE DAY OF PLATING

No. of days after plating	Cell volume Non-irradiated	Irradiated
6	$(1 \cdot 68 \pm 0 \cdot 82) 10^4 \mu^3$	$(9 \cdot 6 \pm 1 \cdot 71) 10^4 \mu^3$
7	$(1 \cdot 75 \pm 0 \cdot 57) 10^4 \mu^3$	$(7 \cdot 45 \pm 1 \cdot 35) 10^4 \mu^3$

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SOIL SCIENCE

Free Monosaccharides in Soil Organic Matter

IN view of the vital importance of carbohydrate fraction of soil organic matter on physical and biochemical properties of soils, a number of workers[1–4] have elucidated the composition of soil polysaccharides by their work on the hydrolysates. The consensus of opinion is that the sugar residues comprise at least 5 per cent of the soil organic matter and the polysaccharides are composed of galactose, glucose, mannose, arabinose, xylose, and rhamnose. Brown, Robinson and Johnson[5] examined the effect of D-xyloketose on the roots of plants and observed that even a minute concentration of the carbohydrate can perform important physiological functions. The present work was designed to determine the presence of free monosaccharides in soils.

Four widely different pedological, vegetational and climatic type soils, namely, a half-bog soil from southern Delaware (Pocomoke), a prairie soil from Illinois (Sable), a red-yellow podzol from North Carolina (Cecil), and a tropical latosol from Brazil (samples passing through 60 mesh sieve) were used for the investigation. 1-kgm. samples of soil were extracted with 1 litre of 80 per cent alcohol five times at room temperature. The filtrates were concentrated under vacuum at 45° C. and reduced to dry residue by lyophilization. The residue was extracted with three 10-ml. portions of dry pyridine, and the pyridine was removed under reduced pressure. The pyridine-soluble material was extracted with 5 c.c. of 50 per cent aqueous isopropanol and centrifuged for 15 min. at 2,000 r.p.m. The supernatant was dried *in vacuo* at 45° and the residue taken up in 5 ml. of 50 per cent isopropanol. The centrifuging, decanting and drying steps were repeated a number of times. The residue was extracted with 1 ml. of 50 per cent aqueous isopropanol and was then used for the qualitative chromatographic determinations. 25λ of this solution was spotted for paper chromatography against known solutions of monosaccharides. The solvent was 4 : 1 : 5 *n*-butanol/acetic acid/water (organic phase in trough and aqueous phase in chamber). Ascending chromatography was used and the dried chromatograph was dipped in 2 per cent aniline trichloroacetate (in glacial acetic acid). This was air-dried and then oven-heated (110° C. for 5 min.). A similar determination of sugars was also made by the descending paper chromatographic technique using pyridine, butanol, glacial acetic acid and water (6 : 4 : 1 : 3) as solvent. The concurrent results by aforementioned two techniques are given in Table 1.

Table 1. FREE MONOSACCHARIDES PRESENT IN FOUR TYPICAL SOILS

Monosaccharides	Pocomoke soil	Sable soil	Cecil soil	Brazil soil
Galactose	—	–	–	—
Glucose	+	+	+	+
Mannose	–	–	–	–
Arabinose	–	–	–	–
Xylose	–	–	–	–
Fucose	–	–	–	–
Rhamnose	–	–	–	–

Sable soil and Brazil soil have two more same unidentified monosaccharides.

The present results indicate that common sugars exist in free condition in soil organic matter. It is very significant to note that glucose was observed in all the four samples. Further work is in progress on the determination of these sugars in widely different soil samples and to investigate the importance of this observation.

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Alteration in the Surface Properties of Soils by Ion Exchange Resins

WHEN soils suspended in glass-distilled water were labelled with phosphorus-32, the rapidly exchanging fraction of the isotopically labile phosphate reacted during the first 10 hr. according to a first-order mechanism, either by 'film' diffusion or by 'mass action'. Suspensions of the soils with mixed resins (Na^+ : 'Zeokarb 225', and Cl^- : 'Amberlite *IRA* 401') behaved quite differently. The rate at which this fraction of the soil phosphate was exchanged by chloride ions from the strongly dissociating resin exchanger was governed by 'bulk' or 'intra-particle' diffusion during 4 days. The mixed resins removed little or none of the remaining 'slowly' exchangeable part of the isotopically labile soil phosphate. (In the absence of resins, this 'slow' fraction exchanged isotopically according to a single 'bulk' diffusion mechanism.) Phosphate diffusion within the resin particle was not the factor controlling the rate of exchange in mixed suspensions of soil and resin because exchange equilibrium was reached in less than 15 min. with Cl : 'Amberlite *IRA* 401' alone in phosphate solutions.

A typical soil in water exchanged 4·8 mgm. of phosphorus per 100 gm. soil isotopically during 10 hr by first order kinetics. When reacted with the 'mixed' resin system the soil exchanged a maximum of 7·3 mgm phosphorus for Cl^- ions, of the same specific activity as the 'rapid' isotopically labile phosphate, in 4 days by 'bulk' diffusion kinetics. The apparent diffusion coefficient, D_i, for the movement of phosphate ions through soil particles of weighted mean diameter 2·9μ in the clay + silt fraction was 3·0 ± 0·5 × 10^{-10} cm.^{2} hr.$^{-1}$.

This evidence suggests that poly-electrolyte anions, products of the chemical and abrasive decomposition of resins, are formed when resin and soil are suspended in water. Phosphate ions held on the soil surface initially must diffuse to the soil solution through multi-layers of such surface-active poly-electrolyte anions on the soil. This leads to the large differences observed in both the mechanisms and the specific rate of exchange of phosphate ions in soil suspended in water with and without resins.

Anomalous surface effects on glass in conductivity water, prepared by using 'mixed bed' ion-exchange resins, have been attributed to weakly basic poly-electrolytes released from the resins[1]. These contaminants appear to be removed by passing through 'Millipore' filters[2], but their size prevents them from penetrating resin particles of opposite charge[3]. Similarly, non-chelating anions like monovalent phenyl-barbiturate 'block' soil pores and decrease the rate of isotopic exchange of the soil phosphate[4].

Increasing use is being made of resins to extract nutrients from the soil and to supply nutrients to plants in studying plant nutrition. Our work indicates that caution is needed in interpreting results from such experiments especially when considering rates of reaction and rates of uptake by plants.

In the example given here the 'slow' fraction of the isotopically labile phosphate, 12·8 mgm. phosphorus per 100 gm. soil, exchanged isotopically during 10–288 hr. with an internal self-diffusion coefficient, D_i^*, equal to 0·8 ± 0·1 × 10^{-10} cm.^{2} hr.$^{-1}$. This is a quarter of the diffusion coefficient obtained by 'mass flow' in the resin : soil system for the phosphate exchanging for Cl^- ions, suggesting that little of this 'slow' fraction would be extracted by the 'mixed' resins.

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PSYCHIATRY

Isolation and Characterization of a Compound from the Urine of Schizophrenics

THE systematic screening of schizophrenic and normal urine in this laboratory has led to the identification of a biologically active compound in urine of schizophrenics. This compound is of particular interest since it does not have the usual sympathomimetic actions of the closely related catecholamines, but does have a depressant effect on the central nervous system[1].

Samples of urine were collected from a series of 19 schizophrenic patients and 14 normal subjects. Neither patients nor controls had been treated medically for at least 48 hr. and had received no tranquillizing drugs for a minimum period of two weeks. The diagnosis of schizophrenia was confirmed by two psychiatrists. The normal controls were selected from the surgical wards and hospital staff. The urine was customarily collected for a period of 20 hr., from noon–8 a.m., and was analysed immediately after the collection. Occasional urines were successfully refrigerated for 48 hr.

A creatinine determination was carried out initially and a volume of urine equivalent to 300 mgm. of creatinine constituted a sample. The urines were treated with barium chloride until no further precipitate formed. The sample was adjusted to 500 c.c. with distilled water and the urine was acidified to *p*H 2 with concentrated hydrochloric acid. Some samples were hydrolysed at this *p*H for 30 min. at 100° C. without effect on the unknown. The sample was then extracted three times with chloroform to remove interfering compounds. The aqueous portion was adjusted to *p*H 9 with 2 *N* sodium hydroxide and the urine was extracted three times with chloroform. These extractions were done quickly inasmuch as the unknown was readily oxidized in base. The organic phase was dried with anhydrous sodium sulphate and evaporated to suitable volumes for application for paper chromatography (an equivalent of 100 mgm. creatinine per spot). The chromatography was developed in butanol/acetic acid/water (4 : 1 : 1) for a period of 16 hr. and the compounds were located with ninhydrin-pyridine reagent. A blue-purple spot appeared repeatedly at R_F 0·60 which was not observed in extractions from samples of normal urine. Further investigations revealed that the application of a modified Ehrlich's reagent (2 gm. *p*-dimethylaminobenzaldehyde dissolved in 90 c.c. acetone and 10 c.c. concentrated hydrochloric acid) to the strips over the purple spot produced by dipping in ninhydrin-pyridine resulted in an immediate pink spot turning to orange on standing. This colour corresponded to the purple ninhydrin spot at R_F 0·60. In this test the strip was first dipped in ninhydrin-pyridine reagent (9 : 1), dried and heated at 80° C. for 1 min. at which time the ninhydrin colours appeared. The strip was then dipped in the modified Ehrlich's reagent. The appearance of a pink or orange spot after the purple ninhydrin spot fades is specific for β-phenylethylamine and other β-phenylethylamino compounds with a free amino-group and substituted only on the ring[2]. These compounds do not react with Ehrlich's reagent alone. Further characterization of the unknown spot revealed that it had an R_F of 0·79 in isopropanol/ammonia/water (200 : 10 : 20). It also produced a purple colour with ninhydrin-acetate reagent and did not fluoresce in the ultra-violet after this treatment. It should be noted that there is occasionally present a purple ninhydrin-pyridine reacting spot slightly slower but not always separated from the unknown. This interfering material has a strong red fluorescence in ultra-violet light: (360 mμ) after reaction with ninhydrin-pyridine and produces a blue colour rather than pink with modified Ehrlich's over ninhydrin.

The unknown did not react with diazotized sulphanilic acid reagent, nitraniline or 2,6-dichloroquinone chlorimide-borate buffer. It also resisted hydrolysis with hydrochloric acid at *p*H 2 for 30 min. These reactions demonstrate that the compound is not phenolic in character nor is it a conjugated phenol. The unknown substance produces a pale brown colour when the strip is sprayed with iodine in chloroform, and does not react with *p*-anisidine amyl nitrite reagent.

After a study of the β-phenylethylamines the above reactions were found to be consistent only with those for authentic 3,4-dimethoxyphenylethylamine (K. and K. Laboratories). This compound has the same R_F values as the unknown substance in the solvent systems described. Also, when 3,4-dimethoxyphenylethylamine is chromatographed with the unknown substance, it enlarges the unknown spot by an appropriate amount.

A dinitrophenyl derivative was made from the unknown substance and the reference compound and chromatographed in methanol/water (8 : 2). The R_F for the dinitrophenol-derivative of both the unknown and the reference was 0·60. Both the unknown and the reference were oxidized to 3,4-dimethoxyphenylacetic acid with potassium permanganate. The R_F of the unknown substance and the reference both coincided with authentic 3,4-dimethoxyphenylacetic acid (butanol/acetic acid/water, 4 : 1 : 1, R_F 0·91).

Identical spectra were obtained from hydrochlorides made from both the unknown and the reference compound (absorption max. 234 mμ and 279 mμ, water).

Thus far, we have demonstrated the presence of a compound, 3,4-dimethoxyphenylethylamine, in the urine of 15 patients with a confirmed diagnosis of schizophrenia. In 2 additional patients a substance which had a bright red fluorescence in ultra-violet, after the application of ninhydrin-pyridine, interfered with the procedure. In 2 patients 3,4-dimethoxyphenylethylamine could not be detected. As yet we have not been able to identify this compound in the urine of the normal subjects examined.

It is necessary that these results be interpreted with considerable caution at the present time. Since we have succeeded in identifying the unknown compound, we plan to investigate additional normal controls inasmuch as we are now able to formulate more specific and sensitive methods. Also, many other possible experimental variables remain to be examined. If this compound does in fact represent an abnormal metabolite it would suggest the presence of a 4,*O*-methyltransferase in schizophrenic patients. It has already been demonstrated that 3,4-dihydroxyphenylethylamine can be metabolized by the action of 3,*O*-methyltransferase in brain homogenates[3]. Recently it has also been shown that 3,4-dihydroxyphenylethylamine can undergo 4,*O*-methylation in liver homogenates *in vitro* in the presence of *S*-adenosyl methionine[4]. We are conducting further investigations to determine the significance of 3,4-dimethoxyphenylethylamine and its possible enzymatic origin.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, June 4

University of London (at the London Hospital Medical College, Turner Street, London, E.1), at 5.15 p.m.—Prof. F. Halberg (University of Minnesota Medical School): "External and Internal Timing in Organisms".*

Monday, June 4—Thursday, June 7

British Electrical Power Convention (at the Dome, Brighton)—Fourteenth Annual Meeting. Theme—"The British Electrical Industry in the World Scene".

Tuesday, June 5

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

University of London (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof J. S. Webb· "Applied Geochemistry in Mineral Exploration" (Inaugural Lecture).

Science Forum (at Friends House, Euston Road, London, N.W.1), at 7.30 p.m.—Prof. J. Rotblat: "The Pugwash Conferences".

Wednesday, June 6

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. A. E. Mourant. "Blood Groups, Populations, and Races".*

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. F. G. H. Blyth: "The Structure of the North-eastern Tract of the Dartmoor Granite".

British Nuclear Energy Society (at the Institution of Civil Engineers, 1–7 Great George Street, London, S.W.1), at 5.30 p.m.—Dr. J. M. Kay and Dr. S. C. Curran: "Nuclear Education" (two papers).

University of London (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. John Chadwick: "The Decipherment of Linear B—The First Ten Years".*

Women's Engineering Society, London Branch ((at "Hope House", 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Annual General Meeting.

Thursday, June 7

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof F. S. Dainton, F.R.S., and Mr. D. B. Peterson: "Forms of H and OH Produced in the Radiolysis of Aqueous Systems"; Mr. M. Z. Hoffman and Prof. G. Porter, F.R.S.. "Radiationless Conversion from the Triplet State".

Mineralogical Society (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—General Meeting.

University of Leeds (in the Arts Lecture Theatre, The University, Leeds), at 5.15 p.m.—Prof. P. P. Ewald, F.R.S. (Polytechnic Institute of Brooklyn): "William Henry Bragg and the New Crystallography"* (First Bragg Lecture).

Institution of Polish Engineers in Great Britain, Group for Economic, Social and Management Studies (at 55 Princes Gate, Exhibition Road, London, S.W.7), at 7 p.m.—Mr. M. C. C. M. d'Abranyi. "English Versus Continental Attitudes in Education".

Chemical Society (at Burlington House, Piccadilly, London, W.1.), at 7.30 p.m.—Scientific Papers.

Friday, June 8

Biochemical Society (in the Department of Biochemistry, The University, Edinburgh), at 10 a.m.—Scientific Papers.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Head (with an honours degree in mathematics, suitable teaching experience, and preferably industrial or research experience in a related field) of the Department of Mathematics (Grade III), Enfield Technical College, Queensway, Enfield, Middlesex—The Chief Education Officer (F.1), Middlesex County Council, 10 Great George Street, Westminster, London, S.W.1 (June 7).

British Nylon Spinners Post-doctoral Fellowship in Physics—The Registrar, The University, Bristol 8 (June 8).

Lecturer (with a good honours degree in physics or electrical engineering, together with experience in the field of communications, rather than in electronics) in Telecommunications—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (June 8).

Demonstrator in Agriculture, for duties on the University Farms—The Registrar, The University, Leeds 2 (June 9).

Demonstrator or Assistant Lecturer (medically qualified and/or a good honours degree in physiology) in Physiology—The Registrar, The University, Manchester 13 (June 9).

Lecturer in Theoretical Chemistry—The Secretary of University Court, The University, Glasgow (June 9).

Lecturers (2) in the Department of Mathematics—The Secretary, The University, Edinburgh (June 9).

Research Assistant (with postgraduate experience of research in mycology and plant pathology, preferably to Ph.D. level) in the Department of Botany, to participate in a research project in plant pathology which is supported by a grant from the Agricultural Research Council—The Registrar, The University, Manchester (June 9).

Lecturer or Assistant Lecturer in Analytical Chemistry—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (June 13).

Lecturer (with a good honours degree, and preferably a special interest in the design aspects of digital computers, or in low temperature developments) in Electrical Engineering—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 15).

Lecturer in Philosophy—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (June 16).

Lecturer or Assistant (with special interests in economic statistics and econometrics) in the Department of Statistics—The Secretary, The University, Aberdeen (June 16).

Research Assistant in Physiology—The Registrar, The University, Sheffield (June 16).

Research Assistant (science or medical graduate) in the Department of Physiology, for the study of contact inhibition and invasiveness of malignant cells—The Registrar, The University, Bristol (June 16).

Lecturer or Assistant Lecturer (Probationary) (man or woman graduate) in Psychology—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 22).

Assistant Lecturer in Pure Mathematics—The Registrar, The University Sheffield (June 23).

Chair of Geology; and Lecturer or Assistant Lecturer in Geology at the University College Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 23).

Senior Lecturer or Lecturer (preferably specialist in pure mathematics) in the School of Mathematics, The University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (June 29).

Lecturer in Mathematics at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

Lecturer in Physics at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, June 30).

Senior Lecturer or Lecturer in Mathematics at Wollongong University College, The University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C 2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (June 30).

Senior Lecturers or Lecturers (with a special interest in agronomy, animal husbandry, agricultural botany, agricultural zoology, agricultural chemistry or soil science) in the Faculty of Agriculture, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 30).

Teaching Fellow (with an honours degree or equivalent qualification) in the School of Chemistry, University of New South Wales, to undertake research for a higher degree, preferably in organic chemistry, and also some teaching duties—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (June 30).

Professor or Senior Lecturer (with a first degree in agriculture, or preferably horticulture together with M.Sc. and/or Ph. D. degrees, with preference for a Ph.D. in a strictly horticultural subject) in Horticulture at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 30).

Lecturer (with a science degree specializing in geology, and research experience to Ph.D. or comparable level) in Geology (Palæontologist) at the University of Queensland, Brisbane—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, July 31).

Assistant Master to teach Chemistry up to G.C.E. "A" and "S" levels—The Headmaster, Wrekin College, Wellington, Shropshire.

Chemist (with a first- or good second-class honours degree in chemistry, or an equivalent qualification, and preferably experience in the inorganic analytical field and a knowledge of geochemical techniques) in Nyasaland, to take charge of the Geological Survey Department's chemical laboratory and to undertake assaying and chemical testing of rocks, including full silicate rock analyses—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. *T*/RC 218/110/01.

Lecturer, and an Assistant Lecturer (Grade B) to teach Chemistry mainly to G.C.E. "A" level and Ordinary National Certificate courses—The Principal, Cambridgeshire College of Arts and Technology, Collier Road, Cambridge.

Lecturer (man or woman, with good academic qualifications, and preferably with experience in schools) to teach General Science and Biology—The Principal, Avery Hill Training College, Bexley Road, Eltham, London, S.E.9, quoting Ref. TS.3/T/1109/5.

Master to teach Mathematics, mainly to Sixth Form—The Headmaster, Latymer Upper School, London, W.6.

REPORTS and other PUBLICATIONS

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Great Britain and Ireland

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SALARIES AND THE EXPANDING BRITISH UNIVERSITIES

THE motion which was moved by the Leader of the Opposition, Mr. Hugh Gaitskell, in the House of Commons on April 5 was concerned with two distinct but related issues arising out of the statement which the Chief Secretary to the Treasury, Mr. H. Brooke, made on March 14. The rejection of the recommendations of the University Grants Committee regarding the provision for university expansion raises the whole question of the scale of university expansion in the quinquennium 1962–67 and indeed of higher education in general in that period. The statement regarding the salaries of university teachers bears no less closely on that expansion, because, even before the statement from the Exchequer, the limiting factor in the rate and scale of university expansion was not so much the financial resources available as the ability of the universities to recruit teachers of the requisite capacity and to retain their services in the face of competitive demands.

To look first at this question of university staff may perhaps help to put the problem of university expansion and its finance in proper perspective, particularly as even the debate on April 5 scarcely removes the doubts expressed in the universities that neither Parliament nor electorate is fully convinced of the importance of the universities or appreciates their significance in relation to national survival. Moreover, the question of recruiting and retaining staff touches closely a further matter which in recent months has been causing serious concern outside as well as within the universities—the question of the scale and balance of university research. The research effort of the universities is a matter of staff as well as of facilities and the two are largely interdependent.

It is, in fact, the more important that the implications of the Government decision not to authorize an increase of more than 3 per cent in the bill for academic salaries at present, although the scales will be reviewed afresh next year, should be clearly understood in that, as Mr. Gaitskell rightly said, university teachers do not much like getting involved in politics and are normally disinclined to make a fuss about salaries and conditions of service. Indeed, even now, the dissatisfaction of the teachers themselves seems to centre as much on the disagreement between the Government and the University Grants Committee revealed in Mr. Brooke's statement as on the treatment of their claim for an increase in salaries. Mr. Gaitskell, nevertheless, showed clearly that it is the very moderation and restraint with which this claim, originally advanced in November 1959, for an increase of 15–20 per cent has been put forward, although in the meantime further increases have been awarded both in the Civil Service and to lecturers in technical colleges, that has put them in a relatively unfavourable position. The University Grants Committee, after hearing evidence, agreed to present a further claim on behalf of the university teachers in April 1961, and only submitted the claim in July 1961, as was indicated in the Committee's survey published last September.

It is in these circumstances that it is difficult to accept the Government's rigid application of the pay pause or to entertain the confidence which Mr. Brooke expressed on April 5 that neither recruiting nor staff numbers would suffer. Mr. Brooke himself admitted that the university teaching staff had been treated less generously than teachers, and that the former stood at some disadvantage in the negotiating arrangements. He could, however, scarcely have hoped to command assent to his view that the universities now offer a much better career prospect than the colleges of technology.

Nevertheless, there is substance in the argument that rigid comparison between the remuneration of different posts is full of difficulties, and it is hard to say what is proper remuneration of high intellectual attainment in any sphere. University salaries are unlikely ever to compete in such terms with posts in industry, and in some respects university posts are unique and owe their attractiveness to the nature of the work and conditions. If, however, the basic remuneration falls below a certain level that attraction ceases to function, and it is a key contention in the present situation that the starting level for an assistant lecturer of £800 or £1,050 for a lecturer, compared with £1,600 in a college of technology has been allowed to fall below the point at which that attraction can exert its normal and reasonable effect.

Mr. Brooke claimed indeed that in recent years recruitment to the higher ranks of the Civil Service had been feeling the competition of the attraction of a university appointment, rather than vice visa; but there is no evidence one way or another in the recent report of the Civil Service Commissioners. Moreover, it is all to the good that there should be a certain amount of interchange, as was strongly emphasized in the Zuckerman Report, and from that point of view alone, the gap between scales in the Civil Service and in the universities should not be allowed to become too wide. Further, in reaffirming that the 3 per cent increase in salary bill had been fixed in accordance with national considerations which extended far beyond the position of the universities, Mr. Brooke added that, while it was not for the Government to say how the 3 per cent increase should be distributed, the Government was prepared to accept the recommendation of the University Grants Committee that the increase should be concentrated on lecturers and assistant lecturers.

In concluding the debate on April 5, the Financial Secretary to the Treasury, Sir Edward Boyle, pointed out that it should not be supposed that the cost of salary increases had to be met out of the ordinary recurrent grant. During the present quinquennium, earmarked grants for salaries had already been made twice and the present award would require a further addition to the £8·75 million earmarked in the present year. He agreed that the real test of a university salary scale must be whether it attracts and retains the right numbers of staff. There was nothing else like universtiy work, and one could not arrive at right figures for salaries simply by means of external comparison.

Sir Edward Boyle spoke at the end of a debate in which, however, not only Mr. Gaitskell but also other speakers, such as Mr. J. Grimond, Mr. F. Mulley and Mr. F. Willey, had quoted instances of the universities losing staff to the colleges of technology as well as overseas. Mr. Willey, in particular, referred to the evidence recently submitted to the Robbins Committee by the Institute of Physics and the Physical Society on the loss of physicists to the United States and Canada. Apart from the list of some seventy British physicists now holding senior permanent appointments in America appended to this report, the report supplied disturbing evidence that conditions in British universities were for the first time failing to attract physicists of the front rank and that at a time when the projected expansion called for a net annual increase of some seventy-five physicists.

This is partly due to insufficient salaries, but only partly in the sense that is represented by the 3 per cent award. It arises to some extent out of the low proportion of senior posts, and this is a problem that affects the science departments at least generally. Sir Howard Florey put the point plainly in his anniversary address to the Royal Society on November 30, 1961. It is the promotion prospects that, coupled with the low salaries for lecturers and assistant lecturers, are responsible for the present anxiety over the staffing of British universities, and this anxiety found free expression at the discussion on the university teaching profession at the Home Universities Conference last December. Dr. Chilver flatly questioned whether it was reasonable to accept a proportion of 30 per cent of all staff for professors and other senior staff, and pointed out that the proportion of professors throughout the universities appeared to be only three-fifths of what it was before the Second World War. Prof. R. L. Brett's analysis of the returns from the University Grants Committee suggested that the chances of a lecturer or assistant lecturer becoming a professor were only about half what they were in the pre-war years: the ratio had fallen from 1 professor to 1·7 lecturers in 1937–38 to 1 to 3·5 in 1959–60.

The difficulty is not simply one of wide discrepancies in prospects between one faculty and another, but also avenues of promotion being blocked by age structure and mobility between one university and another being restricted. Mr. D. W. Reece, of the Association of University Teachers, was obviously most disquieted about the situation in December, and a memorandum submitted in March by the Manchester Local Association to the Robbins Committee argued that, quite apart from the widening of the gap between university salaries and those in comparable professions, the barriers to promotion and the 2: 7 ratio of senior to junior posts were already responsible for some migration of staff. Nor is it very likely that the development of the new university institutions will improve the situation, and, in any event, it must be remembered that, altogether apart from the salary scales in force, the universities have to budget for the appropriate rises on those scales in their recurrent expenditure each year. Taken with the automatic increase in expenditure on non-academic salaries—administrative, technicians-clerical, maintenance and the like—arising out of scales over which the universities have no control, as well as out of deliberate efforts to improve conditions which are responsible for waste of highly trained manpower and in which the universities often compare badly with research institutions either in Government or in industry—much of the 55 per cent increase in the Government recurrent grant will be absorbed in such ways before the universities can begin to plan to meet the needs of 35 per cent more students. The universities are already seriously considering, for example, a marked increase in the number of technicians available for the science departments.

This aspect was virtually ignored by both Mr. Brooke and Sir Edward Boyle from the Government side, although it was strongly put by Mr. G. Johnson Smith in the debate, who pointed out that the universities were facing a most significant rise in unavoidable charges, which were likely to absorb the extra money and leave none available for expansion. He welcomed Mr. Brooke's statement that if building costs became inflated the Government intended to insulate the universities by increasing non-recurrent grants for the building programmes, but this appears to be the only point at which the Government appears to have faced fairly the full implications of the request to the universities to provide for an expansion to 150,000 students by 1966–67. Even so, it seems optimistic to assume that any adjustment of the non-recurrent grants previously arranged is contemplated sufficient for the rise in costs which has already occurred and to falsify the belief of the Committee of Vice-Chancellors and Principals that the building programme to increase the number of places to 150,000 cannot be completed by 1966–67.

Apart from this, however, the Vice-Chancellors and Principals do not believe that this target can be achieved by that date with the limited recurrent grants now proposed by the Government. To disregard the other obligations of the universities in an attempt to come as near as possible to this target would inevitably result in a wholly unacceptable deterioration of standards, not only in staff–student ratios and research but also in academic and residential accommodation. The staff–student ratio has some bearing on the general question of staff, for the full impact of the salary situation cannot be appreciated except in the context of the past decade or more—a period of unprecedented expansion in which university teachers have often been desperately improvising and looking forward for improved conditions in which fuller justice could be done to the university ideal. Still more is this true of research, and here one of the keenest notes of anxiety in the universities is as to the effect of the Government decision on the scale and quality of university research.

Before the decision was announced, concern had been freely expressed within and outside the universities as to the adequacy of the support provided for academic research, and some fear expressed that the coming quinquennium might be unduly stringent. In the debate on April 5, the Government was taxed with unloading research on the universities from the Department of Scientific and Industrial Research, the Agricultural Research Council and the Medical Research Council. Neither Mr. Brooke nor Sir Edward Boyle replied to this charge, which can perhaps well await the report of the inquiry into the organization of civil research which the Minister for Science has already implemented. Meanwhile, however, the need for the universities to have available adequate funds for research in their own right cannot be too strongly emphasized, and this is a vital factor in their ability to recruit and attract staff of the highest quality.

The scale and quality of the research facilities available are a dominant factor in building up a university science department of high quality as is well brought out by the Institute of Physics and the Physical Society in their evidence to the Robbins Committee, and it would be foolish to deny that it affects the migration to the United States of leading scientists. None the less, it is difficult to assess how serious is such migration. Probably no salary scale that is practicable in British universities can have much effect provided the scale does not compare too unfavourably with those existing in comparable professions in Britain. The nature of the facilities available and the scale of financial support are far more likely to be determinants. It would appear that the Government has given far too little weight to the need for the universities to increase the scale of their research effort in asking them to attempt to increase the number of students by 35 per cent with the recurrent grants now announced.

While there is substance in the charge that the Government in willing the end has failed to will the means, there is rather more to be said for the Government than many of its critics have conceded, and probably the most serious charge to be brought against the Government is that it has not been entirely honest. As Mr. Brooke quite rightly emphasized, the final responsibility for deciding how much Britain can spend on university education and on higher education generally must rest with the Government: it cannot be bound to accept the advice of the University Grants Committee in that respect. It is bound, however, to ensure that the Committee does not spend its time unprofitably, and when for good and convincing reasons the Government believes that economic circumstances put a strict limit on the nation's expenditure in that direction, good management would have suggested that the University Grants Committee should have been advised before and not after they formulated their quinquennial proposals.

It was also appropriate that both Sir Edward Boyle and Mr. Brooke should point out that university expansion does not provide the only means of providing increased opportunities in higher education. It is too readily assumed that a university education is necessarily the best means of developing the talents of the gifted adolescent. Quite apart from the 22,000 places which will be available in the colleges of technology, other institutions of higher education have an important contribution to make, and the Robbins Committee is at present endeavouring to assess the relative significance of the various alternatives. Nor is the present structure of university education to be taken as sacrosant. Even without sacrifice of academic standards, it is possible that substantial economies in the cost per student could be achieved, quite apart from the use of new methods of teaching on which research is only now being prosecuted.

All this comes within the purview of the Robbins Committee, but in the meantime it is probable that, with the development of the new universities, the cost per student may rise appreciably in the near future. It is only right that the Government should have clearly in mind the possibility of the present expenditure on education rising to more than £1,000 million a year in a few years time and that university education should not be treated as something apart. What is not reasonable is that while these questions are being explored with what can only be assumed to be the fullest possible expedition, the Government should take action which not only threatens to prejudice the work of the Robbins Committee but also to cause frustrations and waste that endanger the effectiveness of expansion in any way.

This, however, is exactly what it is manifest that the Government's handling of the whole situation has done. They have failed either to take the University Grants Committee into their confidence or, noting an unequivocal warning which the Zuckerman Report addressed to them only last August, to hold their hand and take decisions on the basis of the Robbins report when it is received. In consequence, they have brought into question the adequacy of the administrative arrangements through the University Grants Committee which they profess to respect and have created feelings of anxiety and frustration among its members and the responsible authorities in the universities which can scarcely conduce to efficiency. Thus, there are grounds for asking whether the Government really believes that the universities and the colleges of technology, for example, are a real factor in industrial and economic development.

Nor is this all. However reasonable in theory the Government's objectives, the execution of that policy in practice could scarcely have been more inept. Besides bringing organization into disrepute, it has contrived to create feelings of frustration and exasperation among a body of responsible men and women whose whole attitude to questions of salary has been marked by restraint and patience. If this were not enough, it also threatens to cause further deterioration in the conditions of service and general attractiveness of academic life, which to many of them are far more determinant than salary. There is no sign that the Government even yet appreciates the seriousness of the situation or the urgency of, at the least, being ready to overhaul the scales of salary at an appropriate moment by the proper means with the utmost despatch. On the contrary, it appears all too likely that the situation is being allowed to drift while tendencies develop which it will be far from easy to dispel and which may prevent all hope of achieving anything like the target of university expansion which even the Government has set within the next decade. Plausible as were some, even most, of the arguments presented for the Government in the debate on April 5, they fail altogether to take account of the mischief wrought by the Government's own handling of the whole question—its refusal to recognize that it is the putting of pressure on the universities which limit their ability to deal according to their own judgment with the problems that confront them, its unwillingness to face the existing situation and provide what is required while the Robbins Committee provides the facts on which a long-term policy can be based, or to say honestly that it believes Britain to be too poor to provide for university expansion to meet the needs of an expanding population except by a drastic cut in standards both in the universities themselves and those who teach and work in them.

FABRICATION OF REACTOR FUELS

Fuel Element Fabrication with Special Emphasis on Cladding Materials

(Proceedings of a Symposium held in Vienna, May 10–13, 1960.) Vol. 1. Pp. xi + 538. 100*s*. Vol. 2. Pp. xii + 384. 71*s*. 6*d*.; 10 dollars; 260 Austrian schillings (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1961. Published for the International Atomic Energy Agency.)

THE primary functions of the cladding on a fuel element are to prevent (*a*) the escape of fission products into the coolant, and (*b*) attack of the fuel by the coolant. A most important factor, however, is to keep the ratio of the volume of canning material to that of the fuel as low as possible, and hence to reduce the parasitic loss of neutrons to a minimum while ensuring complete integrity of the can throughout the life of the fuel element. The object of the symposium, arranged by the International Atomic Energy Agency, was to review the work being undertaken at present on fuel elements and cladding materials in particular, to reduce costs in fuel-cycle and to improve the economics of atomic power.

Volume 1 of the proceedings contains twenty-eight papers from nine countries collected under two general headings "Characteristics of Cladding Materials" and "Fuel Element Fabrication". The subject-matter ranges over problems associated with the more popular canning materials, including stainless steel and alloys based on zirconium, aluminium, magnesium and beryllium, with the greatest accent on zirconium cans for steam and water-cooled reactors.

Canadian experience of the behaviour of zirconium alloys is described in two papers which discuss the pick-up of hydrogen during corrosion and its effect on the mechanical properties, the advantages of Zr–Nb–Sn alloys over zircaloy-2, the consequence of neutron irradiation and the possible economical advantages of modifying the design and methods of fabrication of zirconium-clad uranium dioxide fuel elements. The development of zircaloy-4 in the United States to decrease the hydrogen pick-up on corrosion in water and steam up to 400° C. is described, and a Swedish paper outlines the possible improvement in the mechanical properties of zircaloy-2 by suitable heat-treatment. Another Swedish author details pickling treatments for zirconium components and two German papers survey the production and welding of zircaloy tubes. Workers from the United States describe the fabrication of flat-plate elements of uranium dioxide clad in zircaloy which are pressure-bonded and include chromium or graphite diffusion barriers to prevent interaction between fuel and can.

Swedish tests on various aluminium alloys indicate that Al–Mg–Si alloys may be suitable for application in water-cooled reactors at 160° C., whereas Al–Mg and Al–Mg–Ni alloys are susceptible to intercrystalline corrosion under these conditions. The fabrication and testing carried out in the United Kingdom of material testing reactor fuel elements are described both for standard components and for hollow elements which can be used for irradiation esperiments in a fast neutron flux. As an alternative to the standard "picture frame" technique described by the British, Belgian authors give details of the rolling of material testing reactor fuel elements from Al–U billets around which the aluminium sheath has been cast. The present state of knowledge on the suitability of sintered aluminium powder as a canning material is summarized by European authors, including its formability, mechanical properties and compatibility with both uranium and uranium dioxide fuels and with steam at 500° C.

Several papers deal with the production and impregnation of graphite for high-temperature gas-cooled reactors, including one on the German manufacture of spherical graphite elements. A Belgian paper describes the fabrication of elements for an experimental swimming pool reactor which consist of uranium dioxide particles dispersed in impregnated graphite. The varied applications of austenitic steels are well illustrated by papers dealing with elements for helium-cooled reactors (with can temperatures up to 700° C.), organic liquid moderated reactors and liquid sodium cooled reactors. Workers in the United States describe the hot extrusion of stainless steel clad elements in which from 91·3 to 100 per cent theoretical density of the uranium dioxide fuel has been obtained. Other papers include details of French fabrication of elements canned in magnesium *ZA* alloy (containing 0·6 per cent zirconium), of British facilities for fabrication of beryllium components, including the precautions necessary to shield the operators from hazards arising from the toxicity of the metal, and of the plant available in Trombay, India, for manufacturing various types of fuel elements.

With such a miscellany of topics as those covered by the papers, a subject-index is essential and this, along with an author-index, is provided at the end of the volume. As is usual for a symposium of this kind, there is a wide variation in the quality of the papers presented, but nevertheless there is a wealth of new data on many of the materials and processes discussed. The full text of most of the papers is in English with a few others in French, but abstracts are provided in each case in English, French, Russian and Spanish.

Volume 2 includes the remaining twenty-two papers presented at the symposium by authors from nine countries under three main headings, "Quality Control and Inspection", "Corrosion and Radiation Damage" and "Economics and General Trends in Development". The most interesting and novel inspection technique described is in a British paper which details work on microradiography of a variety of components both before and after irradiation. Other papers deal with the inspection of zircaloy tubes for the Swedish heavy-water-cooled reactor, helium leak-testing of elements for the French *G*2 reactor and the types of defect observed in swaged stainless-steel-clad uranium dioxide elements by Japanese workers.

On compatibility problems, Australian authors describe tests on stainless steel in carbon dioxide, and discuss the mechanism of the oxidation of beryllium in wet carbon dioxide and the factors that affect breakaway oxidation, while a French paper deals with the oxidation of magnesium alloys in carbon dioxide. A German paper reports on an investigation of the diffusion-rate of certain metallic fission products through sintered aluminium products, and the effect of various alloying additions on the creep properties of this cladding material at 350° C. and its corrosion behaviour in water at 300° C. and in steam at 400° C. The general effects of radiation on various metals and alloys are summarized by a British author; these include the formation of dislocation loops in aluminium and copper and of helium gas bubbles in beryllium, copper and aluminium as identified by electron microscopy of both replicas and thin sections. American experience is given of the irradiation behaviour of various canning materials with special reference to the variability of the effect of irradiation on the pick-up of hydrogen in zirconium alloys. The examination of irradiated Calder-type fuel elements at Windscale has made a big contribution to the improvement of the design of fuel elements for British civil power stations and the facilities for doing this tedious work are described.

In a paper from the United States on the economic factors associated with design variations in fuel elements for water reactors, it is concluded that there is little advantage in using annular uranium dioxide pellets with stainless steel as the cladding material. A development gained in the United States is described for coating uranium dioxide particles with metals prior to compacting to increase thermal conductivity and retention of fission product gases. Descriptions of other bonding techniques for improved performance are given by the Belgians (bonding uranium fuel to an aluminium can with nickel) and the Italians (uranium dioxide particles dispersed in liquid lead in a sintered aluminium product canned fuel element for organic liquid-cooled reactors). The French detail the fabrication on fuel elements for gas-cooled reactors and discuss the problems associated with the manufacture of their 'snow crystal' elements, which consist of clusters of aluminium tubes containing uranium dioxide. Two papers illustrate the considerable effort being directed in the United States towards improved methods of fabricating fuel elements containing uranium dioxide, of which the most promising technique appears to be the vibration compacting of uranium dioxide powder followed by swaging; the novel use of yttrium as a can for liquid fuels is also mentioned. A historical description of fuel element research and fabrication in the United Kingdom includes cost estimates of the various phases of the development work required.

As with Volume 1, the papers vary in quality and detail and include data which have been published previously. However, taken as a whole, they give a reasonably clear picture of the effort required to ensure the highest integrity in fuel elements and to develop a real measure of improvement in the processes used for their manufacture. Both author- and subject-indexes are provided.

B. W. Mott

IONIZATION PHYSICS

Ionization Phenomena in Gases

By Dr. Gordon Francis. Pp. vii+300. (London: Butterworths Scientific Publications, 1960.) 60*s*.

Dr. Francis has excellent qualifications for writing a book on ionization phenomena in gases and has made good use of them. His book is written mainly for those finishing their degrees and those beginning research but will in fact, by virtue of the choice of material and breadth of treatment, be eagerly read by a much wider public with an interest in the behaviour of ionized media. Throughout the book the emphasis is on the understanding of the physical principles of ionization processes and the linking together of a wide range of physical phenomena involving ionization.

The first chapter is a brief one devoted to the nuclear atom, spectra, spectroscopic notation and the concepts of excitation, ionization and the conduction of electricity in a gas. Chapter 2 gives an account of fundamental collisional, excitation, ionization and deionization processes in gases and at electrode surfaces and treats the behaviour of swarms of charged particles in gases. The succeeding chapters are devoted to detailed accounts of several special and important subjects in ionization physics and their applications. Thus, in the third chapter the similarity principle in discharges is dealt with at length and some applications to a number of types of discharge are examined. Alternating and high-frequency discharges at low and high gas pressures in various electric field configurations are treated extensively in the succeeding chapter and applications considered include nuclear particle detection, high-temperature welding and plasma diagnostics. Excitation and ionization in the upper atmosphere, the reflexion, scattering and absorption of radio waves and the physical processes responsible for these phenomena are examined in Chapter 5, which also includes a short account of aurora. The sixth chapter deals with some selected topics of very recent investigations in thermonuclear and plasma physics. The theory of the high-current pinched discharge, the phenomena of the collapse of the pinched discharge, skin effect and instabilities are developed in a clear manner. Some of the practical devices aimed at producing high-temperature gas discharges and controlled fusion reactions are described. The final chapter is a highly concentrated treatment of oscillations and waves in ionized media. Six very useful appendixes are included in the book; these give the Periodic Table and electron configuration of the elements, critical potentials of elements and compounds, some physical constants, a neat derivation of the Boltzmann transfer equation and finally, specialized references to very recent developments in branches which could not be treated at length in the book.

Dr. Francis's book is well written in a refreshing and stimulating manner. It contains a wealth of information which will not be found in many other books and which can otherwise be obtained only by extensive literary research. The book can be highly recommended to anyone with an interest in ionization physics.

C. GREY MORGAN

BILIARY CIRRHOSIS OF THE LIVER

Biliary Cirrhosis

By Sir Roy Cameron and P. C. Hou. (Pathological Monographs, No. 1). Pp. x+214. (Edinburgh and London : Oliver and Boyd, Ltd., 1962.) 63*s*. net.

THIS volume is the first of a series of monographs on clearly defined pathological subjects under the general editorship of Sir Roy Cameron and Prof. G. Payling Wright. In their general introduction to the series, they stress the indebtedness of pathology to the 'basic' sciences of biochemistry, biophysics and genetics. Both the editors are eminent pathologists, and it is doubtless due to their innate modesty that they refrain from pointing out that this intellectual traffic has not been entirely one way; pathology and medicine in general have provided fruitful stimuli to research in the more basic sciences in the past, as it is to be hoped they will continue to do in the future.

Sir Roy himself is joined by Prof. Hou Pao-chang, until recently professor of pathology in the University of Hong Kong, in the authorship of this excellent first monograph. Biliary cirrhosis is a term covering many pathological pictures in the liver which are by no means obviously interrelated. Nevertheless, the title gives to pathologists a clear idea of the ground to be covered, and it will be invaluable to have available in this convenient form the history of clinical, morphological, physiological and experimental research into this complex subject, and the present status of knowledge about it. The authors make clear where the gaps in knowledge lie, and this book will be essential reading for future investigators in this field of study.

Part 1, consisting of five chapters, gives a full but succinct account of the anatomy, embryology and physiology of .the biliary apparatus ; a particularly useful section is devoted to the experimental work which has been done on bile-duct regeneration, and in which Sir Roy Cameron himself has played a distinguished part over the past thirty years.

Part 2 is concerned with the pathology of human biliary cirrhosis. It recapitulates (Chapter 6) earlier classifications, and gives sound reasons for not adding to their number. Chapters 7 and 8 deal with secondary (obstruction of extra-hepatic ducts) cirrhosis and with congenital types. Chapters 9–12 are concerned with the various forms of primary biliary cirrhosis, that is, those where extra-hepatic biliary obstruction is absent. One wonders if 'primary' is really the best adjective available for this group, but it has become hallowed by convention. The possible part played by virus hepatitis is discussed on pp. 158–163. Rather surprisingly, a whole chapter is devoted to the somewhat mysterious entity described by Hanot more than 80 years ago. The closing chapter deals with parasitic cirrhosis, a subject to which Prof. Hou himself has made important contributions.

The book is beautifully produced, the bibliographies comprehensive, and the illustrations clear. Only one misprint has been detected. The price, as usual nowadays, is too high.

J. W. ORR

ESSAYS ON EVOLUTION

Evolution und Hominisation

Festschrift zum 60. Geburtstag von Gerhard Heberer. Herausgegeben von Gottfried Kurth. Pp. x+228. (Stuttgart: Gustav Fischer Verlag, 1962.) 48.50 D.M.

A *FESTSCHRIFT* dedicated to a distinguished scientist when he retires, or when he reaches the age of seventy or eighty, or on his death, has not been uncommon in the past. But here we have a *Festschrift* to celebrate the sixtieth birthday of a palæontologist. Dr. Gerhard Heberer. All honour to him that his work should thus be recognized at such an early age; clearly his colleagues at the University of Göttingen and elsewhere hold him in high esteem and affection. But it is not easy to review a mixed collection of essays such as the present volume offers for the reason that they are so various in their themes, though broadly speaking their content is covered by the title "Evolution and Hominization". (Parenthetically, it is to be hoped the term "Hominization" has not come to stay—it was originated by Teilhard de Chardin and inevitably tends to convey an element of the mysticism that is so apparent in the posthumous publications of this Jesuit Father—himself also a distinguished palæontologist.)

Dr. G. G. Simpson writes on "Some Cosmic Aspects of Evolution" and extends his thesis of the opportunistic factor in the evolutionary process to a surmise on the possibility of life in other worlds than our own. Ernst Mayr discusses the problem of chance and plan in evolution, Dobzhansky and Drescher write on the genetic basis of 'quantum evolution' with particular reference to the evolutionary potentialities of modern man (der Mensch passt öfter die Umwelt seinen Genen an, als dass er seine gene der Umwelt anpasst), varying opinions are expressed by other authors on the antiquity of early man, but in some cases these are already out of date (an exception is the important paper by von Koenigswald on the absolute age of *Pithecanthropus*), while other essays deal with the australopithecines, early members of the genus *Homo* in Africa, and so forth.

But it has to be admitted that much that this volume contains is not new—some of the essays are but little modified versions of publications that have already appeared in the last few years. This raises the question whether the days of *Festschriften* are not passing, for with so many publications of carefully co-ordinated symposia on so many different subjects, disjointed mixtures of essays no longer serve so useful a purpose in scientific literature. Observations of importance included in such essays are likely to be overlooked unless they are also published in recognized scientific journals (as, in fact, they almost always are). The eagerness to honour a distinguished scientist which his friends and colleagues show is, of course, easy to understand. But with the already voluminous and unwieldy profusion of scientific literature, the growing custom of honouring an eminent scientist by, say, dedicating a volume of a scientific journal to his name, has much to commend it.

Progress in Non-Destructive Testing

Vol. 3. Edited by Dr. E. G. Stanford and J. H. Fearon. Pp. vii + 227 + 15 plates. (London: Heywood and Co., Ltd., 1961.) 55*s*. net.

THIS volume contains articles on seven topics, three of which are concerned with X-rays. Recent developments in X-ray spectrochemical analysis have been concentrated on finding the most efficient and speedy detectors. The article includes a discussion of the accuracy now obtainable and also a description of the X-ray microanalyser, which will show, by simultaneous display on two oscilloscopes, an optical image of a small area and the distribution of various chosen elements.

X-ray microscopy has developed to the stage where sub-microscopic flaws, inclusions and lattice defects, including dislocations, may be located and identified. The use of the xerographic process, associated with X-rays, can save time compared with the standard radiographic process. The nature and preparation of the semiconductor plates and their response to X-rays are discussed at some length.

The visco-elastic properties of plastics under dynamic loading can be interpreted in terms of storage and loss moduli—the latter giving the "mechanical internal energy loss". These moduli are functions of frequency and of temperature. Their relationship to molecular structure and their measurement are discussed in an article.

Optical methods can be used for testing flatness, surface layer strains, homogeneity of thickness or refractive index and for revealing atomic lattice defects. While on the other hand magnetic hysteresis methods can give results which correlate with various other properties, such as composition or mechanical strength. The two methods are dealt with in separate chapters.

The use of electromagnetic testing for such purposes as the detection of local defects, cracks, flaws, inclusions, etc., has required a different theoretical approach from that given by Maxwell's equations.

K. J. PASCOE

Astronomical Dictionary in Six Languages

English/Russian/German/French/Italian/Czech. By Josip Kleczek. Pp. 972. (New York and London: Academic Press, Inc.; Prague: Publishing House of the Czechoslovak Academy of Sciences, 1961.) 25 dollars.

THIS volume, the first of its kind, will be of great use for two categories of persons; for trained translators who are not acquainted with astronomical terms and also for astronomers with a slight knowledge of one of these languages. The author and his co-workers are to be congratulated on the completion of this book which has been prepared with great care. On the pages 16–575 the words and terms are given in six languages. They have been subdivided into 34 sections, the first of these being "General", the last "Astronautics". Within each section the words have been numbered. The choice of the sections is somewhat arbitrary, but this has no influence on the usefulness of the dictionary.

The last 400 pages of the book consist namely of six parts in which all the words have been arranged alphabetically in each language with references to the section and number in the first part. This makes the book equivalent to thirty bilingual dictionaries. It is a handsome volume, and the paper and printing are excellent.

P. TH OOSTERHOFF

The Economics of Education

By John Vaizey. Pp. 165. (London: Faber and Faber, Ltd., 1962.) 21*s*. net.

IN this book Mr. Vaizey follows his survey of *The Costs of Education* with a lively survey of existing economic thought about education. Beginning with a brief review of what leading economists from Adam Smith and David Hume to Marshall have written about education in general, he surveys in successive chapters comments made on specific aspects, such as education as consumption or investment, the returns to education, expenditure on education, the finance of education, the productivity of education, man-power, teachers and their salaries, and the comments of economists on the schools. Two chapters on education in economic growth extend the survey to the under-developed countries, particularly Nigeria and Pakistan, in which he comments on the appalling waste of talent caused in Britain by the desire of successive Governments to keep down taxes, lead him to a very brief concluding chapter on the role of education in a modern economy. The value of this brief but comprehensive survey is enhanced by an excellent select bibliography, which, however, omits Joyce Alexander's *Scientific Manpower*. For its chapter on "Scientific Womenpower" this little book is more relevant to the British context than the American publication entitled *Womenpower* listed by Mr. Vaizey.

R. BRIGHTMAN

Religion, Art, and Science

A Study of the Reflective Activities in Man. By Prof. John Macmurray. (The Forwood Lectures, 1960.) Pp. 78. (Liverpool: Liverpool University Press, 1961.) 12*s*. 6*d*. net.

THIS little volume consists of four lectures given before the University of Liverpool in 1960. The titles are: (1) "Science Out of Bounds"; (2) "Contemplation and Communion"; (3) "Religious Reference"; (4) "Christianity for the Future". The author stresses in particular the part played by action; it is what people do that is important. There is an excellent discussion of self-transcendence (as opposed to ego-centricity) which derives, one suspects, from the writer's study—as far back as 1927—of the relation of this quality to genius. Religion must incorporate both science and art into a universal fellowship.

There are two matters on which comment may be admissible. The first is that dogma seems to be judged a trifle harshly. After all, it means an opinion, which may harden into a tenet or even into an assertion. Science, like other intellectual activities, has its dogmas, and it is difficult to see how religion can get on without at least some of them, if it is to supply mankind's innermost needs.

The other point is that science appears to be taken almost entirely in the Baconian sense of man's conquest of Nature for material ends, including the acquisition of power. (It is true that much of the early history of the Royal Society would support this view.) But it is simply not the case for the highest achievements of pure science, for example, the works of Eddington and Heisenberg, which approximate more to poetry than to anything else, and have nothing in common with technological triumphs.

Nevertheless, these addresses are fascinating, and most timely: scholars will welcome their advent in print.

F. I. G. RAWLINS

FATE OF DIETARY FAT IN THE BODY*

By PROF. A. C. FRAZER
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THE first Lord Leverhulme pioneered the supply of fats for human consumption on a large scale. The object of this article is to consider what happens to these fats in the body after they are eaten, what effect different dietary fats may have on the body and what the important properties of a food fat may be from the health point of view.

Fats with differing properties are assimilated to a different extent. Short-chain fats tend to be absorbed better than long-chain compounds. This has been ascribed to their melting-points being higher than body temperature, so that crystallization occurs. This is not the whole explanation, however, since erucic acid with a melting-point below body temperature is also selectively rejected. Degree of saturation is also important. Progressive hydrogenation of a fat leads to a corresponding decrease in assimilability, unless a sufficient amount of a carrier oil vehicle is also provided. In patients with defective absorption there is a much greater increase of saturated fats in the stools when the steatorrhœa is aggravated and the administration of more unsaturated dietary fat results in improvement in the steatorrhœa. These points may be related to the peculiar properties of stool fats. They commonly contain 30–40 per cent of stearic acid, which is a higher proportion of this fatty acid than is found in any other natural animal fat. A significant amount of this stearic acid may be formed by hydrogenation, some may be accounted for by selective rejection; possibilities of synthesis and excretion must also be considered. A large amount of hydroxy-fatty acids, such as occurs in castor oil, may interfere with absorption owing to poor emulsification; if a suitable oil vehicle is provided to ensure fine dispersion, good absorption occurs and the cathartic effect of the castor oil is abolished.

Dietary fats are partially hydrolysed by pancreatic lipase. Selective action on the fatty acids in the 1 and 3 position occurs. Thus, the nature of the dietary fat may affect the nature of the monoglyceride formed. In *in vitro* investigations different monoglycerides have different activities in micelle formation and glyceride resynthesis; it is not known, however, whether this has any significance *in vivo*. The fatty material is finely dispersed in the intestinal lumen by means of a triple system consisting of fatty acids, monoglycerides and bile salts. This system is an effective emulsifying system under intestinal conditions; it is also able to form micelles. The particulate fatty material in the lumen ranges from large droplets down to invisible micellar particles. It is not known at what point the particles become absorbable. For a long time the critical diameter has been considered to be less than 0·5μ; recent investigations suggest that it may be in the region of 0·1μ.

The finely dispersed fatty material enters the intestinal cell by passing down the intermicrovillous crypts, as revealed by electron microscope studies. Many invaginations may be seen at the bottom of these crypts; but the precise mechanism of absorption is still under discussion. Visible particulate fat does not appear to enter the substance of the microvilli. During its passage through the intestinal wall the fatty material is changed. Mono- and di-glycerides are converted back to triglycerides, and phosphatides are formed in significant quantities; short-chain fatty acids and glycerol are not re-incorporated into triglycerides and pass into the body in the portal vein.

Two pathways of glyceride biosynthesis have been demonstrated in the intestinal cell. One involves phosphatidic acid as an intermediate; but the other does not. This second pathway may be important, since present evidence still suggests that every molecule of fat absorbed does not pass through a phosphatide stage. Nevertheless, the addition of glycerophosphate, choline, or phosphatidyl choline does increase the amount of glyceride fat absorbed in 3 hr. The precise significance of phosphatides in the fat-absorption mechanism is still obscure. In addition to changes in composition, the fat particles also change their surface properties during absorption. The fat in the chyle after absorption is stabilized with lipoprotein. If treated with phosphatidase D, the chylomicrons flocculate and coalesce. Phosphatidyl choline appears to be essential for their stability in the presence of plasma proteins. Thus, one important function of the phosphatide formed in the intestinal cells may be the provision of the lipoprotein stabilizing film.

The blood fat is carried as lipoproteins. The greater the amount of glyceride present, the lower the density. These lipoproteins can be characterized in various ways; one method extensively used is ultracentrifugation. The glycerides in the lipoproteins during absorption closely resemble those fed, except that short-chain fatty acids (less than 10 carbons) have largely disappeared. The alimentary hyperlipidæmia after a moderate fat intake reaches a peak in 3–3½ hr. and returns to normal levels in 5–6 hr. The essential change is in the glyceride-level. The extent and duration of the alimentary hyperlipidæmia is greater in man and the dog than in the rat; almost no significant change occurs in the guinea pig. Unsaturated fats cause a shorter hyperlipidæmia than saturated fats. Old people may have a prolonged hyperlipidæmia. The administration of pancreatic lipase with the fat affects the amplitude of the hyperlipidæmia; it can also be decreased by the administration of heparin and a number of other substances, and enhanced by the administration of protamine. Further information on the effects of different dietary fats on alimentary hyperlipidæmia might be interesting.

The effect of heparin is due to liberation into the blood of a lipase, which hydrolyses chylomicrons Provided that a fatty acid acceptor, such as albumin, is present, this lipase can cause disappearance of the lactescence in hyperlipidæmic blood. This or a similar enzyme is normally present in muscle, adipose

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tissue and other cells; it is, however, absent from liver cells. This lipase acts better on chylomicrons, possibly due to the presence of the lipoprotein stabilizing film; it has therefore been called 'lipoprotein lipase'. It appears to facilitate the removal of glyceride fat from the blood, but hydrolysis is not an obligatory step, since liver and possibly other cells take up glycerides without previous hydrolysis. In essential hyperlipidæmia, a genetically determined inborn error of metabolism, blood glyceride accumulates because of slow clearing. In three patients with this condition, a deficiency of lipoprotein lipase was demonstrated; but this does not seem to be the explanation of all forms of this disease. More resistant fat particles might also be present and these have been shown to occur under certain circumstances.

Dietary fat has been shown to affect the resting blood lipid-level. This is also affected by age, sex and genetic factors. The ingestion of a relatively high proportion of polyunsaturated fatty acids causes a reduction in the level of cholesterol and β-lipoproteins. The body is able to synthesize fatty acids *de novo* from small fragments. In this way the saturated and monoethenoid fatty acids can be formed. There is difficulty, however, in forming such fatty acids as linoleic and linolenic acid. These are important because the nature of the further polyunsaturated fatty acids formed in the body is dependent on the nature of the starting material. Oleic acid when further desaturated gives rise to 5,8,11-eicosatrienoic acid, whereas linoleic acid yields arachidonic acid and linolenic acid gives docosa pentaenoic and hexaenoic acid. Arachidonic acid is an essential constituent of phosphatides in mitochondria and other cell structures. If there is an insufficient amount of arachidonic acid it will be replaced by a trienoic acid, which is an unsatisfactory substitute—a syndrome known as the essential fatty acid deficiency syndrome results. The linoleic acid must be in the *cis cis* form for it to function as an essential fatty acid. The effect of polyunsaturated fatty acids on blood cholesterol and β-lipoproteins is not, however, confined to the linoleic acid family. Many other polyunsaturated fatty acids, including many fish oils, are equally effective.

Glycerides enter liver cells without previous hydrolysis. The liver is well equipped to carry out any modifications in the fatty acids which may be required. Much of the fatty acid may be oxidized; the remainder is converted into various esters or other lipids. The liver endeavours to keep the level of free fatty acids under control, and it maintains a state of equilibrium with the blood lipids. Glyceride may accumulate in the liver under conditions that involve interference with phosphatide formation; these include choline deficiency from any cause, unsaturated fatty acid deficiency and various vitamin deficiencies. The glyceride which accumulates in greatest quantity is dietary fat.

Adipose tissue takes up dietary fat. Its composition closely resembles the dietary fat if the fat intake is high. Adipose tissue cells can also synthesize fatty acids *de novo* from glucose and shorter-chain fatty acids. Fat is continually being mobilized from the fat depots; their half-life in the rat is 10 days, but it differs with different fats. The main form in which fat is mobilized is as fatty acid; this circulates in the body as non-esterified fatty acid Adrenocorticotrophic hormone; adrenaline and growth hormone stimulate mobilization; insulin and glucose inhibit it.

Lipids are deposited in the walls of blood vessels; these deposits increase with age. More lipid may be deposited in people with high blood fat-levels. Since a high intake of polyunsaturated fatty acids reduces blood fat-levels, this is an important dietary consideration. An adequate intake of polyunsaturated fatty acids, a sufficient amount as *cis cis* linoleic acid, should be maintained. The taking of large quantities of polyunsaturated fatty acids may be a useful therapeutic measure in people with raised blood lipid-levels and severe atheromatous changes, but there does not appear to be a good case for excessive intake in normal people. The link between atheroma and coronary thrombosis and myocardial infarction is tenuous. Controlled trials are needed to establish whether patients with these heart diseases derive any benefit from the ingestion of polyunsaturated fatty acids. There is certainly no justification on present evidence for attributing changes in the incidence of these disorders to dietary factors alone, or in proposing any major change in the national diet.

However, it is the duty of all concerned with food supplies to ensure that as much as possible is discovered about the effects of food on the body. The intake of important nutrients, such as *cis cis* linoleic acid, should be safeguarded. The effects of processing or storage on food materials should be studied and kept under close review. Only by this means can the potential benefits resulting from the work of men such as the first Lord Leverhulme be satisfactorily and safely realized.

TRACE CONSTITUENTS OF BUTTERFAT

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FIFTEEN years of research on flavour in the Unilever laboratories in the Netherlands and in England has brought to light an interesting series of trace constituents in butterfat, knowledge of which, on one hand, throws considerable light on the nature of butter aroma and, on the other, points to unexpected bypaths in biological synthesis or breakdown in the ruminant.

The investigation was commenced at a time when liquid/liquid partition chromatography opened up a new phase in the study of lipids. This was followed, in 1952, by the introduction, by James and Martin[1], of gas/liquid chromatography, which provided very high resolving power and required only micro-amounts of substances for analysis. In the same period, the development of spectroscopic techniques, such as

Table 1. (*a*) SATURATED FATTY ACIDS IN BUTTERFAT

Type		Known up to: 1940	1955	1960
Straight chain	Even	C_4–C_{26}		C_2
	Odd		C_{11}–C_{15}	C_{17}–C_{23}
Branched chain	Even (iso)		C_{14}	C_{16}
	Odd (iso)		C_{13}–C_{17}	
	(ante-iso)		C_{15}–C_{17}	
Poly-branched				C_{20}

(*b*) UNSATURATED FATTY ACIDS IN BUTTERFAT

Type		Known up to: 1940	1955	1960
Mono-	Even	C_{10}–C_{18}*	C_{18} isomers	Geometric C_{12}, C_{14}, C_{16} isomers C_{20}–C_{24}
	Uneven			C_{15}–C_{23}
Di-		C_{18}†	Conjugated	
Tri-		C_{18}	Conjugated	C_{20}
Tetra-		C_{20}	Conjugated	C_{22}
Penta-				C_{20}–C_{22}
Hexa-			C_{22}	

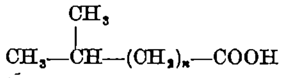
* Apart from *cis* Δ^9-, also *trans* Δ^{11} C_{18}.
† Linoleic acid containing iso-linoleic acid

infra-red, nuclear magnetic resonance, and mass spectrometry, made it possible in many cases to characterize the molecular structure of compounds when only minute amounts were available.

In Unilever, Schogt and De Bruyn[2] developed a method for the efficient isolation of volatile components from fats and oils, known as the high vacuum degassing method. This technique permits the direct, quantitative transfer of volatile components from oils and fats to the gas-chromatographic column, and from there to the spectroscopic apparatus, without loss or contamination.

Lipid chemistry has greatly profited from these developments. This is illustrated, for example, by the great advances made in the knowledge of the fatty acid composition of butterfat, as shown in Table 1, compiled from literature data.

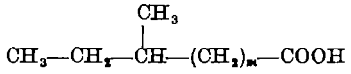
The iso-acids and ante-iso-acids described by Shorland *et al.*[3], namely:



$$CH_3—\overset{CH_3}{\overset{|}{C}H}—(CH_2)_n—COOH$$

iso[C : *13*, 14, *15*, 16, *17*]

and



$$CH_3—CH_2—\overset{CH_3}{\overset{|}{C}H}—(CH_2)_n—COOH$$

ante-iso[C : *13*, *15*, *17*]

are widely represented in butterfat, and we ourselves (Schogt *et al.*) have been able to identify the tetramethyl-palmitic acid with a terpenoid structure[4]. These acids occur in butterfat in concentrations of about 1,000 p.p.m.

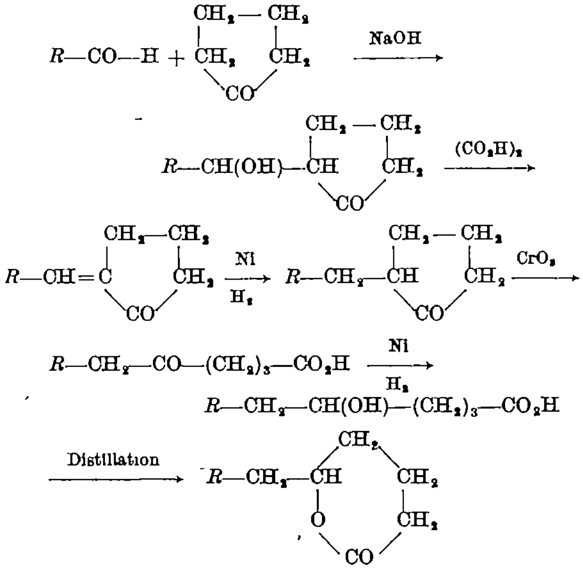
In this article we shall restrict ourselves to trace constituents which have been detected in butterfat either in a free or combined form in concentrations in the order of 10 p.p.m. or less. In particular, the detection and identification of both saturated and unsaturated aliphatic δ-lactones, unsaturated γ-lactones, δ-hydroxy acids, δ-keto acids, β-keto acids and methyl ketones will be described.

δ-lactones. In the course of our investigations we have applied many different methods for the isolation of crude lactone fractions from butterfat, namely: (*A*) methanol extraction; (*B*) molecular distillation; (*C*) steam distillation; (*D*) weak-alkali extraction; (*E*) binding by means of basic ion-exchanger; (*F*) vacuum degassing.

These we have applied singly, or in various combinations, and supplemented them—when the presence of lactones was indicated—by the following operations: (1) saponification; (2) unsaponifiable components extracted; (3) hydroxy-acids lactonized; (4) higher fatty acids removed (urea); (5) lower fatty acids removed (soda).

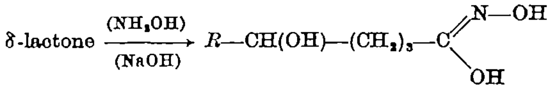
The probability that lactones were present was disclosed initially by repeated titration of a typical concentrate, which exhibited spontaneous partial re-esterification when the soap was split and the acids recovered. Classification as δ-lactone type followed readily when infra-red spectrometry became available. The close resemblance between synthesized δ-dodecalactone, used as model substance and a lactone fraction from butterfat obtained by microdistillation, is shown in Fig. 1. Careful examination of infra-red spectra of various butter lactone preparations, however, revealed that we were dealing with a mixture of lactones of different chain lengths.

A range of δ-lactones was prepared in the laboratory, for use as model substances to aid identification of the butterfat lactones. They could be synthesized in various ways, but we will describe here only the method which proceeds via the δ-keto acid, as this acid has played an important part in further development of the subject.



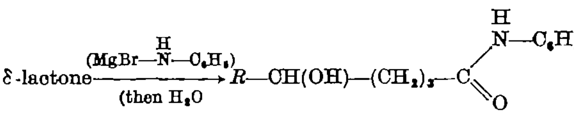
Direct chromatographic separation of the mixture of lactones was not feasible since there was a tendency for the lactone ring to open in solvent systems and for some inter-esterification to take place. To obviate this, and also make chromatographic separation easier to follow, the following derivatives were prepared and examined:

(*a*) hydroxamic acids;
(*b*) anilides.



$$\delta\text{-lactone} \xrightarrow[(NaOH)]{(NH_2OH)} R—CH(OH)—(CH_2)_3—C{\overset{\displaystyle N—OH}{\underset{\displaystyle OH}{\lessgtr}}}$$

hydroxamic acid



$$\delta\text{-lactone} \xrightarrow[(\text{then } H_2O)]{(MgBr—\overset{H}{N}—C_6H_5)} R—CH(OH)—(CH_2)_3—C{\overset{\displaystyle \overset{H}{N}—C_6H_5}{\underset{\displaystyle O}{\lessgtr}}}$$

anilide

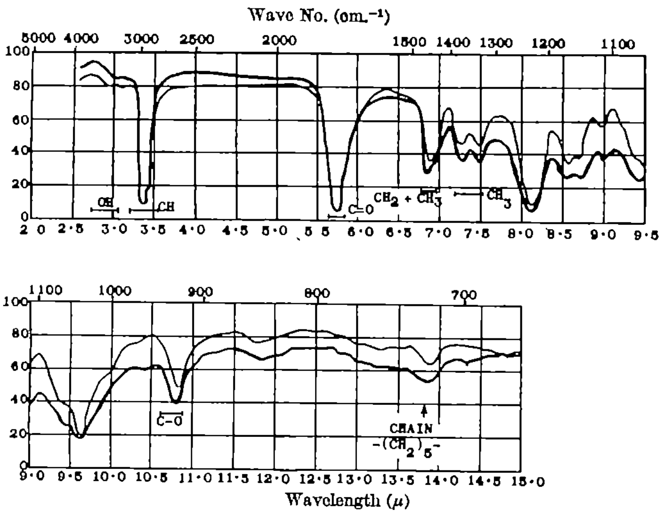


Fig. 1. Infra-red recording of a lactone fraction from butterfat. Thick line, lactone from butterfat; thin line, synthetic δ-dodecalactone

Of the two, the anilides proved to be the more satisfactory, both in the ease and reliability of preparation and in the wealth of information they provided.

The hydroxamic acid mixture, separated by paper chromatography in the solvent system benzene/ethanol and developed by ferric chloride in alcohol, showed only the even series: C$_{10}$, C$_{12}$, C$_{14}$ and possibly C$_{16}$.

The anilides were chromatographed over silica-gel columns in the system cyclohexane/aqueous alcohol and they were more finely resolved than the hydroxamic acids (Fig. 2). Apart from relatively major zones corresponding to lactones with 8, 10, 12, 14 and 16 carbon atoms, there were also other minor zones which corresponded nominally to lactones with uneven numbers of carbon atoms. The concentrattions of these components were of the order of a few per cent of those of the main components. Further investigation brought to light a number of interesting lactones; for example, fraction No. 4 was found to contain, *inter alia*, saturated δ-undecalactone, and fraction No. 3, an unsaturated δ-dodecene-9-lactone, which was found later to be identical with a lactone synthesized by Haverkamp Begemann. In subsequent work, a lactone mixture obtained by vacuum degassing of butter, was separated directly by gas chromatography (Fig. 3). This has also led to the isolation and identification of the unsaturated γ-dodecene-6-lactone.

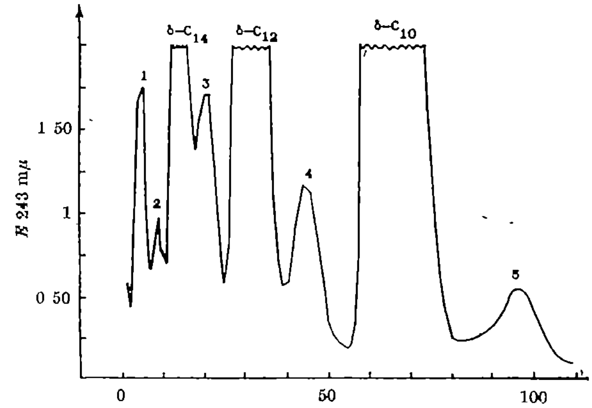


Fig. 2. Partition chromatogram of hydroxy-acid anilides from heated butterfat

However, no quantitative yardstick could be obtained in this way: the ratio of the amounts in which the various components were obtained was greatly influenced, in high vacuum degassing, by differences in volatility, and, in alkali extraction, by differences in the rates of hydrolysis and differences in solubility.

Furthermore, the method of extraction took no account of the possible natural state of the lactones in the butterfat.

Circumstantial evidence on the natural state of the lactones was obtained by an investigation in which a concentrate (obtained via methanol extraction plus molecular distillation) was subjected to liquid/liquid partition chromatography in the system light petroleum/aqueous methanol. Little free lactone was disclosed. Acid fractions yielded some lactone after ring-closure, but they did not account for the quantity of lactones to be expected. A number of fractions, however, had the infra-red characteristics of hydroxy glycerides and these, on hydrolysis, yielded further quantities of lactones. Analysis of one such fraction gave: total combined fatty acids 57 per cent: combined lactonizable acids 22 per cent.

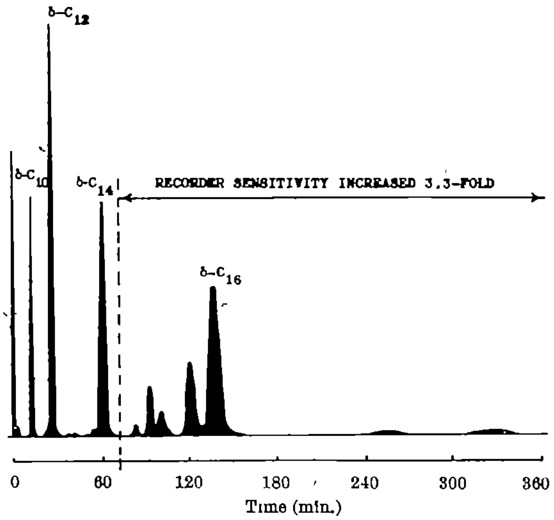
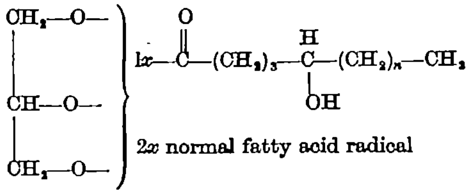


Fig 3. Lactones from butter. Immobile phase, silicone oil; temperature, 185° C.

To put the matter on a more quantitative basis the well-known isotope dilution method was applied. A number of lactones, which were labelled with carbon-14 in the carboxyl group, were synthesized.

The interesting fact was then observed that the lactone content of butterfat increased if the fat was heated for some time at 140° C. This is illustrated in Table 2.

We would direct attention to the low free lactone content of fresh cream fat. The conclusion to be drawn again is that δ-hydroxy acid must be present in bound form. The most logical supposition is that the δ-hydroxy acid, in combination with two normal fatty acids, is built into the glycerides, from which lactone can be split off by heating.



We have prepared a number of model glycerides, namely: δ-hydroxy-acid mono- and tri-glycerides, and mixed δ-hydroxy-acid normal tri-glycerides, and studied their breakdown pattern on heating (Fig. 4). They are heat-labile, the mono-glycerides more readily so than the tri-glycerides. (For this reason it is better that the synthesis should proceed via the δ-keto glyceride.)

Table 2. LACTONE CONTENT OF VARIOUS FATS BEFORE AND AFTER HEATING AT 140° C.

Fat phase of	C_{10}	C_{12}
Fresh butter	5 (10)	
	4 (10)	10 (25)
	3 (10)	5 (28)
	9 (14)	16 (30)
Non-pasteurized cream (fresh)	3 (20)	4 (27)
Non-pasteurized cream (soured)	3 (19)	5 (31)
Pasteurized cream (fresh)	6 (19)	11 (38)
Pasteurized cream (soured)	6 (18)	13 (35)
Beef tallow	0·2 (0·5)	0·6 (2·3)

These facts together may not be wholly conclusive, but they provide good circumstantial evidence that glycerides of the structures described are present in butterfat.

We come now to what is perhaps the most important characteristic of the lactones of butterfat—and indeed of milk fat—in so far as it points unequivocably to how they originate. It became evident by observation that the anilides of the naturally occurring lactones deviated, in respect of both melting-point and X-ray diffraction data, from the anilides of synthesized lactones.

From these data we concluded that the lactones represented only one of the two optical antipodes, although measurements of rotation on admittedly minute quantities of anilide gave (α_D) values of the order of only 1°. However, the finding was confirmed by measuring the optical rotation of a more adequate quantity of lactone isolated from butter. (The rotation of the hydroxy acids cannot be measured since spontaneous partial ring closure leads to spurious values. However, for the potassium salt of the decanoic acid we obtained an (α_D) value of $-4°$.) As the highest value for the rotation (α_D) = + 37·8° was found.

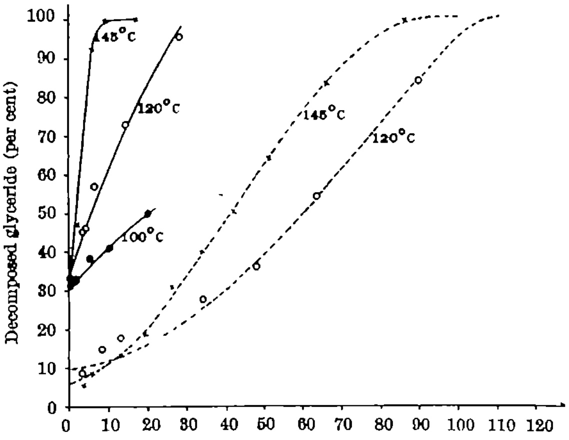


Fig. 4. Decomposition of δ-hydroxydecanoic acid glycerides (measured with the aid of lactone absorption at 926 cm.$^{-1}$) —— monoglyceride; – – – triglyceride

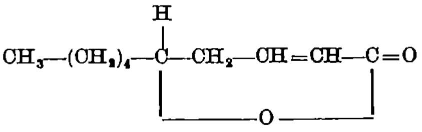
Table 3. MELTING POINTS AND X-RAY DATA OF HYDROXY ACID ANILIDES

		Melting point (°C.)	X-ray analysis Longest observed spacing (Å.)	Chief side spacings (Å.)	
C_{12}	From butter	91–95	21·6	4·63 4·28	4 06 3·66
	Synthesized	104–104 5	15·7	4·50	4·02 3·82
C_{10}	From butter	110–111	18·5	4·71	4·38 4 10
	Synthesized	98 5–99 5	14·2	4·60	4·12

As early as 1940, Th. M. Meyer[5] described the optically active "massoi lactone" and the saturated δ-decalactone obtained from it by hydrogenation.

Massoi lactone occurs in the bark of *Cryptocaria massoia* (Lauraceae), which grows in New Guinea.

Massoi lactone



$$[\alpha_D] = -87{\cdot}6°$$

$$\text{Hydrogenated}: [\alpha_D] = +44{\cdot}6°$$

In our own investigations, slightly deviating values for (α_D), namely, $-$ 84·6° and + 45·5°, were found.

We have also separated the synthetic lactones, by means of cinchonidine, into the optical antipodes. It was then found that the melting-points and X-ray diagrams were in better agreement with the observations made on anilides from butter lactones.

This was also true of the anilide prepared from dihydro-massoi lactone.

If the preponderance of the homologous series with an even number of carbon atoms already indicated the biological formation of the lactones, there was certainly no further room for doubt once their optical activity had been discovered.

At this point mention must be made of a series of articles by Patton, Keeney and Tharp[6], who during the past ten years have published a number of the facts given above. In the course of their work, the compounds originally regarded as off-flavours have been identified as lactones.

The detailed investigation described in the present paper (on the basis of which patents applied for as early as 1952 have been granted), as well as our finding that the lactones are optically active, precludes the possibility of their formation by autoxidation of certain precursors.

It is especially interesting in this connexion to report the discovery by Tuynenburg Muys, De Jonge and Van der Ven[7] that various micro-organisms are capable of converting δ-keto acids into optically active δ-hydroxy fatty acids and lactones. Of these micro-organisms we mention the following:

Moulds, *Cladosporium butyri;* yeasts, *Saccharomyces cerevisiae, S. fragilis, Candida globiformis, C. dattila;* bacteria, *Sarcina lutea.*

Most of these organisms, including *Saccharomyces cerevisiae*, produce lactones which are identical with those occurring in butter; others, including *Clado-*

sporium butyri and *Sarcina lutea*, produce lactones with opposite rotation.

The lactones so prepared have the following optical rotations.

		(α_D)
Lactone mixture from butter :		+37·8°
Homologous series of lactones obtained by micro-biological reduction :	C_8	+58·4°
	C_{10}	+56·0°
	C_{12}	+48·8°

In addition, it has been found that pigeon- and bovine liver can also reduce δ-keto acids. The reducing enzyme present in yeast is located in the mitochondria and it has been found that the reduction proceeds via the coenzyme A ester of the keto-acid. Study of the exact nature of the enzyme is still in progress; it is possibly identical with, or closely related to, enzymes known in the field of biological fatty acid synthesis or breakdown. One might speculate whether, in the biosynthesis, δ-keto acids result from two consecutive additions of acetate, the first of which escapes reduction to β-hydroxy-acid–coenzyme-A. The very interesting and recent discovery of traces of δ-keto acid in the glyceride portion of butterfat (a few p.p.m.) suggests that the lactones are indeed formed via δ-keto acids.

Further evidence, pointing to the presence of other bound keto acids, was obtained during a study of the volatile carbonyls in butterfat.

It was found then that for a certain class, namely, the methyl ketones, precursors must be present in the fat. On complete removal of the volatile carbonyls by high-vacuum degassing, and subsequent steam distillation, at 180° C., of the fat, a homologous series of methyl ketones, with an odd number of carbon atoms, was detected in the distillate.

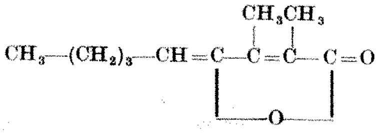
Table 4. METHYL KETONES FROM BUTTERFAT BY STEAM/DISTILLATION AT 180° C. (in p.p.m.)

C_{15}	Methyl-tridecyl ketone	42
C_{13}	Methyl-undecyl ketone	22
C_{11}	Methyl-nonyl ketone	13
C_9	Methyl-heptyl ketone	9
C_7	Methyl-pentyl ketone	12

This evidence suggested that β-keto acids, a normal intermediate in the biological fatty acid cycle, were present in bound form in butter. This was confirmed by isolating pyrazolones, which formed by allowing Girard-*T* reagent to react with butterfat. In addition, synthetically prepared glycerides containing β-keto acids were submitted both to hydrolytic heating and to treatment with Girard-*T* reagent, with similar results to those obtained with butterfat.

γ-Lactones. The presence of minor quantities of normal aliphatic γ-lactones has been detected in butterfat concentrates; isomeric conversion from γ-lactone during isolation is unlikely. But any account of butter lactones would be incomplete without mention of an entirely unique γ-lactone: unique because, in this case, we are not concerned with a homologous series.

The substance in question, to which we have given the name 'bovolide', has the following structure:

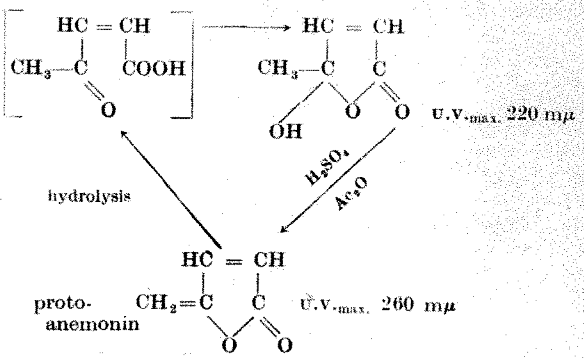


$$\mathrm{CH_3{-}(CH_2)_3{-}CH{=}C{-}\overset{CH_3}{C}{=}\overset{CH_3}{C}{-}C{=}O}$$

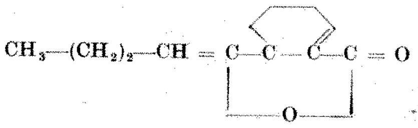
It is the enol lactone of

2,3-dimethyl-4-keto-nonene-2-acid

The compound has a characteristic triene ultraviolet absorption at 270 mμ and occurs in an amount of about 0·5 p.p.m. in butter. It has a strong odour reminiscent of celery. When it is saponified the absorption at 270 mμ disappears and does not return on acidification. The lactone can be regenerated only by the action of a strong dehydrating agent, such as potassium hydrogen-sulphate. The free acid readily converts into a hydroxy-lactone or pseudoacid, analogous with the behaviour of proto-anemonin, as was described by Shaw[8] in 1946.



The analogy with compounds from celery is striking; compare, for example, the lactone of sedanonic acid, in which, however, the double bond is not in the lactone ring.



The origin of this lactone is still not known; we suspect that it comes originally from the fodder and we assume that, in view of the absence of a homologous series, it is not connected with the normal biological fatty acid synthesis.

The work described in this article was a collaborative effort in which the following took part, in addition to the authors: J. C. M. Schogt, Miss P. Haverkamp Begemann, G. Lardelli, G. Tuynenburg Muys, A. P. de Jonge, B. v.d. Ven, A. Francke, G. J. v. Beers, A. S. M. v.d. Zijden, G. Dijkstra, P. Roele, P. D. Harkes, K. de Jong, Miss J. Koster (Vlaardingen, The Netherlands); R. Wellwood, W. T. Weller, J. Clifford, D. Chapman, J. Devine, J. R. Edisbury (Port Sunlight, England); V. Lamberti (Edgewater, New Jersey, United States). Fuller details of the findings will be given in a series of forthcoming publications.

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RELATION BETWEEN STRUCTURE AND SEQUENCE OF HÆMOGLOBIN

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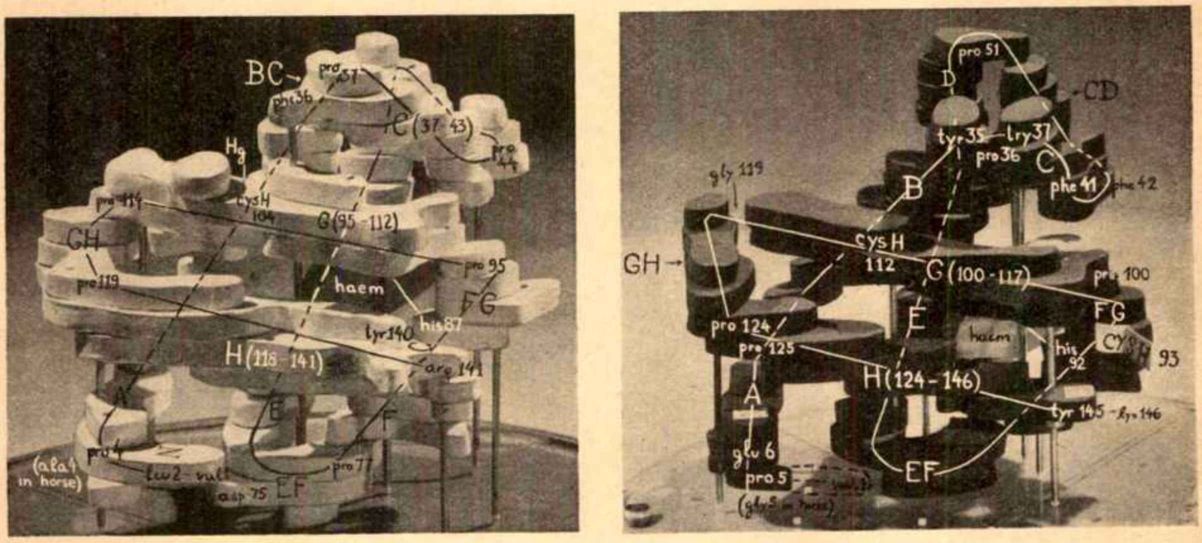
WHEN the tertiary structure of horse hæmoglobin was first published, its amino-acid sequence was still unknown, and the resolution of the X-ray analysis was too low to allow side-chains to be seen[1]. Since then, the amino-acid sequences in the two different chains of human hæmoglobin and in one of the chains of horse hæmoglobin have been elucidated[2-5]. In addition, most of the structure of sperm whale myoglobin is now known in atomic detail[6]. Thanks to the close similarity in tertiary structure between sperm whale myoglobin and horse hæmoglobin the position of almost any residue of a given sequence number can be found by looking up the co-ordinates of the corresponding residue in myoglobin and then transferring those co-ordinates to the model of hæmoglobin. This procedure can be checked, because the positions of certain residues can be found directly in the electron-density maps of hæmoglobin, despite the low resolution, and can then be compared with those inferred by analogy with myoglobin. For example, the cysteines are found by labelling them with mercurials; the indol rings of tryptophan are recognizable as bulges of high electron density protruding from the main chain; by contrast, glycines in non-helical regions sometimes show up by causing a marked constriction in the roughly cylindrical cloud of high electron density which represents the polypeptide chain.

A year ago, Watson and Kendrew published a preliminary comparison of the structure and sequence of hæmoglobin, but at that stage only parts of the chemical sequence were known[7]. In the light of the further knowledge now available, many interesting new features reveal themselves.

In the discussion which follows, the tertiary structures of equine and human hæmoglobin will be assumed to resemble each other closely. This has not yet been proved; but is very probable in view of the similarity in amino-acid sequence of these two hæmoglobins, and in view of the correspondence between the tertiary structures of the different species of myoglobin and hæmoglobin so far examined[9,10].

Chemical Identification of Chains in the Crystallographic Model

The two different chains of horse hæmoglobin have the *N*-terminal sequences valyl-leucyl and valyl-glutaminyl, and are similar in composition to the valyl-leucyl and valyl-histidyl-leucyl chains of human hæmoglobin[4,5], which have become known respectively as the α- and β-chains. Smith and Perutz have shown the valyl-glutaminyl chain to correspond to the black chain of the crystallographic model[8], from which it would follow that the black chain also corresponds to the β-chain of human



a *b*

Fig. 1. *a*, A model of the cloud of high electron density representing the α-chain of horse hæmoglobin. The letters *A*, *C*, *E*, *F*, *G* and *H* refer to various regions of chain identified as right-handed α-helices in myoglobin. The numbers in brackets give the sequential residue numbers associated with them. *EF*, *FG*, *GH* refer to corners or non-helical regions. Except for proline 4_α all the residues marked in this figure occupy the same sequential positions in human and equine hæmoglobin. Note the proximity of aspartic acid 75 to valine 1, the *N*-terminus

b, The β-chain. The residues marked on the model are taken from the sequence of human hæmoglobin. Cysteine 112_β is known to be replaced by another residue in equine hæmoglobin; there may be some other differences between the two hæmoglobins which affect this figure and which are not yet known. Note the position of glutamic acid 6, the residue which is replaced by valine in sickle-cell hæmoglobin; also the bulges marking the positions of tyrosine 35, tryptophan 37 and of phenylalanines 41 and 42, and the gap which marks the position of glycine 119 in the non-helical region *GH*. The hæm group lies at the back of the models

Table 1. ABBREVIATED AMINO-ACID SEQUENCES OF VARIOUS HAEMOGLOBIN CHAINS, ILLUSTRATING POINTS MENTIONED IN THE TEXT
For the α-chain of horse hæmoglobin only those residues are shown which differ from the corresponding ones in human hæmoglobin. In the β-chain of horse hæmoglobin only the *N*-terminal sequence is shown which differs from human hæmoglobin in three places. The vertical lines indicate tryptic peptides the composition of which is either the same as that of the corresponding peptide of human hæmoglobin or differs from it by only one amino-acid residue. In the γ-chain only those residues are shown which differ from the corresponding ones of the β-chain or (bracketed) where only the composition, but not the sequence of the peptide is known

Human α		Horse	Human β		Horse	Human	Remarks
No.	Residue	α	No.	Residue	β	γ	
			1	val		gly	
1	val		2	his	gluN		These residues form a hook at the *N*-terminal end which has no counter-part in myoglobin
2	leu		3	leu	│	phe	
3	ser		4	thr	ser		
4	pro	ala	5	pro	gly	glu	
5	ala		6	glu			glu 6β is replaced by valine in hæmoglobin S
14	try		15	try			Tryptophan responsible for photodissociation effect
15	gly		16	gly			
36	phe		35	tyr			
37	pro		36	pro			Proline in *CD* corner
38	thr		37	try			Tryptophan replaced by lysine in 'fast' horse hæmoglobin
44	pro		43	glu		asp	
45	his		44	ser			
46	phe		45	phe			
│	│		46	gly			α-Chain shortened by one residue
47	asp		47	asp			
48	leu		48	leu			
49	ser		49	ser			
50	his		50	thr		ser	
│	│		51	pro		ala	Proline at *N*-terminal end of helix *D* in β-chain
│	│		52	asp		ser	
│	│		53	ala			α-Chain shortened by five residues
│	│		54	val		ileu	
│	│		55	met			
51	gly		56	gly			
52	ser		57	asp			
53	ala		58	pro			Proline in corner *DE* in β-chain
54	gluN		59	lys			
55	val		60	val			
56	lys		61	lys			
57	gly	ala	62	ala			
58	*his*		63	*his*			Histidine on distal side of hæm
59	gly		64	gly			Glycine essential for short contact between helices *E* and *B*
60	lys		65	lys			
61	lys		66	lys			Three lysines in neighbourhood of distal histidine forming Braunitzer's 'basic centre'
76	met	leu	81	leu			
77	pro		82	lys			
78	aspN	gly	83	gly			Proline in non-helical region *EF* of α-chain
86	leu		91	leu			
87	*his*		92	*his*		his ?	Hæm linked histidine
88	ala		93	cysH		cysH?	Reactive cysteine in β-chain
89	his		94	asp		asp ?	
95	pro		100	pro			Proline in corner *FG*
104	cysH		109	val		val?	Unreactive cysteine in α-chain
107	val	ser	112	cysH	not cysH	thr	Unreactive cysteine in human β-chain
114	pro		119	gly			Proline in non-helical region *GH* of α-chain
115	ala	asp	120	lys			
116	glu	asp	121	glu			
117	phe		122	phe			
118	thr		123	thr			
119	pro		124	pro			Prolines at *C*-terminal end of helix *H*
120	ala		125	pro		glu	
139	lys		144	lys		arg	
140	tyr		145	tyr			Tyrosine forming hydrogen bond with main chain carbonyl in *FG* corner
141	arg		146	his			

hæmoglobin. We therefore have the following relations:

	N-*Terminal sequences*		*Crystallographic model*
	Horse	*Human*	
α-Chain	valyl-leucyl	valyl-leucyl	white chain
β-Chain	valyl-glutaminyl	valyl-histidyl-leucyl	black chain

Table 1 lists those parts of the sequences of human and equine hæmoglobin which are discussed in this article, while Fig. 1 shows the tertiary structures of the α- and β-chains with the positions of certain residues superimposed. The sequences of the α- and β-chains are similar, but to bring them into register all along their length appropriate gaps have to be left in certain positions. Thus a gap of one, and another of five, residues have to be assumed between residues Nos. 46 and 51 in the α-chain in order to keep its sequence in register with that of the β-chain. These gaps evidently correspond to a shortening of the α-chain which should be noticeable in the model. By looking up the positions of the corresponding residues in myoglobin the shortening can be traced to the non-helical region *CD* and the helical region *D* which lie in the loop at the top of the models in Fig. 1. It will be noted that in the white chain this loop is cut short compared with the black chain, showing that the shortening of the α-sequence has its corollary in the electron density maps.

Co-ordination of Hæm Groups

The hæm-linked residue in myoglobin has been identified as histidine. The α- and β-sequences each show a histidine in the corresponding position (87_α and 92_β), thus confirming that histidine is the hæm-linked residue in hæmoglobin, as had long been predicted from physico-chemical data[12-14]. Watson and Kendrew have pointed out that a second histidine occurs on the distal side of the hæm group in all three chains (62 in myoglobin; 58_α and 63_β); this histidine is not in contact with the iron atom, but is within hydrogen-bonding distance of the hæm-linked water molecule, and is the residue which is replaced by tyrosine in two of the forms of hæmoglobin M (ref. 15). This may be the second histidine in close proximity to the iron atom, but not directly linked to it, the presence of which has long been surmised[11–13,16,17]. It is most satisfactory to find these expectations confirmed by crystallographic analysis.

Position of Cysteines

All vertebrate hæmoglobins appear to contain at least one pair of cysteines the sulphydryl groups of which are reactive in the native protein. Their positions can be determined by X-ray analysis, using mercurials as a label. From the X-ray results, combined with model building, it was possible to show that in horse hæmoglobin the reactive cysteine is the residue immediately following the hæm-linked histidine in the β-chain, and a cysteine in this position has since been found by chemical sequence work in human hæmoglobin (93_β). The histidine and cysteine are part of an α-helix, and the side-chain of cysteine points in the direction away from the iron atom.

Horse hæmoglobin contains one other pair of cysteines which is unreactive in the native protein[18,19], but can be labelled with mercurials under appropriate conditions. This is attached to the helical regions *G* of the α-chains, and lies tucked away in the interior of the molecule, sandwiched between neighbouring sub-units. The position deduced by labelling with mercury corresponds exactly to that inferred for residue No. 104_α, the sequential position of the corresponding cysteine in human hæmoglobin, by analogy with the myoglobin structure.

Adult human hæmoglobin, but not fœtal human or horse hæmoglobin, contains a third pair of cysteines (112_β), the position of which in the model is similar to that of 104_α in that it is part of the helical region *G* and lies in the interior of the molecule. This pair is also unreactive in the native protein[19,20]. Neither of the unreactive pairs has any contact with the hæm group, nor are they in contact with each other or with residues of any chain other than their own. Thus their function is obscure. Their lack of reactivity in the native protein can be attributed to their situation in the interior of the molecule, though covering of the sulphydryl groups by immediate neighbours cannot be excluded at this stage.

Tryptophan

As has already been mentioned, the indol groups of tryptophan are recognizable as marked bulges of high electron density protruding from the main polypeptide chain, even at a resolution of only 5·5 Å. Tryptophan 14_α and 15_β appear in the exact positions occupied by tryptophan 12 in myoglobin and are part of the helical region *A*. The indol ring lies in the interior of the molecule, sandwiched between helices *A* and *E*; it is almost parallel to the plane of the porphyrin ring, but separated from the latter by helix *E*, and lies at a distance of 14 Å. from the iron atom. Since this is the only tryptophan which all chains of hæmoglobin and myoglobin so far analysed have in common, it is probably the residue responsible for the photodissociation observed when carbon monoxyhæmoglobin is illuminated at the ultra-violet absorption band of tryptophan. In this process a quantum must be transferred from the indol ring, across helix *E*, to the porphyrin ring. However, the photodissociation of carbon monoxide is unlikely to be of biological significance, and the common occurrence of tryptophan in this position suggests that it may actually play a part in the oxygen-combining activity in a manner which we cannot yet guess. The β-chain of human hæmoglobin contains another tryptophan in position 38. This is also present in the slow component of horse hæmoglobin, but in the fast component it is replaced by lysine[21]. Myoglobin contains a second tryptophan in position 5 which is absent in hæmoglobin. Evidently these tryptophans are not essential.

Prolines and Corner-turning

The two chains of human hæmoglobin each contain seven prolines which all lie in corners or non-helical regions of the chain, as had been suggested by Watson and Kendrew[7]. However, there are only three positions (the corner *CD*, the *N*-terminal end of helix *G* and the corner *GH*) where prolines occur both in myoglobin and in the α- and β-chains. The fourth proline of myoglobin (98) occurs in a position where the hæmoglobin chains have none, and curiously enough, the prolines which occur in each of the chains of hæmoglobin do not all occur at the same corners. For example, in the neighbourhood of the short helical region *D* the α-chain has no proline and the β-chain has two (51 and 58). On the other hand, the α-chain has a proline near the *C*-terminal end of the region *C* (44) where the β-chain has none. Admittedly, the regions *C*, *CD* and *D* are those where the two chains also differ most markedly in structure, but there is also proline 77_α which occurs in the non-helical region *EF* and has no counterpart in the β-chain. In the γ-chain of human fœtal hæmoglobin, which replaces the β-chain in the adult form, the prolines in positions 5 and 51 are replaced by glutamic acid and alanine respectively[22]. The two chains of horse hæmoglobin contain only six prolines each, and differ from the human chains by the absence of prolines 4_α and 5_β at the *N*-terminal end of helix *A*; these are replaced respectively by alanine and glycine. These results suggest that the corner-turning mechanism which depends on the presence of proline is readily replaced by others, and is therefore not of great evolutionary importance.

The correspondence between structure and sequence found in this work provides an independent check for the interpretation of the X-ray results. If the meaning of the electron density maps of either hæmoglobin or myoglobin had been misinterpreted, the correspondence between the observed and predicted positions of cysteine and other residues could not have been found.

A full account of this work, giving a complete description of the methods and results of the X-ray analysis, has been published by Cullis, Muirhead, North, Perutz and Rossmann[23].

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OBITUARIES

Dr. Ernst Schwarz

SYSTEMATIC mammalogy suffered a severe loss with the death on September 23, at Bethesda, Maryland, of Dr. Ernst Schwarz. Although in recent years Schwarz had retired from active work in systematic mammalogy to devote his energies to public health problems his name will long be remembered for his earlier fundamental contributions to the diagnosis of a very wide range of mammalian groups from marsupials to primates.

Schwarz was born at Frankfurt am Main on December 1, 1889. His academic studies (1907-12) were pursued at the Universities of Berlin, London and Munich, and he graduated Ph.D. at the last mentioned in 1912. During 1912–15 he was in charge of the mammal collections at the Senckenbergische Naturforschende Gesellschaft in Frankfurt, thereafter (1915–18) transferring his interests to the clinical laboratory of the University Hospital in the same city. His interest in medical science was continued after the First World War when, in 1919, he became research assistant to the Paul Ehrlich Institute for Experimental Therapy, where his interests were directed towards cancer research. In 1925 he returned to mammalogy, spending the next four years in research at the Zoological Museum of the University of Berlin. During 1929–33 he was custodian of the Natural History Museum of the University of Greifswald, but fled from Germany to London on the advent of the Nazi régime. He spent the next four years attached unofficially to the mammal room of the British Museum, whence many of his best-known papers emanated. In 1937 he transferred to the U.S. National Museum in Washington, where he remained for ten years, with numerous intermediate assignments such as a guest lectureship at the U.S. Navy Medical School at Bethesda (1942–43) and later (1947–53) to the Graduate Medical School, U.S. Naval Hospital, Bethesda. During 1948 he was organizing, planning and participating in the U.S. Navy African Expedition. In 1950 he went on a mission to Venezuela studying bubonic plague, becoming a member of the World Health Organization Commission on this disease. His last years, 1956–59, were spent as a guest research worker at the Armed Forces Research Institute of Pathology at Washington, where he was attached to the Section of Neuropathology.

Schwarz was decorated with the Medal of King Leopold II of Belgium and with the Distinguished Service Medal of the United States.

As a systematic mammalogist, Schwarz is considered a lumper; in fact, he was perhaps overzealous in this direction, as witnessed especially by his treatment of the *Colobus* monkeys—a fact he himself admitted after the publication of his studies. His very last publication dealt with pygmy marmosets, where here too he oversimplified the problems. The position is tersely assessed by Booth, who declared that "Schwarz's descriptions are meticulously careful, but his conception of the species is, in the case of the Primates, often too broad".

Of some sixty-eight scientific papers published by Schwarz, about two thirds were devoted to the systematics of mammals, living and extinct. The remainder deal with problems of applied medicine dating from his early interest in carcinogenesis and some few to general problems of distribution, genetics and evolution.

Deep sympathy goes to his widow, Henrietta E. Schwarz, whom he married during his period in London, and who was a constant help to him in his work.

W. C. OSMAN HILL

Dr. N. K. Sen

DR. N. K. SEN, who died on March 21, was born on February 26, 1917, the eldest son of late Jitendra Prosad Sen, subordinate judge, who was the son of the well-known Dewan Bahadhur Sarada Prosad Sen of Dacca. Dr. Sen graduated with honours in botany from the Presidency College, Calcutta, in 1936, and M.Sc. in 1939.

During 1939–41, he was lecturer in botany, Jagannath College, and during 1942–43 in the Dacca Intermediate College and the Rajsahi College (now in East Pakistan). He joined the Botany Department of Presidency College, Calcutta, as a lecturer in botany in 1943 and stayed there until 1948. For two years (1948–50) he was a graduate student, University of California. In 1951 he was awarded the Ph.D. degree in genetics by that University. In 1951 he was also awarded D.Phil. (Science) degree of the University of Calcutta.

In 1952, Sen joined the Agricultural Engineering Department (Applied Botany Section) of the Indian Institute of Technology as assistant professor of applied botany. He published more than fifty scientific papers on genetics, cytogenetics and breeding of pulses.

He started the first course in India on applied botany. He was the editor of *Bulletin of the Botanical Society of Bengal* (1961–62), and first editor of *Bijnan Parichaya*, a scientific Bengali monthly, published from Dacca and founded by Prof. S. N. Bose, Prof. P. N. Mazumdar, Prof. S. G. Mukherjee, the late S. K. Sen, etc., under the inspiration of the late poet Rabindranath Tagore.

R. M. DATTA

NEWS and VIEWS

The Queen's Birthday Honours List

The following names of scientists and others associated with scientific work appear in the Queen's Birthday Honours List:

K.C.B.: G. E. Godber, chief medical officer, Ministry of Health, Ministry of Education and Home Office.

K.B.E.: Sir Charles Harington, director, National Institute for Medical Research; F. W. G. White, chairman, Australian Commonwealth Scientific and Industrial Research Organization.

Knights: B. de Bunsen, principal, Makerere College, East Africa; T. F. Fox, editor of the *Lancet*; J. F. Lockwood, Master of Birkbeck College, University of London; J. T. Randall, professor of biophysics, King's College, University of London; F. J. Schonell, vice-chancellor, University of Queensland.

C.B.: A. W. Stableforth, director, Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food; R. W. Sutton, superintendent, Services Electronic Research Laboratory, Admiralty.

C.M.G.: J. C. F. Hopkins, director, Commonwealth Mycological Institute; D. Rhind, adviser on agricultural research, Department of Technical Co-operation; P. R. Stephenson, lately director, Desert Locust Survey; L. T. D. Williams, director-general of Atomic Weapons, Ministry of Aviation.

C.B.E.: A. C. Best, chief scientific officer, Meteorological Office; F. C. Fraser, keeper of zoology, British Museum (Natural History); A. C. Frazer, professor of medical biochemistry and pharmacology, University of Birmingham; T. R. R. Mann, director, Agricultural Research Council Unit of Reproductive Physiology and Biochemistry, Cambridge; J. M. Robertson, Gardiner professor of chemistry, University of Glasgow; T. L. Viney, director (works and buildings), engineering group, U.K. Atomic Energy Authority.

Ministry of Aviation Appointments :

Mr. F. H. East

Mr. F. H. East has been appointed head of Weapons Project Group, Weapons Department, Royal Aircraft Establishment, Farnborough. Mr. East was educated at the Skinners' Company's School, Tunbridge Wells, and at University College, Exeter. After taking a University of London degree in physics in 1940 he joined the Research Department of the Royal Aircraft Establishment in Exeter, where he was engaged on defence system research and the early experiments on proximity fuzes. He transferred to the Armaments Department of the Royal Aircraft Establishment in 1942 and worked on sighting systems for aircraft guns and rockets. He was given a permanent appointment to the Scientific Civil Service in the post-war reconstruction scheme and was responsible for the development of fuzing systems for a variety of weapons. He paid a number of visits to Australia to assist in the planning of facilities at the Woomera range and to carry out some of the early trials. In 1954 he joined the team at the Royal Aircraft Establishment responsible for the design of advanced weapon systems, and in 1957 was appointed assistant director of Air Armament Research and Development in the Ministry of Supply. He has been closely associated with the U.S./U.K. *Skybolt* project since its inception. He was elected an Associate of the Institute of Physics in 1941, an Associate Fellow of the Royal Aeronautical Society and a Member of the Institution of Electrical Engineers in 1958.

Mr. A. Stratton

Mr. A. Stratton has been appointed head of the Research and Assessment Group, Weapons Department, Royal Aircraft Establishment, Farnborough. Mr. Stratton was educated at the Skinners' Company's School, Tunbridge Wells, and University College, Exeter, obtaining honours in physics. He joined the Royal Aircraft Establishment in October 1939, his wartime field of work being research and development on radio proximity (*V.T.*) fuzes. This work was recognized by the awards of the United States Medal of Freedom with Bronze Palm and the sum of £2,000 by the Royal Commission on Awards to Inventors. He was appointed senior scientific officer in the post-war Reconstruction Competition (1946) and promoted to principal scientific officer in 1948. In 1954 he was appointed superintendent of Instruments Division in the Instrument and Air Photography Department of the Royal Aircraft Establishment, with the rank of senior principal scientific officer, where he worked on aircraft instruments and inertial navigation. He has published a number of papers in these fields. He was elected a Fellow of the Institute of Physics in 1945, an Associate Member of the Institution of Electrical Engineers in 1953 and a Member of the Institute of Navigation in 1960. In 1951 he obtained an M.Sc. (London) degree in mathematics and in 1957 a B.Sc. (Oxon.) *ad eundem*. He is at present chairman of Convocation, University of Exeter.

Electrical Engineering at Manchester :

Prof. E. H. Rhoderick

Dr. Emlyn Huw Rhoderick has been appointed to a newly created chair in electrical engineering in the Faculty of Technology, Manchester College of Science and Technology. Dr. Rhoderick was educated at Jesus College, Oxford, where he graduated with honours in the School of Natural Science (Physics) in 1941. In 1941 he was employed as junior scientific officer (afterwards scientific officer) at the Radar Research and Development Establishment, Malvern, where he was engaged on the development of microwave radar. He became a research student at Trinity College, Cambridge, in 1945, working at the Cavendish Laboratory in the field of nuclear physics, and graduated Ph.D. (Cambridge) in 1948. During 1948–54 he held the post of lecturer in natural philosophy at the University of Glasgow, where in the last three years he acted as head of the Physics Honours Class. For the academic year 1960–61 he studied as a Fulbright Fellow at Columbia University, New York, working on the radio-frequency spectroscopy of atoms. Since 1955 Dr. Rhoderick has worked at the Services Electronics Research Laboratory (Admiralty), Baldock, as principal and senior principal scientific officer, working on semi-conductors, and on superconductivity in thin films, recently as leader of a research group. He is also at present editor of the *Services Electronics Research Laboratory Technical Journal*.

Scientific and Technical Personnel in the United States

Scientific Manpower Bulletin No. 17 (April 1962), issued by the National Science Foundation, summarizes characteristics of scientists reporting to the National Register of Scientific and Technical Personnel, 1960. The median annual salary of United States scientists in 1960 was 9,000 dollars—a 14 per cent increase on the 1956–58 average. Of the 201,292 reporting, almost half were in private industry or self-employed, 28 per cent were employed by educational institutions, and 16 per cent by Government. Almost 75,000 were employed in research, development or design, nearly 30,000 in teaching and 49,000 in management or administration. The age group 20–29 claimed 18 per cent, 30–39, 40 per cent, and 40–49, 24 per cent. Chemists (53,000), biological sciences (27,000), engineering (24,000), physics (20,000) and the geological sciences (17,000) claimed the largest numbers. Some 25,000 scientists are at present reporting for 1962.

Overseas Students in Britain

THE Association of Universities of the British Commonwealth reports 13,385 students (1,862 women) enrolled for full-time study or research in United Kingdom universities at the beginning of the academic year 1961–62, compared with 12,410 in 1960–61 and 11,284 in 1959–60. Of these, 8,086 came from other parts of the Commonwealth, India sending 1,660 and Nigeria 1,124; 297 were studying agriculture and forestry; 3,415, arts; 165, dentistry; 2,157, medicine; 1,984, science; 2,316, social studies; 2,958, technology; and 93, veterinary science. Of the 5,716 postgraduate students, arts (1,639), medicine (875), science (1,152), social sciences (879) and technology (990) claimed the largest numbers, and most came from India (892), the United States (658), Canada (442), Pakistan (347) and Australia (290). Of the total, 5,850 held an award of some kind and 2,750 were living in college or hall of residence and 10,635 in lodgings or with friends or relatives. There were also 1,981 students enrolled for part-time study or research, of whom 948 came from other parts of the Commonwealth and 956 were postgraduate.

Degree Failures in British Universities

IN a written answer in the House of Commons on May 16, the Minister of Education, Sir David Eccles, gave the number of students with State Awards who failed their first degree examinations at the Universities of Cambridge and Oxford as 13 out of 1,381 in 1956–57 and 28 out of 1,516 at other universities; in 1958–59 the corresponding figures are: 14 out of 1,844 and 37 out of 1,331; and in 1960–61, 11 out of 2,048 and 40 out of 1,507. For Local Education Authority Awards at all universities the figures are: 1956–57, 599 out of 11,121; 1958–59, 697 out of 13,553; and 1960–61, 757 out of 16,022.

Geophysics and Oceanology in the Indian Ocean and Persian Gulf

THE Survey Ships H.M.S. *Owen* and H.M.S. *Dalrymple* returned to the United Kingdom on May 31 after a series of surveys, respectively in the Indian Ocean and Persian Gulf, as a result of which it has been discovered that the East African Continent may extend underwater almost as far as the Seychelles, nearly 1,000 miles from the existing coastline. Work carried out by H.M.S. *Owen* has revealed an offshore zone 200 miles wide and extending about 2,000 miles from Madagascar to Socotra. The zone is characterized by a striking absence of gravimetric and magnetic relief, suggesting that the whole zone may be underlaid by a wedge of sedimentary rocks, several miles in thickness. Geologists working on land in East Africa and Madagascar have suspected that the eastward-tilted Continent might continue beneath the deep water of the Indian Ocean, and the findings of H.M.S. *Owen* this year appear to confirm this. This work has been part of the British contribution to the International Indian Ocean Expedition, jointly sponsored by the International Council of Scientific Unions and by Unesco, and has two objects: the scientific exploration of the marine biology, water circulation and submarine geology of the Indian Ocean; and the encouragment of marine sciences in the countries bordering the Indian Ocean, the rising populations of which may soon compel them to turn to the sea for food. The scientific programme for H.M.S. *Owen* was planned by a Committee set up by the Royal Society at the invitation of the Hydrographer of the Royal Navy, and its execution was entrusted to the Department of Geophysics, University of Cambridge. H.M.S. *Dalrymple* has carried scientists from the Imperial College of Science and Technology, and during the season in the Middle East has obtained approximately 5,500 miles of magnetic profiling using a nuclear spin magnetometer. Although recording took place whenever possible, attention was mainly concentrated on the John Murray Ridge in the North Indian Ocean and its extension in the Gulf of Aden. For this special purpose the ship steamed along six tracks specially chosen to cross the Ridge, while all other profiles were obtained during normal working of the ship.

The City Museum, Sheffield

THE annual report for the year ending March 31, 1961, of the City Museum, Sheffield, records several new displays (Pp. 16+4 plates. Sheffield: The City Museum, 1962). In the Archæology Gallery, a four-year task has been brought to completion by the re-arranging of British material dating from the Palæolithic to Mediæval times. When the headquarters of the York and Lancaster Regiment were transferred from Pontefract to Sheffield in 1960, space for part of the extensive regimental museum was allocated at the Weston Park Museum. In the Natural History Galleries a series of new displays, designed to promote interest in bird protection and the conservation of wild-life in general, were introduced. A group of cases illustrating the National Parks of the Peak District, the Highlands of Scotland and East Anglia proved popular.

Dauntney's School Natural History Society

THE report of Dauntney's School Natural History Society covers the period 1959–61 and consists mainly of summaries of the individual field studies carried out by boys and submitted to the Cambridge examiners under their field work scheme (Pp. 44. West Lavington, Near Devizes: Dauntney's School Natural History Society, 1962). Most of their allotted time is devoted to community studies in woodland and fresh water near the school, and to work in the biological gardens, but after an introductory term boys are encouraged to select for study topics in which they are particularly interested. The subjects studied include *Basidiomycetes* on tree-stumps in Erlestoke Woods by H. T. H. Cromack; the size of *Gammarus pulex* in fast- and slow-running streams by K. Houston; the growth of *Limnaea pereger* in a wild population by F. P. H. Huntingdon; the performance of *Endymion non-scriptus* in open and shaded areas by D. S. I. Taylor; and the effects of temperature on air breathing in *Limnaea stagnalis* (L) by R. L. Page. The report also contains details of the School's biological gardens and the meetings held by the Society.

Electrical Engineering *Conference Report Series*

As one of the several alternative methods for the rapid publication of the proceedings of conferences and symposia, the Papers Committee of the Institution of Electrical Engineers has launched a new series, *Conference Report Series* (1. Pp. ii+27. London: Institution of Electrical Engineers, 1962. 5*s*.). The first number contains a report of the proceedings of the symposium on nuclear electronics held during November 20–December 1, 1961, at Savoy Place, London, which was organized by the Measurement and Control Section of the Institution in conjunction with the British Nuclear Energy Conference. A conference on the same subject was held by the International Atomic Agency at Belgrade during May 1961. The Institution's symposium was held in order to enable those who were not fortunate enough to attend the Belgrade conference to hear and discuss reports on the material presented. Copies of the papers presented at that conference are not yet available, but the proceedings are to be published this year by the Agency. There were three sessions at the symposium devoted to radiation detectors, electronic circuits and techniques, and radiation monitors and instruments, respectively. Contributions included reports on the Belgrade conference by W. Abson, K. Kandiah and D. Taylor, and brief notes by J. B. Birks on improved organic scintillator detectors; Prof. A. W. Merrison and others on data-logging equipment for nuclear physics experiments; and D. E. Barnes on plutonium monitoring.

Standardized Genetic Nomenclature for Mice

THE Committee on Standardized Genetic Nomenclature for Mice has prepared two listings of inbred strains of mice, arranged alphabetically according to standardized designations. These appeared in *Cancer Research* (12, 602; 1952; and 20, 145; 1960). While virtually all investigators who maintain colonies of inbred mice have accepted the standardized nomenclature in theory, a few of them are careless in practice. Of those investigators who do not raise their own animals, some are often ignorant of the rules and may copy incorrect usage from other sources; others who know the rules may at times be careless. The Committee feels that it is as important to use correct designations for strains as to spell ordinary words correctly, and has now made available reprints of the second listing. Copies of this can be obtained from: Dr. Mary F. Lyon, Radiobiological Research Unit, Harwell, Didcot, Berks; Dr. N. N. Medvedev, Laboratory of Inbred Animals, Academy of Sciences of the U.S.S.R., Moscow, Solyanka 14; or Miss Joan Staats, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.

Fluorescence Microscopy in the Diagnosis of Pulmonary Tuberculosis

THE demonstration of acid-fast bacilli in smears of sputum remains the most rapid laboratory technique for a presumptive diagnosis of pulmonary tuberculosis. The widely used and classical staining technique of Ziel-Neelsen has limitations, however, in Africa, where there are limited laboratory facilities and where there is a shortage of trained laboratory staff. The technique may not always be accurate when used in the large out-patient clinics, for technicians are pressed for time and long and tiresome scanning of smears is often necessary before the presence or absence of bacilli can be assumed. Thus, a more efficient method is required. A method of fluorescence microscopy for the detection of acid-fast bacilli was introduced by Hagemann in 1937 and has been developed by others over the years. The method consists of staining bacilli by the fluorescent dye auramine, to which they are acid- and alcohol-fast, and which has the property of converting rays at the lower end of the spectrum to light of longer wave-length. When auramine-stained bacilli are subjected to a source of ultra-violet light which has been passed through a filter to absorb blue-violet and ultra-violet rays, they fluoresce; if a yellow filter is placed in the eyepiece they appear yellow against a dark field. The fluorescence is clearly visible microscopically, and a magnification of 200–300 times is sufficient to recognize acid-fast bacilli. A paper by Dr. W. J. Bell and Dr. P. P. Brown, of the West African Council for Medical Research, Tuberculosis Research Unit, Ghana, presents evidence for the accuracy of the method of fluorescence microscopy when used by junior technicians in Africa (*Cent. Afric. J. Med.*, 8, No. 1; January 1962).

Medical Research Council : New Research Group

THE Medical Research Council is to establish a Research Group on the Relationship between Organic and Functional Mental Disorders in the Department of Psychological Medicine, King's College, Newcastle-upon-Tyne (University of Durham), under the honorary direction of Prof. Martin Roth.

The International Society on Toxinology

A NEW international society devoted to the advancement of knowledge on the plant and animal toxins was recently formed in Atlantic City, New Jersey. The new society is known as the International Society on Toxinology and will bring together biochemists, pharmacologists, toxicologists, physiologists, immunologists and physicians with interests in the toxins derived from the tissues of plants and animals. Founding officers are: *President*, F. E. Russell, Los Angeles; *Secretary-Treasurer*, P. R. Saunders, Los Angeles; *International Co-Chairmen*, H. Michl, F. Ghiretti, A. de Vries, B. N. Ghosh, S. A. Minton, H. A. Reid, T. M. Yanagita, J. M. Gonclaves, J. F. Gennaro and M. Whiting. The office for the new Society is Box 323, 1200 N. State Street, Los Angeles 33, California. The journal *Toxicon* will become the official organ of the new group and will carry notes and announcements of the Society's activities.

Beit Memorial Fellowships

THE following elections to Beit Memorial Fellowships have been announced: *Junior Fellowships*,

P. P. Dendy, to investigate the effects of irradiation with a microbeam of ultra-violet light on the protein metabolism of cells, at the Department of Radiotherapeutics, University of Cambridge; D. Gompertz, to investigate the effect of pituitary growth hormone on fat synthesis, at the Department of Biochemistry, University College, London; M. R. Lee, to investigate the control of the generation of vasoactive polypeptides in the blood, at the Department of the Regius professor of medicine, the Radcliffe Infirmary, Oxford; Dr. G. F. Rowland, to investigate nucleic acid synthesis in the isolated pre-cancerous nucleus, at the Department of Chemical Pathology, University College Hospital Medical School, London; Dr. Sheila M. Day, to investigate the factors that influence the binding of amines in normal and neoplastic mast cells, at the Department of Pharmacology, Royal College of Surgeons of England, London; E. A. Newsholme, to investigate the importance of the 6-phosphofructokinase reaction in the regulation of muscle glycolysis, at the Department of Biochemistry, University of Oxford; J. S. Wigglesworth, to investigate the cytology of the human placenta in normal and abnormal pregnancy, at the Department of Morbid Anatomy, University College Hospital Medical School, London.

The Royal Institution: Open Days

Open days at the Royal Institution are being arranged this year for June 14–15. To mark the centenary of the birth of Sir William Henry Bragg (1862–1942), who was director of the Davy Faraday Research Laboratory from 1926 until 1942, X-ray crystallographic laboratories in Britain are providing a series of exhibits which illustrate the historical development of the subject. The main emphasis of the research work at present proceeding in the Davy Faraday Laboratory is on the application of X-ray diffraction methods to the study of biological molecules, and a number of rooms in the Laboratory, where some of the latest work in this field can be seen, will be open to visitors. The exhibition will be open each day from 10 a.m. to 1.0 p.m. and from 2.30 p.m. to 6.0 p.m. Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W.1.

Course on Aerial Photogrammetry

A theoretical and technical course on "Aerial Photogrammetry", arranged by the French National School for Geographic Sciences, will be held at Saint-Mandé, near Paris, during June 18–July 27. The programme of lectures will include: general problems of aerial photogrammetry; problems concerning aerial surveys; principles, construction and adjustment of plotting machines; problems concerning mean scales (1/5,000 to 1/50,000) and small scales (1/100,000 and 1/200,000) plotting; practice of aerial triangulation; mechanical and electronical methods for compensation of blocks in aerial triangulation. There will also be practical instruction on the use of instruments and visits to different places concerned with photogrammetrical activities. Further information can be obtained from the Director, l'Ecole Nationale des Sciences Géographiques, 2 Avenue Pasteur, Saint-Mandé (Seine).

Conferences on Dermatoglyphics

On the occasion of the Second International Conference on Human Genetics, held in Rome during September 6–12, 1961, an organization devoted to research on finger-prints, palms and soles (dermatoglyphics) was formed. Its principal objectives are to promote activity in this field, to distribute information on current investigations, to standardize methods and to hold meetings for the presentation of reports and discussions. Interested workers are invited to communicate with any member of the Organizing Committee: *President*, Prof. Harold Cummins, Tulane School of Medicine, 1430 Tulane Avenue, New Orleans 12, Louisiana; *Chairman*, Dr. José Pons, Universidad de Barcelona, Barcelona; *Secretary*, Mr. Abhimanyu Sharma, 40/*C* Hanuman Lane, New Delhi-1; Dr. Mariassa Bat-Miriam, Israeli Institute for Biological Research, P.O.B. 19, Ness Ziona; Dr. Sarah B. Holt, University College, Gower Street, London, W.C.1; Commissioner C. H. Vogelius Andersen, Danish Identification Bureau, Politigaarden, Copenhagen V; Dr. Margaret Weninger, Universitat Wien, Van Swietengasse 1, Wien IX; Prof. Masaya Yasunaka, Nagasaki University School of Medicine, 1699 Sakomoto-machi, Nagasaki-City.

Announcements

Under the Statute of the Royal Society which provides for the election of persons who either have rendered conspicuous service to the cause of science or are such that their election would be of signal benefit to the Society, the Right Hon. Harold Macmillan has been elected a Fellow of the Royal Society.

Consequent on the acquisition of the shares of J. W. Towers and Co., Ltd., by A. Gallenkamp and Co., Ltd., the name of J. W. Towers and Co., Ltd., has now been changed to A. Gallenkamp and Co. (Northern), Ltd. The two companies will in future both trade under the registered business name of 'Gallenkamp. (Reg. trade mark)'.

The British Cast Iron Research Association is holding open days during July 3–4. Visitors will be able to see the Association's new laboratory, which is to be inaugurated by the Marquess of Exeter on July 3, and a representative selection of the Association's work will be on show. Further information can be obtained from the Publications Officer, British Cast Iron Research Association, Bordesley Hall, Alvechurch, Birmingham.

A symposium on "Sonar Systems", sponsored jointly by the British Institution of Radio Engineers, the Institute of Physics and the Physical Society and the Electrical Engineering Department of the University of Birmingham, will be held in the University of Birmingham during July 9–12. Subjects under discussion will include: propagation; survey equipment; sonar for fisheries; arrays and signal processing. Further information can be obtained from the British Institution of Radio Engineers, 9 Bedford Square, London, W.C.1.

The annual meeting of holders of Nobel Prizes will be held in Lindau during June 24–29. This year the lectures will be given by physicists, among whom will be: Prof. N. Bohr (Copenhagen); Prof. H. C. Urey (California); Prof. R. Hofstadter (California); Prof. J. Franck (North Carolina); Sir John Cockcroft (Harwell); Sir Edward Appleton (Edinburgh); Dr. J. Bardeen (Illinois); Prof. M. Born (Bad Pyrmont); Prof. W. H. Brattain (New Jersey); Prof. W. Heisenberg (Munich). Further information can be obtained from Hans Kiderlen, Nonnenhorn/Bodensee, Uferstrasse 65*a*.

POTATO DISEASES

POTATO production is beset with persistent troubles which all add to uncertainties of cropping, so potently felt in Britain at present. In addition to vagaries of weather, it sustains the heaviest loss from disease of any single crop—28 per cent of total value in west Scotland, and only about half this loss is controllable by economic methods known at present. Here is a challenge, and it is most opportune that the Pathology Section of the European Association for Potato Research arranged a meeting at the Edinburgh School of Agriculture during March 26–29. It was particularly well organized by Dr. A. E. W. Boyd, chairman of the Section, with the assistance of Dr. J. H. Lennard as secretary.

The first session discussed black scurf (*Rhizoctonia solani*) and common scab (*Streptomyces scabies*), two 'nuisance' diseases which reduce market value in times of plenty, out of all proportion to their minute effect on nutritive quality though *R. solani* can also reduce the yield. R. E. Labruyère reported the appearance of both diseases in newly reclaimed virgin polders in the Netherlands; infection by *R. solani* (though not of scab) could best be prevented by treatment of seed tubers with organo mercury compounds. In Sweden, G. Smedgård had used thiram powder (TMTD) as a control of black scurf on the tubers, with increases of yield between 8 and 16 per cent. Elizabeth G. Gray reported that fumigation of washed or brushed tubers with trichloroethylene greatly reduced germination of sclerotia, but warned that the bacterial disease blackleg could be increased by washing and chemical treatment. Organo-mercurial compounds are toxic, and D. C. Graham has investigated the use of safer organo-tin substances; one gave good control of *R. solani* but is at present uneconomic. Soil treatment with PCNB was found by O. Wagn to control both scab and black scurf in Denmark. B. G. Lewis described his experiments on moisture-level in scab infection, a subject on which much information is needed. Control by resistant varieties would be ideal though commercial stocks are all more or less susceptible, but R. K. McKee described the testing of new varieties.

Potato root eelworm, *Heterodera rostochiensis*, is not so much a cause of present loss as a huge menace for the future. The present policy is to avoid potato growth in infested ground; but the amount of eelworm-free land is diminishing exponentially. Again the ideal solution would be to produce resistant varieties, and suitable genes occur in certain wild *Solanum* varieties. There are, however, resistance-breaking biotypes of the parasite, as described by J. M. Dunnett (Scotland) and C. A. Huijsman (Netherlands). An extensive survey by F. G. W. Jones, followed by detailed assessment, showed that resistance derived from *S. andigenum* would be immediately useful on about half the potato fields of Britain, that from *S. multidissectum* on about one in ten and that from *S. vernei* on about nine out of ten. The possibility of obtaining clones resistant to more than one biotype has been investigated by H. W. Howard, using hexaploid *S* × *juzepczukii*, but so far no seedlings with dual resistance have occurred. H. den Ouden showed that eggs from cysts less than a year old gave lower rates of reproduction than those from more mature cysts, and E. Dunn described how 'pre-conditioning' at 32° C. stimulated greater larval emergence than at lower temperatures. In direct control, J. E. Peachey found the most satisfactory assay of performance was by root invasion and that sodium methyl dithiocarbamate was the best of 12 compounds tested. J. Grainger described practical 'disease escape' where first-early potatoes can grow before the eelworm becomes active at a soil temperature of 7° C. A suitable variety must be able to devote a large proportion of its dry weight to tuber production during low temperatures and short, dull days, and, so far, none better than the variety Epicure has been found. He also demonstrated a new type of tractor-mounted machine for mixing the cheaper non-volatile control materials very intimately with the soil mass, thus bringing the possibility of control sufficiently economic for field use.

An urgent problem with bacterial disease is to exclude from temperate countries the pathogens *Pseudomonas solanacearum*, causing brown rot, and *Corynebacterium sepedonicum*, giving a tuber ring rot Bacteriologists held a separate session under the chairmanship of Dr. D. C. Graham as a result of the European Plant Protection Organization conference on this subject in Paris, 1960. A. C. Hayward reported four strains of the brown-rot organism and the discovery of such variants makes it more imperative than ever to avoid the introduction of this disease on ware potatoes imported during the present potato shortage in Britain. International quarantine arrangements make a co-operative approach impossible, so the value of such consultations as were held in Edinburgh is greatly enhanced.

Late blight, *Phytophthora infestans*, is the most serious potato disease at present. G. A. de Weille showed that bright sunshine reduced spore germination by lowering air humidity and by direct inactivation. Sunshine and rainfall forecasts can be used to predict the progress of crop infection. I. F. Storey found that only some 'Beaumont' periods actually forecast the first appearance of the disease, and V. Mélard compared genetically produced field resistance of the foliage with hypersensitive resistance and correlated both with tuber resistance, where the disease causes the heaviest loss and that most difficult to control.

The production of virus-free potato seed has helped to stabilize yield more than any other factor, and the process could be carried further if transmitting aphids on the crop could be controlled. Menazon, a systemic insecticide, has been used by J. F. Newman as a tuber dressing to protect the young sprouts for eight weeks, and C. E. Taylor reported a correlation between severity of aphid attack and physiological state of the host plant. Short communications were made by Maria Kostrowicka (unusual symptoms of potato rots), Mary Noble on races of wart disease which can attack 'resistant' varieties, and E. P. Keller on the detection of tuber discolorations by the relative transmission of strong light through them. Visits were made by members of the conference to the Scottish Plant Breeding Station, Pentlandfield, for demonstration of genetic resistance to blight, root eelworm, scab and viruses, and the Agricultural Scientific Services Station of the Department of Agriculture and Fisheries for Scotland, for disease testing, eelworm assessment and experimental washing and disinfection. Visits were also made to the original Wedderspoon plant at Castleton, Eassie, for commercial washing and fungicidal dipping, and a store at Muthill, Crieff, where the palette system of storage is utilized, and where fungicidal dipping of cut ware tubers is carried out before planting. An informal reception by the Potato Marketing Board completed the proceedings.

Shortage of potatoes engenders public clamour and the acceptance of relatively low standards of quality: excess of supply excites less comment, but brings a fussy rejection of tubers with but slight blemish from scab or blight. Neither extreme is good, and the Potato Marketing Board has gone as far as it can by administrative means to steer between plenty and scarcity. The only valid long-term solution requires the technical discovery of an economic method of preserving surplus production for human food in times of shortage, and the Board has initiated a research programme that includes this. Such a technical solution would, moreover, contribute to the larger question of adjusting the world's food supply to its growing population. J. GRAINGER

THE BIOLOGICAL ENGINEERING SOCIETY

THE Biological Engineering Society held its second meeting of the year in the Physiological Laboratory, Cambridge, during March 23 and 24, under the chairmanship of the president, Prof. R. Woolmer. Sir Bryan Matthews, the guest speaker, described some engineering methods in physiology. Sir Bryan gave some instances both of the way in which apparatus has been designed at Cambridge, both past and present, to assist in teaching and research, and of the ways in which advanced engineering principles are found in biological material; the mammalian ear was invoked as an elegant example here.

P. E. K. Donaldson (Physiological Laboratory, Cambridge) described a machine for the automatic simulation of human skills. The simplest skills seem to be those of the $S \to R$ kind, in which S and R can each be described by a single continuous variable. Tracking tasks, and tasks involving the stabilization of unstable systems (for example, bicycle riding) require skill of this class. It has been known for some time that human operators can be represented quite closely by relationships of the form:

$$R = e^{-p\tau} (Ap + B + C/p) S$$

Mr. Donaldson described a machine which automatically forms an image of a human operator performing a simple balancing trick, and in so doing finds values for A, B and C which, on the average, give the best fit. It is hoped to develop this device so as to be able to describe analytically what happens when an individual's skill improves with practice, or when it deteriorates in consequence of various kinds of distraction.

Dr. H. Barlow (Physiological Laboratory, Cambridge) discussed learning machines and generalization. Dr. Barlow felt that designers of existing learning machines, or of proposed learning machines, are trying to achieve too much too quickly. These machines can be regarded as linking units in which input data from the environment are acted on by a number of 'demons' which operate on the inputs in a variety of ways to form the outputs of the machine. Some sort of 'teacher' monitors the outputs in the light of some criteria, 'rewarding' the demons when their output is deemed valuable and 'punishing' them when it is not. Though such systems can be made to work, they are characteristically unable to do any kind of generalization, for example, to respond correctly if gaps or mistakes occur in the input data.

What is needed in order to generalize and fill gaps is knowledge of the statistics of the input. This leads to the idea that linking units should be preceded by a 'precoder', the function of which is to form, from the raw inputs derived from the environment, new signals with known predetermined statistical properties. It is these new signals which are fed to the linking unit. Such precoding may be done by a demon-flogging feedback technique, as is done in the linking unit itself, but with some important differences: the rate of feedback of information is much larger than the 1·6 bits per trial gained in the linking unit (outcome of each trial 'reward', 'punish' or 'neutral', $\log_2 3 = 1{\cdot}6$); the statistical requirement can be built in; and the teacher does not need to have access to the raw inputs. The output of the precoder is simpler than the raw input, since its statistics are now known, and forms a more suitable input to the linking unit.

The dynamics and operating characteristics of a common artificial kidney system were described by H. A. F. Dudley (Department of Surgery, University of Aberdeen). Dr. Dudley has been carrying out some performance measurements on an artificial kidney of the twin-coil type, the channels being 20 m. long. The performance of such a device should be described by:

$$D = V\left[1 - \exp - \frac{PS}{V}\right]$$

where V is the rate of flow of blood, D is the rate of flow of filtered blood in the output, P is the permeability constant for the 'Cellophane' membrane, and S the surface area of the membrane. For small values of V the theoretical performance can be obtained in practice, but for higher values, which are nevertheless within the range of flow-rates possible in human subjects, the artificial kidney falls far short of what may be expected; especially if the output blood pressure is high. The discrepancy seems to be caused, at least in part, by a decline in the surface-to-volume ratio of the blood channel toward the output end. The deficiency can probably be remedied by a change in the design of these instruments; but in any event it is clear that preliminary work with models is necessary to produce an artificial kidney to meet a previously laid down specification.

R. L. Gregory (Psychological Laboratory, Cambridge) gave a talk on "Digital Recording for the Poor Man".

It frequently happens that experimental results are recorded in a manner which retains more information than is actually required. For example, in measuring a quantity by averaging a number of determinations the mean value and the standard deviation may be important, whereas the serial order in which the readings are obtained is not. Mr. Gregory described a simple recorder he has designed in which advantage is taken of the economy possible here. A display is built up by dropping steel balls into cells in a 'Perspex' sheet or block. In one application the recorder builds up, from balls, a histogram showing the distribution of a reaction time. When the stimulus is delivered, a ball is transferred from the magazine to a moving belt, which carries it over a line of cells. When the subject responds, the ball is released and drops in the appropriate cell beneath it. The process repeats with the delivery of the next stimulus. If the reaction time is the same, the new ball will fall into the same cell as its predecessor; in general the time will be slightly different, and the ball will fall into a neighbouring cell. Mr. Gregory pointed out how easy it should be to find the mean and standard deviation of the completed display; the former by finding where it balances on a knife-edge, and the latter by measuring its moment of inertia. So far as the display proper is concerned, there is no reason why the base of the histogram should not be some quantity other than time; the ball dropper can be positioned by an appropriate servo-mechanism. Further, the base of the histogram can be plane rather than linear, so that 'solid' models of joint frequencies may be built up.

Electrochemical recorders and memories were discussed by H. S. Wolff (Bio-engineering Laboratory, National Institute of Medical Research, Hampstead, N.W.3).

Mr. Wolff began with a disavowal; what he was going to talk about were proposals rather than achievements. He needed to produce data recorders very much smaller than those at present in general use, sufficiently small to be worn by human subjects for long periods without causing inconvenience or discomfort. A possible solution was a small electrochemical cell possessing two anodes and one cathode.

One anode would be of simple metal, the other of a long-life radioisotope of the metal, for example, nickel and nickel-64. The quantity to be recorded would control the mark/space ratio of a multivibrator, which in turn would switch plating current between the two anodes. In this way, the quantity to be recorded would be laid down as a fluctuation in the proportion of radio nickel with the depth of the coating built up on the cathode. Such a record could be read back once, by unplating the cathode again and measuring the radioactivity of the electrolyte solution with a suitable detector.

Another problem was some way of storing a number without the continuous expenditure of energy. In contemporary analogue computing practice this is done by a servo-driven potentiometer; this technique works quite well, but is neither very small nor very cheap. An arrangement which would be both small and cheap would be to have a junction photocell with between it and a source of illumination a semi-transparent electro-plated film; the output of the photocell would depend on the thickness of the electro-deposited layer. It remained to be seen whether it would be equally satisfactory in other respects.

"Monkey Perimetry", was the subject of a paper by L. Weiskrantz (Psychological Laboratory, Cambridge). Dr. Weiskrantz and Dr. A. Cowey have been examining the effect of experimentally produced brain lesions on the field of vision of monkeys. To map the boundaries of a monkey's visual field, it is clearly not possible to use the same technique as is used with human subjects. The best that can be done is to train the monkey to respond in a recognizable way if it sees a small light flashed on; then to flash lights on, one by one, a very large number of times and from a number of different directions, recording each time where the monkey was looking. By subtracting the co-ordinates of the monkey's point of regard from the co-ordinates of the light flashed, one can—by knowing also whether the monkey saw the light or not—build up eventually a map of the monkey's perimeter of vision.

The problem was to measure where the monkey was looking. This was solved by having in front of the monkey, in addition to the field of small test lights, four bigger lights permanently switched on; one above and one below the test field in the sagittal plane, and one each side of the test field in the coronal plane. The monkey's eye is photographed during each presentation of a test light. In the photograph the four are seen reflected in the monkey's eye as four bright points arranged round the pupil. By measuring the distances from the bright points to the edge of the pupil, the direction of gaze can be inferred.

Unfortunately, such measurements are sensitive to translations of the monkey's eyeball as well as to rotations; but this difficulty is met by obliging the monkey to look through a peephole. The precision of each determination obtained by the technique is estimated at plus or minus two degrees of arc.

R. H. J. Brown (Zoological Laboratory, Cambridge) described the measurement of the ground reaction of the jumping locust.

Dr. Brown had carried out a rough calculation to establish the peak tension achieved in the extensor muscle of a locust's tibia when it jumps. This came out to about 1·5 kgm. and it was felt that this remarkable figure needed some experimental confirmation; the apparatus described was built in consequence.

The locust jumps off a platform stiffly mounted but capable of movement in the vertical and one horizontal direction; such movement is coupled to two capacitance transducers. In this way the transducers measure the horizontal and vertical components of the locusts's ground reaction in jumping off, effectively, a rigid platform. The damping of the mechanical system is rather below critical, and the resonance occurs at 500 c./s.; the sensitivity is 40 pFs./kgm.

In the electronic gear associated with each transducer, the capacitance change alters the frequency of an inductance-capacitance oscillator and hence the voltage output of a coupled-in tuned detector circuit; this voltage is the output of the device and may conveniently be displayed on a cathode ray oscilloscope. The sensitivity is 20 V. per pF., giving an overall sensitivity in each of the orthogonal directions of 800 V./kgm.

The equipment is not fully developed yet, but preliminary experiments confirm the earlier calculation; the locust can indeed produce a tension of 1·5 kgm. when it jumps.

P. E. K. DONALDSON

MANAGEMENT AND RESEARCH WORKERS

IN both industry and Government, a costly and dangerous trend is emerging—a trend towards a kind of management system the pattern of operation of which is incompatible with that required for highly creative performance by scientific and engineering staff. It is a trend toward tighter budgetary controls and tighter organization of the work. It is brought about by the impact of greater competition and, even more, by faulty information concerning the relative effectiveness of various approaches to the reduction of cost and improved performance.

Prof. Rensis Likert suggests in the March issue of *International Science and Technology* that this is a dangerous trend because tighter controls, while appearing to provide desired results over a short-run period, may damage an organization over a longer span of time. As a counter-measure, he proposes a management system which enables scientists and engineers to function at their most creative level. This is a system which has evolved from an extensive programme of research by the Institute of Social Research of the University of Michigan. From the studies a number of observations concerning the performance of technical people has emerged.

When communications within the organization is frequent, scientists and engineers are likely to perform better. For example, patent applications by technical personnel are higher in such organizations, and employee evaluations of one another are also higher. Frequent communications with colleagues who think differently from one another also improve an organization's performance. Scientists who do communicate with one another, but who are not conscious of a need for competent colleagues—who maintain an independent frame of mind—tend to be better performers than those who are more dependent.

Scientists and engineers who see their administrative chief often perform better than those who do not. Their performance is better still when they can also set their own technical goals, or at least have some influence on their chief in setting those goals. Best performance is observed when the scientist has high self-determination combined with free access to someone in authority. The potentialities of younger subordinates are best developed by the supervisor who can maintain the difficult synthesis of close interest in the young man's work without domination of it. If the technical man's personal motivation is low and, if he is not deeply involved in his work, it is not advisable to allow more than moderate self-determination in his work. If his motivation is high, then full self-determination leads to best results.

These observations point to the important general conclusion that scientists and engineers are likely to be most creative when their supervision is such that they feel substantial freedom in their work—in selecting their problems and goals, in deciding on the approach to achievement, and in interpreting their data—and when they have frequent interaction with their superiors. These findings are valid for the administration of basic research, developmental research, and engineering. Such conclusions are often greeted with scepticism. To some it seems that the conclusions are obvious, needing no research to prove its validity. Many research directors argue that "my people do feel substantial freedom". Prof. Likert counters with examples to show that the true feelings of a manager's subordinates are probably quite different from what he believes them to be.

The other kind of scepticism involves a more traditional bias: if you allow too much freedom people will take advantage and the organization will suffer. Here studies have proved the reverse of this, but they have also shown that it takes time to institute such a programme. During a short period of, say, one year, the kind of programme proposed is likely to prove less productive than one that is oriented round tighter controls.

T. H. HAWKINS

EDUCATION RESEARCH IN BRITAIN

IN reply to a question in the House of Commons on April 19, the Minister of Education, Sir David Eccles, said that he had offered £5,000 a year for three years to the Educational Foundation for Visual Aids towards the running costs of its new experimental development unit; £16,000 over four years to the National Foundation for Educational Research for research into the relative merits of block release and day release; about £12,000 over three years to the University of Sheffield for research into the logical programming of mathematics syllabuses; and about £9,500 over four years to the University of Manchester for a sociological study in grammar schools. He expected that actual grants in 1962–63 for these projects would total about £12,000, and he was considering other applications.

Later, on April 19, in a debate on educational research opened by Mr. J. Boyden, the adequacy of the £20,000 which the Minister was making available for educational research was challenged. Starting with the comment in the Crowther Report that in a consistent programme of educational development almost the first step should be to review the provision for statistics and research, Mr. Boyden strongly urged more generous support for the educational research foundations. He also quoted Sir Howard Florey's appeal to the Parliamentary and Scientific Committee for greater support for university research. The universities needed more accommodation, more qualified and technical assistance and more adequate funds for research. In particular, evidence submitted to the Robbins Committee from the British Association for the Advancement of Science had been concerned with the relative lack of research facilities in sociology, social psychology and social anthropology. The lack of consistent policy in developing the social sciences within the university framework had very greatly added to the difficulties of building up a body of knowledge and methodology suitable for the study of the rapidly changing social conditions in Britain and elsewhere. It was still insufficiently realized that social fact-finding, by means of intensive and extensive field and documentary research, was both essential and very expensive. Finally, besides urging that every major educational undertaking should be so far as possible based on the results of research, Mr. Boyden suggested several questions in which research could provide a guide to policy; he also stressed the need for stable careers in educational research, and thought that a Council for Educational Research was desirable.

Mr. P. Goodhart thought that besides research into the problems of learning there was even greater need for research into the technological problems of British outlook. Mr. S. Irving suggested that one reason for the neglect of educational research was the tendency to regard education as an art and not a science. Supporting the idea of a separate Council for Educational Research, he thought more co-ordination was needed, but that the main effort in research should not come under the establishment responsible for the administration of our educational system. He thought Britain could profitably spend £25,000 a year on such research, and in this view he was supported by Mr. A. Albu, who, however, thought that educational research should be part of a wider field—that of a social science or human sciences research council, rather than a separate educational research council. Mr. F. Willey, supporting the idea of an educational research council, thought that such a council should be responsible to the Lord President of the Council, and thought there should also be a close relationship with the Ministry of Education.

In replying to the debate, which he welcomed, Sir David Eccles agreed that the tendency to regard education as an art instead of a science might be one reason for the neglect of educational research, although more such research was proceeding than was generally known. Referring to the administrative changes which were in progress to expand research and intelligence in the Ministry, drawing largely on the Inspectorate for the staff of the new Curriculum Study Group, Sir David said besides this Group there was a new Research and Intelligence Branch and a much stronger Statistics Branch. For the moment, the main task of the Curriculum Study Group would be to identify gaps in our knowledge which might be filled by research, and the Research and Intelligence Branch would then see which of these gaps could be filled and by whom. Two kinds of project were visualized. The first of these would be organized from outside the Ministry, and here Sir David hoped to gain the assistance of a committee of experienced advisers, while the second would be organized within the Department to assist the Minister in policy decisions. At present the Curriculum Study Group was engaged on a study of examinations below the level of the General Certificate of Education arising out of the Beloe Report.

A new consortium of manufacturers and local authorities under the ægis of the London County Council, called the Science Equipment Consortium, was doing for science equipment what had been done for some building materials by the Architects and Buildings Branch. Besides the field of science teaching being taken up in the Nuffield Foundation project (see *Nature*, 194, 238; 1962) there was the whole field of visual aids. In conclusion, Sir David agreed as to the importance of stable careers for research workers, expressing also the opinion that some interchange between teaching, administration and research would be useful.

THE WOOL INDUSTRIES RESEARCH ASSOCIATION

THE report of the director of research*, Dr. A. B. D. Cassie, for 1961, presented at the forty-third annual general meeting of the Wool Industries Research Association on April 11, refers to continued investigations into the causes of seasonal variation in wool production, including an intensive study of the causes of winter thinning or break in the staple, and of the incidence of shedding of fibres. Much work has been done on the identification and quantitative determination of different kinds of fibres in blends and an automatic amino-acid analyser based on the estimation of amino-acids as their copper complexes has been constructed. Extensive studies on the fractionation of α-keratose and γ-keratose have shown them to be heterogeneous mixtures of similar peptides which are very susceptible to hydrolysis in alkaline media, and α-keratose and γ-keratose are now believed to be sub-units of a protein structure of very high molecular weight. The —SS— and —SH contents of wool have been determined by polarographic methods which measure the uptake of various mercurials by virgin and reduced wool. Evidence has been obtained that the differences in the reactivity of the cystine fractions from wool keratin cannot be attributed to differences in accessibility of the cystine, to whether cystine forms intra- or inter-chain linkages, or to whether cystine is located in the fibrillar structure of the fibre or the matrix surrounding the fibrils. Difficulties in measuring accurately diffusion of dyes in keratin have been largely overcome by using thin uniform sections of horn keratin cut by microtome to a thickness of about 7μ.

Dr. Cassie also refers to further developments with the tension regulator developed for fitting to the Noble comb for controlling feed; to a continuing examination of the fundamental causes of fly waste; to further work on the effect of acid dyeing on the properties of carpet yarns and carpets. A survey of woollen and finishing operations following the tinter was commenced and attention directed to scouring in package form of worsted yarn containing a castor/sperm type combing oil.

In his chairman's address at the annual general meeting, Mr. F. W. Hirst referred to the growth of co-operation and instanced the satisfactory shrink-resistance of knitted garments to-day resulting from the joint efforts of the Hosiery Research Association and the use of a Woollen Industries Research Association shrink-resist process. A Committee of Chairman and Directors of the Textile Research Association had considered ways of promoting closer working of Associations. Healthy co-operation between the industry and the International Wool Secretariat was noticeable, while another example of co-operation was to be seen in the resolution of problems of industrial relations by discussion between management and the Unions.

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* Wool Industries Research Association. Publication No. 218: Report of the Director of Research for 1961. Pp. 41. (Leeds: Wool Industries Research Association, 1962.)

WELFARE OF FARM ANIMALS

THE range of subjects dealt with by the Universities Federation for Animal Welfare, as shown by the Spring issue of the *U.F.A.W. Courier*, appears to be worth special comment in that some of them may be taken for granted by many scientific workers or given less than the necessary attention.

A reprint from *Nature*, September 30, 1961, about the symposium on the assessment of pain organized by the Federation, is apposite in a publication of its own and an article based on a lecture given by E. C. Straiton at its summer congress, 1961, on "Unnecessary Cruelties among Farm Animals", which gives emphasis to matters that are constantly under-emphasized by many people.

Straiton's theme is that farm people often, or more than often, assume too easily that the reactions of animals to pain are vastly less than might be guessed by comparison with those of human beings and that, as a corollary, there is little or no objection to the infliction of cruelty when it is done in the guise of attention to the welfare of the individual. This could be described as a cutting article, and, perhaps, this approach was suitable in order to give emphasis to many practices which are in constant operation, many of them receiving some sort of sanction in that they have been accepted for a very long time. He stated that the vast majority of working farm dogs are cruelly treated, inadequately fed, kept tied up with no water available, very often on a short chain and inadequately sheltered. He gave many instances in his experience of stupid, unskilled use of great force in attempts to calve cows, brutality in castrating farm animals, and other practices. He gave strong personal opinions about discomforts and worse from housing cloven-footed animals on accommodation with slatted floors, about the use of instruments for castrating by crushing the tissues, and about the methods of raising calves for marketing as veal by the use of the new methods designed to provide economical farm practice and at the same time veal of the type that appeals to the retail purchaser.

The issue reports Sir Thomas Dalling's account, given at the summer congress, of the international bodies which are concerned with veterinary activities designed to improve the standards and economy of animal husbandry by attention to animal health in the under-developed countries. W. A. POOL

AGGREGATION OF BLOOD PLATELETS BY ADENOSINE DIPHOSPHATE AND ITS REVERSAL

By PROF. G. V. R. BORN

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IN 1956 it was shown that blood platelets contain adenosine triphosphate (ATP) in extraordinarily high concentrations[1] and that most of this ATP breaks down rapidly when platelets are suspended in plasma which is clotting[2,3] or whenever platelets undergo viscous metamorphosis[4]. The suggestion was made[5] that if this breakdown involved ATP situated on the surface of the platelets, the large net negative charge on platelets[6] might be diminished and their tendency towards adhering to each other enhanced.

Recently, it has been found that platelets can be made to aggregate in plasma and to adhere to other surfaces by adding adenosine diphosphate (ADP) (ref. 7). Twenty-two nucleotides were tested for this effect; it was brought about only by ADP and by deoxyadenosine diphosphate. Since it is likely that ADP is produced by platelets themselves whenever their ATP breaks down, the adhesion and aggregation of platelets that occur in the initial stages of thrombosis may well be initiated by ADP. This effect has therefore been investigated by a method, recently described[8], with which the rate at and extent to which platelets aggregate can be determined quantitatively.

About 80 ml. of human blood was mixed with just enough sodium citrate (3·8 mgm./ml. blood) or heparin (0·01 mgm./ml. blood) to prevent clotting. As cooling increases the tendency of platelets to aggregate, the experiments were done at room temperature of 20°–22° C. The blood was centrifuged at $500g$ for 20 min. The plasma, usually about 35 ml. and containing 10^8–10^9 platelets/ml., was transferred with a siliconed capillary pipette into a plastic measuring cylinder.

A sample of 3 ml. was pipetted into a Spinco centrifuge tube, made of transparent plastic, which was inserted into a Unicam *SP* 400 absorptiometer. Light at a wave-length of 600 mμ was passed through the tube. The dark current was set at infinity and the optical density of distilled water at zero. The optical density of platelet-rich plasma was proportional to the concentration of platelets in it, provided the optical density of platelet-free plasma was subtracted.

The platelet-rich plasma was stirred by a small iron rod covered in polythene which was rotated magnetically. Stirring was stopped when readings were taken. When plasma was stirred gently there was no significant change in the concentration of platelets or in the optical density; this showed that the platelets did not stick to the tube or to the stirrer.

When platelet-rich plasma was stirred vigorously the concentration of platelets usually decreased slightly and so did the optical density (Fig. 1). The decrease was mostly in the first half-hour; after that it was very slow or absent. Presumably a small proportion of the platelets was broken up by vigorous stirring.

All additions were made to the plasma while it was being stirred. When substances were added which caused the platelets to aggregate, the rate and extent of aggregation increased as the rate of stirring was increased up to about 1,000 r.p.m. In the experiments described here stirring was always at this rate.

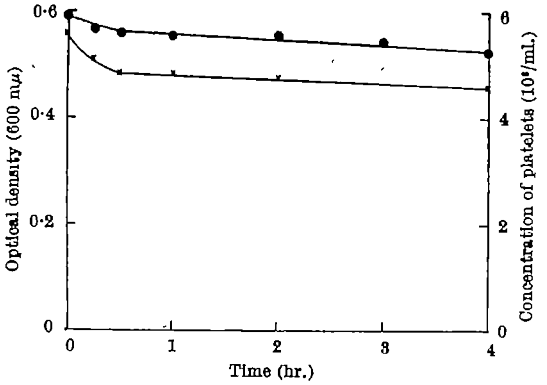

Fig. 1. Effect of vigorous stirring on the concentration of platelets (×) and on the optical density (●) of platelet-rich plasma. In the experiments illustrated by this and by Figs 2-4, human plasma was used containing sodium citrate (3·8 mgm./ml.); and the stirrer in it rotated at 1,000 r.p.m.

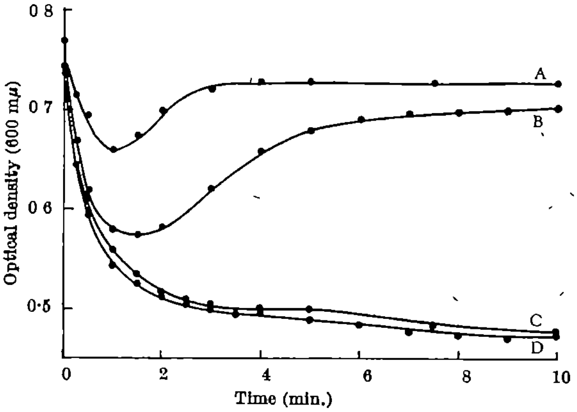

Fig 2. Effect of adding ADP on the optical density of plasma containing 4·75 × 10^8 platelets/ml. ADP was added at 0 min. to give the following concentrations. 2·5 × 10^{-7} M (curve A); 5 × 10^{-7} M (curve B); 1 × 10^{-6} M (curve C); and 2·5 × 10^{-6} M (curve D)

When ADP was added to platelet-rich plasma the optical density decreased immediately; at the same time the platelets could be seen to aggregate. Fig. 2 shows that the rate and the extent of the decrease depended on the concentration of added ADP; both were maximal when the concentration of ADP was greater than 1 × 10^{-6} M. The lowest concentration that brought about a fall in optical density was about 1 × 10^{-7} M, that is, about 0·04 μgm./ml.; this is similar to the ADP concentration which made platelets aggregate under the conditions used by Gaarder *et al.*[7].

Fig. 2 also shows that with the higher concentrations of ADP the optical density remained low and the platelets remained aggregated. However, when the concentration of added ADP was less than about 1 × 10^{-6} M the optical density began to increase again after 1–2 min., and after 10 min. it was almost the same as at the beginning, suggesting that the aggregation produced by ADP was reversible. This was confirmed by microscopy, which showed that as the optical density increased the size of the aggregates diminished until the concentration of single platelets was almost the same as it had been before the addition of ADP. These results suggested that the dispersion of the aggregates might be due to the disappearance of ADP, presumably because it is broken down to adenosine monophosphate (AMP)[9] which, unlike ADP, does not cause platelets to aggregate[7].

The aggregation brought about by ADP is reversed by AMP. In Fig. 3, curve A shows the fall in optical density of a sample of platelet-rich plasma when ADP was added; the platelets remained aggregated for 20 min. Curve B shows the changes in optical density of another sample of the plasma when ADP was added 2 min. after adding AMP at the same molar concentration. The optical density fell rapidly at first, but within 2 min. it began to increase and continued to do so for 20 min.; by then the aggregates had almost entirely dispersed.

It may be concluded that AMP antagonizes the aggregation of platelets by ADP. This strongly suggests that the reversal of aggregation observed with low concentrations of added ADP is due to its breakdown to AMP. The aggregation of platelets by ADP is antagonized also by ATP but less effectively than by AMP; this is shown in Fig. 4.

In human plasma AMP is broken down further to inosine monophosphate, inosine, and hypoxanthine[9]. Whether these substances affect the aggregation brought about by ADP has still to be established.

The mechanism by which ADP brings about the aggregation of platelets is unknown. Experiments performed so far suggest that platelets and ADP interact stoichiometrically and that calcium or magnesium is required as well as ADP. The antagonism of AMP and ATP towards aggregation by ADP may be because they compete either for specific 'aggregating sites' on the platelets or for calcium or magnesium.

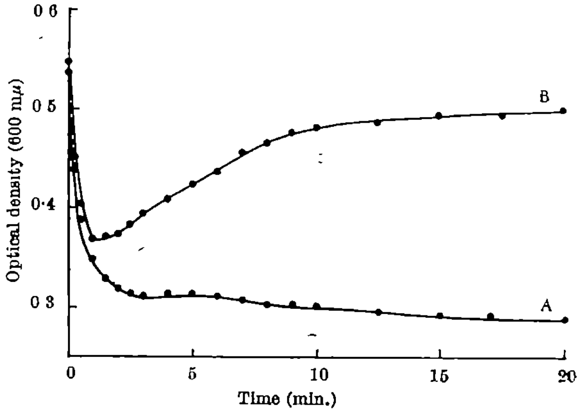

Fig. 3. Effect of adding ADP on the optical density of plasma containing 4·2 × 10^8 platelets/ml., without (curve A) and with (curve B) the addition 2 min. previously of AMP. The concentrations of AMP and ADP were 1 × 10^{-6} M

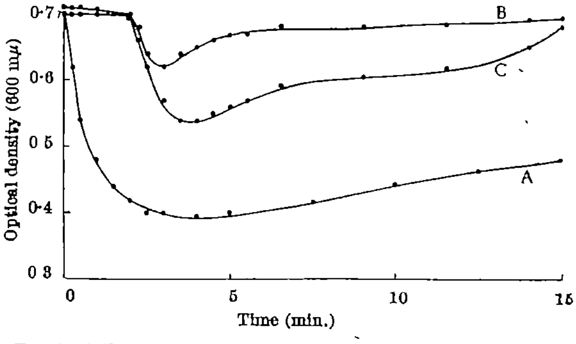

Fig. 4. Effect of adding ADP on the optical density of plasma containing 8·93 × 10^8 platelets/ml., without (curve A) and with the addition 2 min. previously of AMP (curve B) or of ATP (curve C). For A, ADP was added at 0 min. and, for B and C, at 2 min. The concentrations of AMP, ADP and ATP were 2 × 10^{-5} M

The specificity of ADP in making platelets aggregate suggests that this effect may be significant physiologically in the following way: when a blood vessel is injured ATP is broken down in damaged cells which release enough ADP to make a few platelets adhere to the damaged endothelium and to each other. At the same time tissue thromboplastin is released so that a little thrombin is formed. This brings about viscous metamorphosis in nearby platelets[10] during which their own ATP is broken down to ADP (refs. 2 and 11). The ADP, whether it is on the surface of the platelets or released with their granules, causes more platelets to adhere, so that the aggregate of platelets grows until it forms a hæmostatic plug. Pathologically, the aggregation of platelets is an immediate cause of thrombosis. This aggregation may involve ADP if pathological changes cause ADP to be released from cells in the vessel walls or in the blood.

In hæmostasis and in thrombosis platelets remain aggregated for some time before fusing together. Since released ADP presumably disappears by diffusion and by breakdown to AMP, the aggregates should disperse again unless they are maintained by ADP bound to the platelets' surfaces or by its continued release; or by another mechanism altogether.

In citrated plasma platelets can be made to aggregate by adding calcium (without necessarily inducing clotting at the same time[8]). Since calcium is required for aggregation by ADP, it may be that ADP is required for aggregation by calcium, the basic mechanism being the same. Both considerations suggest that the surface membranes of platelets contain ADP.

If it can be shown that ADP takes part in the aggregation of platelets in blood vessels it is conceivable that AMP or some other substance could be used to inhibit or to reverse platelet aggregation in thrombosis.

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PHOSPHORYLASE ACTIVITY IN HÆMIC CELLS

By DENNIS QUAGLINO and Dr. F. G. J. HAYHOE

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In 1955 Takeuchi and Kuriaki[1] described a histochemical method for the detection of phosphorylase in animal tissues, based on the specific staining reaction of newly synthesized polysaccharide derived from glucose-1-phosphate as a result of enzyme activity.

Following the biochemical demonstration by Rohdewald[2] of phosphorylase activity in horse leucocytes, Takeuchi and Kinoshita[3] looked for the enzyme histochemically in blood cells. While phosphorylase activity could readily be shown in the leucocytes of inflammatory lesions, using frozen sections, its presence was less easy to detect in ordinary blood and bone marrow smears. The authors eventually succeeded in obtaining fairly good staining results by fixing the smears for not more than 60 sec. in methanol, incubating in a specially prepared substrate for 2 hr. at 37° C. and staining with iodine, when the newly synthesized polysaccharide stained blue or violet blue. Muscle adenylic acid was found to enhance the reaction, which also required the presence of small quantities of glycogen, acting as a primer.

In human blood and bone marrow smears the enzyme was found in mature neutrophilic and eosinophilic granulocytes, in metamyelocytes, myelocytes and promyelocytes, but not in erythrocytes, monocytes, lymphocytes or basophils. Megakaryocytes showed only a weakly positive reaction. Hæmic cells from pathological conditions were not examined. Takeuchi and Kinoshita concluded that the histochemical demonstration of phosphorylase in blood cells was considerably more difficult than in other tissues, and required the utmost care and skill, and, perhaps because of technical problems, little further work in this field has been reported. According to Pearse[4], however, the method has proved reliable in tissue sections, can be reasonably interpreted, and deserves widespread use. We have recently renewed earlier attempts to examine the enzyme in human hæmic cells, using a modified substrate containing polyvinyl-pyrrolidone and a high concentration of glucose-1-phosphate as suggested by Eränkö and Palkama[5].

Several different fixative procedures were tested, including treatment with methanol for less than 60 sec., as recommended by Takeuchi and Kinoshita[3]. This method gave reasonably satisfactory results; but stronger reactions of similar distribution were obtained after fixation with 70 per cent acetone for 30 sec. at room temperature. It was found that blood and bone marrow smears showed better final preservation if left to dry in air for 3–4 hr. before being fixed. After fixation the slides should not be washed but blotted dry before incubation in the substrate solution. The incubation medium is prepared by dissolving 400 mgm. of glucose-1-phosphate, 40 mgm. of adenosine-5-phosphate, 8 mgm. of glycogen, 720 mgm. of sodium fluoride, and 3·6 gm. of polyvinyl-pyrrolidone in 8 ml. of alcohol and 40 ml. of 0·1 *M* acetate buffer *p*H 5·9 and adding 0·1 ml. of insulin 40 I.U./ml. The solution is then filtered and should be renewed every 4–5 days, especially if used frequently. The fixed smears are incubated in the substrate solution for 2 hr. at 37° C. After incubation the preparations are immersed in 40 per cent alcohol for 2 min., allowed to dry and transferred to 0·32 *M* sucrose solution for 5 min. Finally, the preparations are exposed for 5 min. to Gram's iodine made up in 0·32 *M* sucrose solution, and mounted in iodine–glycerine. When

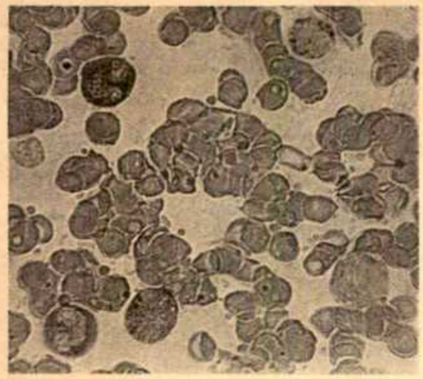

Fig. 1. Two neutrophil polymorphs, an eosinophil, a basophil, a neutrophil metamyelocyte and two negative erythroblasts from normal bone marrow

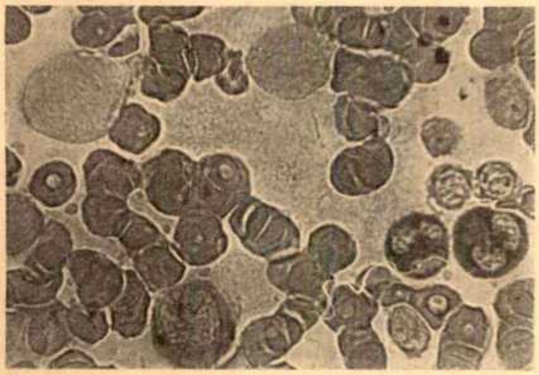

Fig. 2. Neutrophil granulocytes from a normal bone marrow, showing increasing positivity with increasing cell maturation. Two negative erythroblasts are also present

slides are incubated in a solution from which glucose-1-phosphate has been omitted no staining reaction is visible.

Staining reaction and distribution in hæmic cells (Figs. 1–4). A dark blue or violet blue coloration indicates the sites of phosphorylase activity. Positive reactions were found in all cells of the neutrophil granulocyte series, the earliest, myeloblasts and promyelocytes, showing faint diffuse cytoplasmic staining, and later members of the series showing positivity increasing in intensity with cell maturation. Eosinophils were similarly positive, the reaction being localized in the cytoplasm around the specific granules. Basophils contained coarse positive granules. Monocytes and lymphocytes showed variable reactions, some being negative and others having diffuse or granular positivity of varying intensity. Megakaryocytes and platelets showed diffuse positive staining with occasional coarse granules. Cells of the erythroid series appeared normally to be completely negative.

This pattern of distribution follows very closely that of pre-formed glycogen as demonstrated by the periodic acid–Schiff reaction, controlled by salivary amylase digestion, and a similar parallelism has been observed in studying phosphorylase cytochemistry of hæmic cells in pathological states. Thus mature granulocytes in acute infections, in chronic myeloid leukæmia and in the acute leukæmias are generally rich in glycogen and were found to be rich in phosphorylase. In chronic lymphocytic leukæmia again the phosphorylase reaction in lymphocytes showed a close correlation with the extent of positivity to periodic acid–Schiff reagent. Primitive cells from 15 cases of acute leukæmia were examined by both techniques, and once more a parallel was observed between glycogen and phosphorylase content, both in the proportion of cells showing positivity and in the intracellular disposition of the reaction, whether diffuse or granular.

The action of phosphorylase would lead one to expect its distribution in cells and tissues to be similar to that of glycogen; but the similarity observed in hæmic cells is unexpectedly close. That the phosphorylase reaction is not in fact staining pre-existing glycogen is shown by the clear negative results obtained when glucose-1-phosphate is omitted from the substrate; but we are inclined to believe that the existence of pre-formed glycogen in the cell does in some way dictate the apparent cytochemical localization of phosphorylase activity, perhaps by acting as an in-built primer supplementary to the primer included in the substrate mixture. This is not to say that the distribution of cytochemically demonstrable phosphorylase and that of glycogen are necessarily identical; polymorphs, for example, show much greater variability in phosphorylase content than in periodic acid–Schiff positivity while leukæmic primitive cells may show both diffuse and coarsely granular phosphorylase staining when the periodic acid–Schiff reaction shows only coarse granules. Again, in certain cases of acute leukæmia where the majority of primitive cells were entirely negative to periodic acid–Schiff reagent we have observed weak diffuse phosphorylase staining in the cytoplasm of blast cells which must have been negative to periodic acid–Schiff reagent.

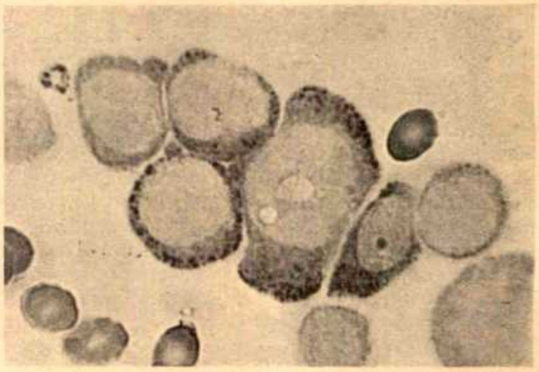

Fig. 3. Lymphoblasts from acute leukæmia showing granular phosphorylase positivity

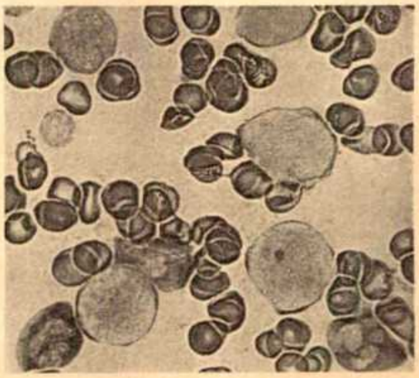

Fig. 4. Weak phosphorylase reaction in leukæmic myeloblasts

For the most part, the iodine staining in this reaction gives a blue colour, but a violet or brownish tinge may be seen occasionally, especially at sites of probable concentration of pre-existing glycogen, such as the coarse granules or blocks which occur in some lymphoblasts. This variation in colour is discussed by Takeuchi[6] and is presumably related to the chain-length of the polysaccharide produced through the synthesizing action of the phosphorylase, and the possible effect of branching enzyme on chains above a critical length.

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HÆMOGLOBIN–LEPORE$_{\text{HOLLANDIA}}$

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In a previous communication Neeb *et al.*[1] reported the occurrence of an abnormal hæmoglobin, identified as Lepore hæmoglobin, in a Papuan family, two members of which suffered from an anæmia which clinically presented itself as thalassæmia major. In members of the family heterozygous for this trait, in addition to about 12 per cent of the abnormal hæmoglobin, there was approximately 2 per cent of Hb–A$_2$, small amounts of fœtal hæmoglobin being occasionally present. In the homozygous carriers of this abnormality there were approximately 26 per cent of the abnormal component, 74 per cent of Hb–F and traces of Hb–A$_2$. To avoid confusion with the originally described Lepore abnormality[2], the Papuan Lepore abnormality will be designated as hæmoglobin–Lepore$_{\text{Hollandia}}$, for short Hb–Lep$_{\text{Holl.}}$, after the town where it was discovered.

The differences between Hb–Lep$_{\text{Holl.}}$ and the normal pigments have been investigated by means of peptide pattern and amino-acid analysis.

Hæmoglobins Hb–Lep$_{\text{Holl.}}$, Hb–A$_1$ and Hb–A$_2$ were prepared by using carboxymethylcellulose (CMC) chromatography[3]. (Hb–A$_1$ and Hb–A$_2$ were obtained from normal adults. No difference, however, could be demonstrated between these and the corresponding components of carriers of the Hb–Lep$_{\text{Holl.}}$ trait.) After concentration of the isolated fractions on CMC, the hæmoglobins were dialysed. The purity of the samples were checked by starch-block electrophoresis at pH 8·6 (ref. 4). Whenever necessary, the hæmoglobin fractions were re-chromatographed on CMC.

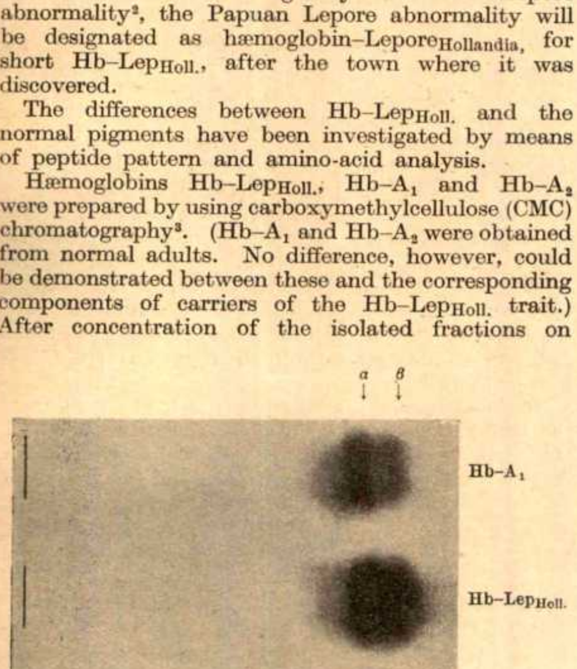

Fig. 1. Electrophoretic behaviour of Hb-Lep$_{\text{Holl.}}$, Hb-A$_1$ and Hb-A$_2$ on starch-gel at pH 1·8

The electrophoretic behaviour of the separate polypeptide chains of the hæmoglobins by means of starch-gel electrophoresis at pH 1·8 (ref. 5) is illustrated in Fig. 1. It may be seen that the α-chains of Hb–Lep$_{\text{Holl.}}$ behave similar to those of the normal pigments, while the non-α-chains of Hb–Lep$_{\text{Holl.}}$ have the same electrophoretic mobility as the β-chains of Hb–A$_1$.

Hæmoglobins Hb–Lep$_{\text{Holl.}}$, Hb–A$_1$ and Hb–A$_2$ were also compared by fingerprinting. Tryptic digests and the fingerprints were prepared in the usual manner[6]. In addition to butanol–acetic acid–water (3 : 1 : 1) as the chromatographic solvent, butanol–pyridine–acetic acid–water (30 : 20 : 6 : 24) (ref. 7) was found to give a clear-cut separation of the tryptic peptides of the hæmoglobins. The fingerprints were also stained for sulphur, histidine, arginine and tyrosine as described[3]. The tracings of the typical fingerprints obtained by using the second solvent combination are presented in Fig. 2. It is evident that peptide Lep$_{\text{Holl.}}$–12 and Lep$_{\text{Holl.}}$–26 resemble those of Hb–A$_2$, while the mobility of peptide Lep$_{\text{Holl.}}$–25 is more or less similar to that of Hb–A$_1$. The arginine-positive peptide A$_1$–26 has a higher anodic mobility and is clearly separated from the adjacent sulphur-containing peptide A$_1$–25, while the peptides Lep–2$_{\text{Holl.}}$6 and A$_2$–26 have lower anodic mobilities than the corresponding peptide 25 of these hæmoglobins and have the same position as the α-chain peptide 23. The mobility of the tryptophan-positive peptide 12 of both Hb–Lep$_{\text{Holl.}}$ and Hb–A$_2$ is markedly different from that of Hb–A$_1$.

In the peptide pattern of Hb–A$_2$ an extra peptide (X) was observed in conformity with the observations of Ingram and Stretton[8]. This peptide could not be observed when butanol–acetic acid–water (3 : 1 : 1) was used as the chromatographic solvent[9]. The absence of peptide X in the peptide patterns of Hb–Lep$_{\text{Holl.}}$ and Hb–A$_1$ is noteworthy.

The differing peptides 25, 26 and 12 of all the three hæmoglobins were isolated by subjecting the tryptic digests to repeated high-voltage electrophoresis (8 kV., 60 min.), followed by chromatography of the appropriate fractions on paper. The purified hæmoglobin peptides were hydrolysed with 6 N hydrochloric acid for 18 hr. under nitrogen and were

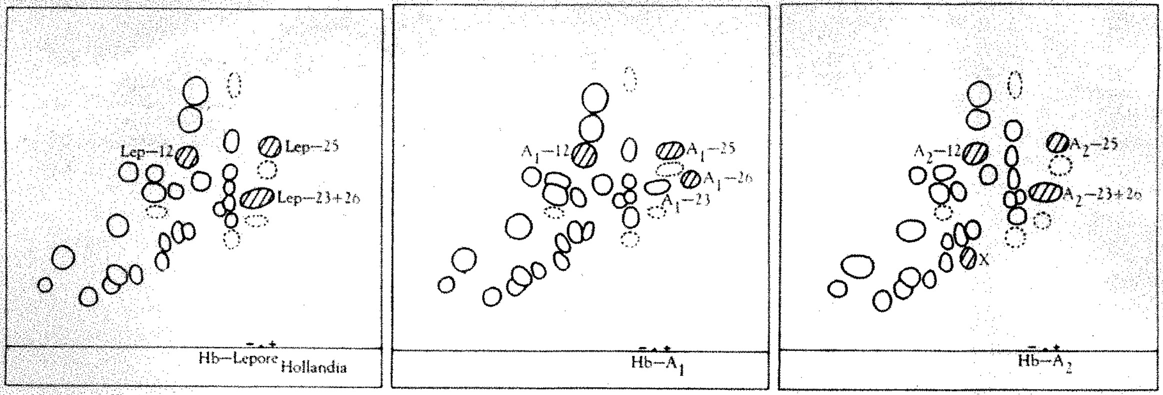

Fig. 2. Tracings of the fingerprints of tryptic digests of Hb-Lep$_{\text{Holl.}}$, Hb-A$_1$ and Hb-A$_2$. Electrophoretic buffer: Michl's buffer pH 6·4. Chromatographic solvent: butanol–pyridine–acetic acid–water (30 : 20 : 6 : 24)

analysed for their constituent amino-acids[3], the results of which are presented in Table 1.

Table 1. COMPARISON OF THE AMINO-ACID COMPOSITION OF THREE DIFFERENT TRYPTIC PEPTIDES OF HB-LEP$_{\text{Holl.}}$ WITH THE SEQUENCE OR COMPOSITION OF THE CORRESPONDING PEPTIDES OF HB-A$_1$ AND HB-A$_2$

Peptide	Amino-acid sequence
A$_1$–25	-phe-phe-glu-ser-phe-gly-asp-leu-ser-*thr*-pro-asp-ala-(val, met)-gly-asp-pro-lys-[10]
A$_2$–25	(phe,phe,gly,ser,phe,gly,asp,leu,ser,*ser*,pro,asp,ala,val, met,gly,asp,pro,lys)[5]
Lep$_{\text{Holl.}}$–25	(phe,phe,glu,ser,phe,gly,asp,leu,ser,*thr*,pro,asp,ala,val, met,gly,asp,pro,lys)
A$_1$–26	-val-aspNH$_2$-val-asp-*glu*-val-gly-gly-glu-ala-leu-gly-arg-[10]
A$_2$–26	-val-aspNH$_2$-val-asp-*ala*-val-gly-gly-glu-ala-leu-gly-arg-[*]
Lep$_{\text{Holl.}}$–26	(val,aspNH$_2$-val,asp,*ala*,val,gly,gly,glu,ala,leu,gly,arg.)
A$_1$–12	-*ser*-ala-val-thr-ala-leu-try-gly-lys-[10]
A$_2$–12	(*thr*,ala,val,*aspNH$_2$*,ala,leu,try,gly,lys,)[8]
Lep$_{\text{Holl.}}$–12	(*thr*,ala,val,*aspNH$_2$*,ala,(i)-leu,try,gly,lys.)

Examination of peptide 25 from all the three hæmoglobins showed that Hb–Lep$_{\text{Holl.}}$–25, like A$_1$–25, contained threonine, which was absent in A$_2$–25. The latter peptide contained a serine more than peptide 25 of Hb–A$_1$ and Hb—Lep$_{\text{Holl.}}$. Examination of peptide 26, on the other hand, revealed that the amino-acid composition of Lep$_{\text{Holl.}}$–26 was identical with that of A$_2$–26, which differs from A–26 by the exchange of glutamic acid in A$_1$–26 by alanine in A$_2$–26. The peptide 12 of Hb–Lep$_{\text{Holl.}}$ likewise had the same amino-acid composition as that of Hb–A$_2$, both having one asparagine more and one serine less than A$_1$–12.

Hæmoglobins Hb–Lep$_{\text{Holl.}}$ and Hb–A$_2$ were analysed for their constituent amino-acids on a Moore and Stein automatic amino-acid analyser (Table 2). The carefully purified hæmoglobin fractions were hydrolysed for 48 hr. in 6 N hydrochloric acid under nitrogen, prior to the analysis.

Table 2. AMINO-ACID COMPOSITION OF HYDROLYSATES OF HÆMOGLOBINS HB-LEP$_{\text{Holl.}}$ AND HB-A$_2$

(Values are given as gm. amino-acid/100 gm. protein)

Amino-acid	Hb-Lep$_{\text{Holl.}}$ 48 hr.	Hb-A$_2$ 48 hr.	Hb-F [11] 22 hr.	Hb-A$_1$ [11] 22 hr.
Aspartic acid	10·6	10·9	9·8	9·6
Glutamic acid	7·0	7·8	7·0	6·6
Glycine	4·3	4·7	4·3	4·3
Alanine	9·3	9·9	8·5	9·2
Valine	8·7	8·9	8·9	10·4
Leucine	14·2	13·7	13·6	13·9
Isoleucine	0·5	—	1·4	—
Serine	3·8	4·1	5·4	4·0
Threonine	5·2	4·8	6·2	5·1
Methionine	1·3	1·3	1·6	1·3
Phenylalanine	8·1	7·7	7·1	7·3
Tyrosine	2·9	2·9	2·5	3·1
Histidine	8·3	8·2	7·6	8·3
Lysine	9·5	10·0	10·0	9·3
Arginine	2·7	3·6	3·1	2·8

It is evident from Table 2 that in agreement with Ingram and Stretton[8] and contrary to a report of Rossi-Fanelli *et al.*[12], no isoleucine is present in Hb–A$_2$. Hb–Lep$_{\text{Holl.}}$, on the other hand, contains appreciable amounts of isoleucine amounting to 2 residues per molecule. To find out which of the sub-units of Hb–Lep$_{\text{Holl.}}$ contained this amino-acid, the purified Hb–Lep$_{\text{Holl.}}$ was separated into chains by the method of Wilson and Smith[13], and the chains were separately analysed for their constituent amino-acids. It was observed that the non-α-chains accounted for all the isoleucine present in the total hydrolysate of Hb–Lep$_{\text{Holl.}}$.

From these results it may be concluded that all the amino-acid differences observed between Hb–Lep$_{\text{Holl.}}$ and the normal pigments occur in the non-α-chains, whether the non-α-chains of Hb–Lep$_{\text{Holl.}}$ are compared with the β-, the γ-, or the δ-chains; in any event, more than a single amino-acid is different. This is contrary to what has been observed for a mutant human hæmoglobin polypeptide chain, where normally only one amino-acid substitution occurs. The amino-acid analysis of the peptides 25, 26 and 12 indicate that the non-α-chains of Hb–Lep$_{\text{Holl.}}$ may bear characteristics of both the β- and δ-chains, whereas the presence of isoleucine in the non-α-chains suggests a faint relationship to the γ-chains of fœtal hæmoglobin.

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A SUB-MOLECULAR MECHANISM OF GEL FORMATION IN SICKLE CELL HÆMOLYSATE

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THE optical rotation of normal adult, sickle cell trait, and fœtal carbon monoxyhæmoglobin was investigated by Havinga[1], who found no significant differences among them using the red light obtained from a cadmium lamp.

The examination of optical rotatory dispersion of normal and sickle cell hæmolysate reported here was undertaken because the abnormal hæmolysate has a negative temperature coefficient of gelation[2], that is, its deoxygenated solution of sufficient concentration gels at 38°, but it liquefies reversibly when cooled to 0°. The primary object of this investigation is to shed some light on the underlying mechanism of gel (sickle cell) formation.

It was known that oxygen, carbon monoxide, alkyl isocyanides, nitrosobenzene, and the like as well as sulphydryl blocking reagents (such as heavy metal ions, *para*-chloromercuribenzoate, *N*-ethylmalimide, etc.) prevent gelling by introducing steric hindrance. The amperometric titration study of sulphydryl groups also suggested conformation change of hæmoglobin molecule with temperature; at 0°, 50 per cent of the groups are sterically hindered, whereas all of them are accessible to the titrating reagent at 38°. As it was suspected, all of them are also titratable if the molecule is loosened with denaturing agent. The energy barrier due to steric hindrance for those SH groups not titratable at 0° amounts to about 2 kcal./mole[3]. Due to conformational changes of hæmoglobin molecules, the reported number of amperometrically titratable sulphydryl groups varies from 2 to 8 per molecule[4]. The present report on the optical rotatory dispersion studies of normal and sickle cell hæmolysates at 0° and 38° is consistent with the notion of dynamic conformational change of the hæmoglobin molecule.

Hæmolysates were prepared in the usual manner[3]. The solution for the optical rotatory dispersion study was centrifuged in a high-speed centrifuge at about 16,000 r.p.m. (32,700*g*) for 30 min. The solution was then filtered through a 'Millipore' filter paper *HA* (pore size, 0·45μ). A recording spectropolarimeter (model No. 260/655/850, Rudolph Instruments Engineering Co., Little Falls, New Jersey) was used, with the following modifications: the light source was changed to an Osram xenon high-pressure lamp, *XBO* 150, the voltage to the photomultiplier tube was increased to gain sensitivity, and the commutator was replaced by a magnetic one. The light path was 1·0 mm. and the half-intensity band-width of 20 mμ was used throughout the study. Molecular rotation was obtained by multiplying the specific rotation by the ratio, mol. wt./100. The concentration of hæmolysates was 11 gm./100 ml. A more dilute solution of 4 gm./100 ml. of hæmoglobin was used to obtain data in the Soret band.

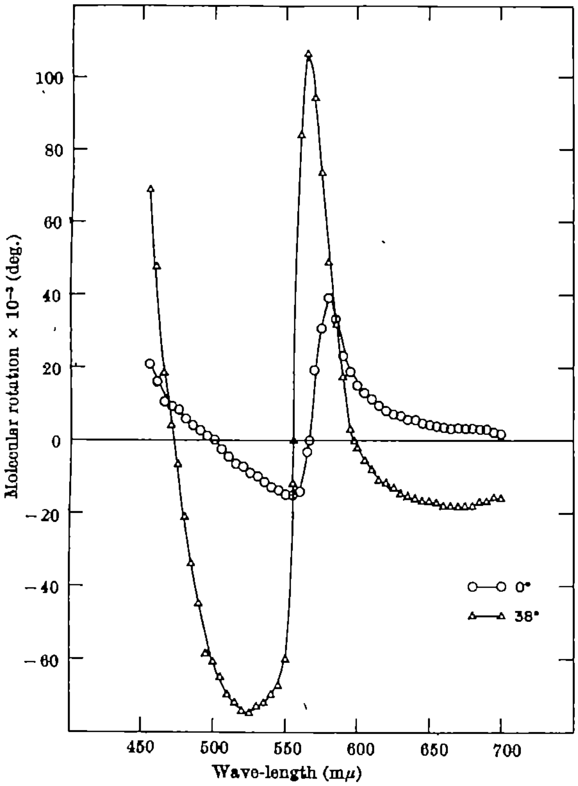


Fig. 1. Optical rotatory dispersion curves for deoxygenated sickle cell hæmolysate at 0° and 38°

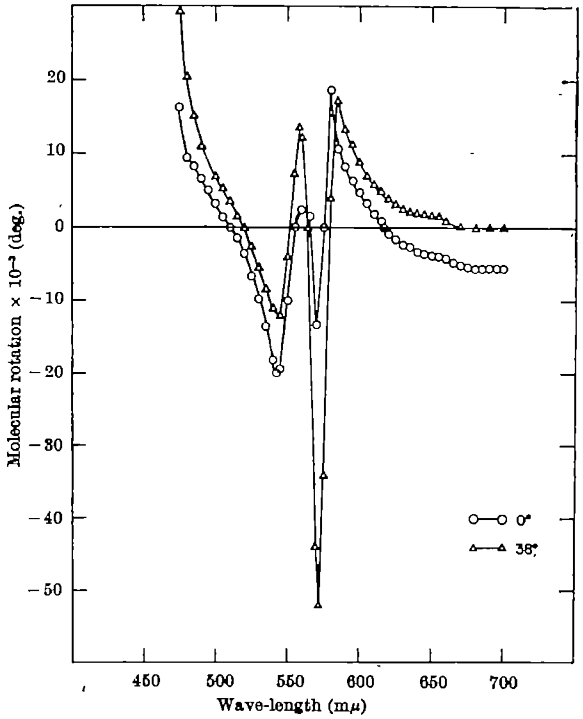


Fig 2. Optical rotatory dispersion curves for oxygenated sickle cell hæmolysate at 0° and 38°

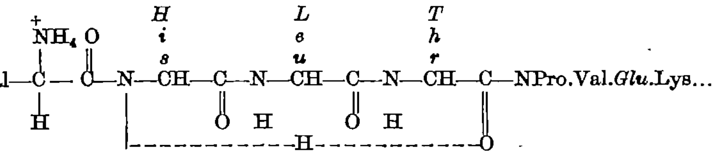
It is generally recognized that optical rotatory dispersion study is one of the best methods for detecting changes in conformation of very large optically active molecules[4]. The present investigation indicates that the sickle cell hæmolysate shows a much greater conformational change with temperature than the hæmolysate of normal hæmoglobin. It was observed that the amplitude of the positive Cotton effect (in the 560-mμ region as well as in the Soret band) at 0° is increased reversibly by about three times when the solution is warmed to 38° (Fig. 1); the hæmolysate of the normal hæmoglobin undergoes but a slight increase in the amplitude of the positive Cotton effect. The oxygenated hæmolysate of sickle cell hæmoglobin shows a similar increase in the amplitude of the Cotton effect, but the magnitude is smaller.

Fig. 2 shows that the oxygenated solution gives a multiple Cotton effect; the amplitude of the positive Cotton effect at 560-mμ region is increased with temperature. The oxygenated hæmolysate of normal red cells undergoes but a slight change in the same conditions.

So far as is known the only chemical difference between the normal hæmoglobin (Hb-A) and the sickle cell hæmoglobin (Hb–S) is in one amino-acid residue of the β-chain[5]. The hæmoglobin molecule consists of two pairs of peptide chains; one pair is designated the α-chain (N-terminal sequence is Val.Leu. . .) and the other the β-chain (N-terminal sequence is Val.His.Leu. . .)[6]. The amino-acid interchange is located in the sixth residue from the N-terminus[7,8]. The amino-acid sequences of the first 8 amino-acid residues from the N-terminal amino-acid of β-chains for Hb–A and Hb–S are as follows[7,8]:

	1	2	3	4	5	6	7	8
Hb-A	Val.	His.	Leu.	Thr.	Pro.	*Glu*.	Glu.	Lys.
Hb-S	Val.	His.	Leu.	Thr.	Pro.	*Val*.	Glu.	Lys.

This interchange of amino-acid residue results in a loss of one negative charge per β-chain on part of Hb–S molecule. Because the molecule is known to have two-fold axis of symmetry[9], these glutamyl residues must exert electrostatic repulsion between the β-chains of Hb–A (near the NH_2-terminal residues). But in Hb–S molecule, the minus charge of the seventh glutamyl might be essentially neutralized by the positive contribution from the epsilon amino-group of the eighth lysyl residue. It appears from model-building experiment with precision components that the amino terminal valyl residue interlocks with the newly introduced valyl residue at position 6 in sickle cell hæmoglobin molecule; this allows cyclization of the peptide chain from histidyl to threonyl by hydrogen bonding as shown schematically below.



$$\mathrm{Val{-}\underset{H}{\overset{\overset{+}{N}H_4}{C}}{-}\overset{O}{\overset{\|}{C}}{-}N{-}\overset{His}{CH}{-}\underset{O}{\underset{\|}{C}}{-}\underset{H}{N}{-}\overset{Leu}{CH}{-}\underset{O}{\underset{\|}{C}}{-}\underset{H}{N}{-}\overset{Thr}{CH}{-}\underset{O}{\underset{\|}{C}}{-}NPro.Val.\mathit{Glu}.Lys\ldots}$$

When the sixth residue is glutamyl as in Hb–A, it appears that this ring closure does not occur. In Hb–S at 38° this segment of the peptide chain is stabilized through this cylization by hydrogen bonding, thus the freedom of rotation about bonds in rings formed is restricted. This is the condition necessary for one of the Kautzmann and Eyring rules which states: "Those influences which tend to restrict freedom of orientation about bonds will tend to increase the order of magnitude of the optical activity"[10]. But at 0° the hydrogen bond under question appears to be transferred in a process of hydration (because hydrophobic bonds are weaker at 0°, but on the other hand, the hydrogen bond approaches its maximum at the ice point) and the chain appears to assume a conformation (of no ring formation) as in the β-chains of Hb–A; thus the numerical value of the optical rotation is diminished.

I thank Mr. Kenneth McCoy, Georgetown University, Washington, D.C., for providing sickle cell hæmoglobin specimens for this work. I also thank Mr. Harold K. Miller for help in making the needed modification on the instrument.

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ANTIGEN- AND ANTIBODY-COMBINING PROPERTIES AND THEIR INFLUENCE ON THE IMMUNE REACTION OF RED CELLS

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HUMAN blood group A erythrocytes from different individuals have been sub-grouped on the basis of their agglutination with human anti-A sera[1]. A_1 cells are more strongly agglutinated than A_2 cells, using anti-A sera; an anti-A serum absorbed with A_2 cells provides a serum with antibodies capable of agglutinating A_1 cells. A_3 cells give microscopic clumps of agglutinated cells surrounded by a large number of free cells. A_4 cells are not agglutinated by most anti-A sera obtained from B individuals, but are weakly clumped by anti-A sera derived from O individuals. Still weaker variants of the A antigen have been described and variously designated A_0, A_x, A_5 and A_6. These differences in the serological properties of the A sub-groups have been shown by numerous family studies to be a stable heritable characteristic of the A antigen[1]. The characteristic agglutination of the human A sub-groups suggested differences in their combining properties.

Studies in this laboratory have been concerned with antibody heterogeneity with respect to combining properties. In the work reported here an attempt

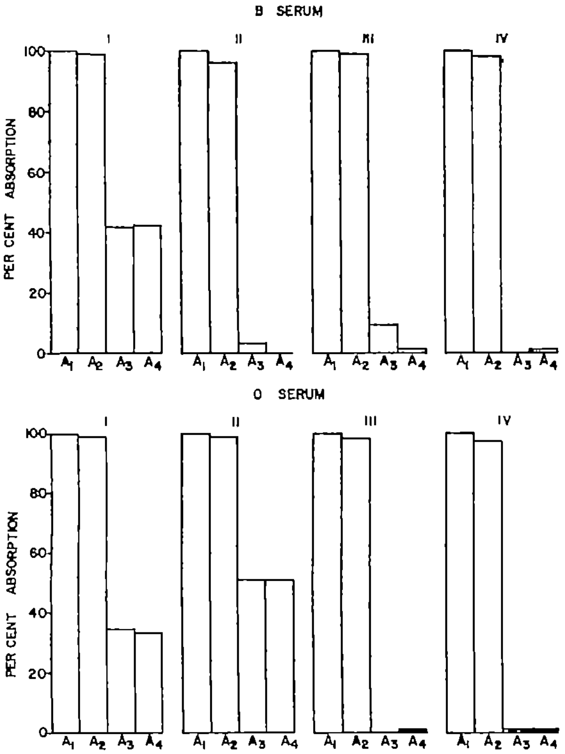

Fig. 1. Comparison of the percentage of agglutinin absorption by different human A sub-groups. Agglutinin determinations made with A_1 cells

was made to demonstrate how differences in antibody-combining properties may be related to differences in the combining properties of the human A sub-groups. These findings have provided information relevant to the general subject of the nature of antigen and antibody-combining sites.

Antibody heterogeneity can be shown to exist within and between human anti-A antibody populations and related to the combining properties of the human A sub-groups. Human anti-A sera were exhaustively absorbed with cells of different A sub-groups and with an O cell control. The amount of antibody activity remaining after absorption was quantitatively determined[2] using A_1 cells, and the percentage of absorbed agglutinin activity was calculated. It can be seen in Fig. 1 that different percentages of antibody activity were removed by different A sub-groups, and that these percentages differed from one antiserum to another. These results suggest a heterogeneity both within and between antibody populations. Qualitative differences in the A sub-group antigens were indicated by the failure of the individual A sub-groups to absorb completely the A antibodies. The absorbed A sub-group sera were also tested for their hæmolytic activity for A_1 cells and for their ability to cross-react with sheep cells. Both hæmolytic activity and cross-reacting antibodies were completely removed by A_1 cells, partially by A_2 cells and little or not at all by A_3 and A_4 cells.

Quantitative differences in the amount of A antigen present on the cells of the different A sub-groups are indicated in Fig. 2, in which the antibody-combining capacity of the A sub-groups has been compared. Such differences in the quantitative absorption of antibodies by the human A sub-group probably contribute to their characteristic serological properties.

Antibody heterogeneity was also examined by a column chromatographic method[3] which takes advantage of the thermal dissociation characteristics of the specific antigen–antibody complex. This method permits quantitative characterization of an antibody population in terms of its combining properties as well as providing a means for isolating antibody fractions differing in their combining properties. The results of a thermal fractionation of a human A iso-antibody population on a column composed of A_1 cells are presented in Fig. 3 and illustrate the extent of heterogeneity existing within this antibody population. The fractionation of different anti-A sera (Fig. 4) indicates the high degree of heterogeneity which may exist between different serum samples.

Fractions obtained by thermal elution were compared for their ability to agglutinate cells of different human A sub-groups, to produce hæmolysis of A_1 cells and to cross-react with sheep cells. The weakly reacting A_3 and A_4 cells could be agglutinated only by that antibody eluted at temperatures above 45° C., even though the serum fractions being compared had equal agglutinin activity for A_1 cells. On the other hand, antibody eluted at temperatures below 15° C. could only produce agglutination of A_1 cells. Anti-A hæmolytic activity and antibody cross-reacting with sheep cells were closely associated with those fractions of antibody eluted at lower temperatures.

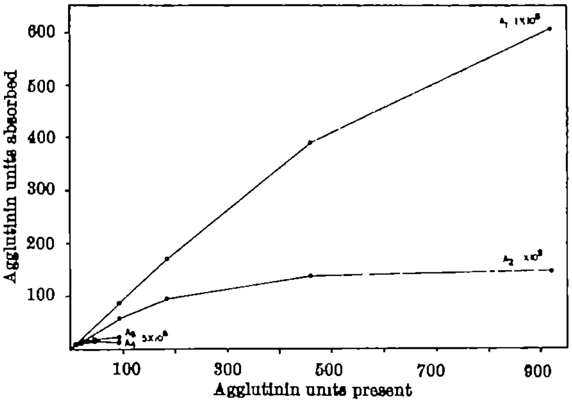

Fig. 2. Comparison of the agglutinin-combining capacity of the human A sub-groups. The human anti-A serum used for this experiment contained antibody, 50 per cent of which was capable of combining with the A_3 and A_4 cells. Varying concentrations of antiserum were absorbed with a constant concentration of cells of different human A sub-groups. The number of unabsorbed agglutinin units was determined on each of the supernates and unabsorbed control sera using A_1 cells for titration. The number of antibody units absorbed was calculated from these data

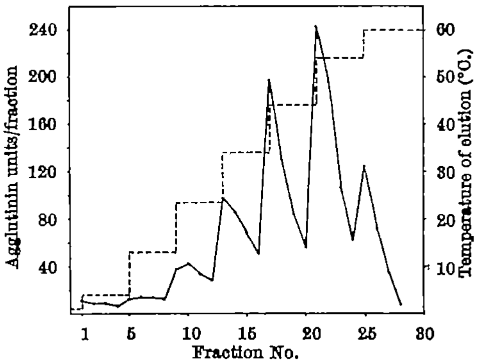

Fig. 3. Thermal fractionation of a human anti-A serum on a column composed of A_1 cells. Agglutinin activity of fractions determined quantitatively (ref. 2)

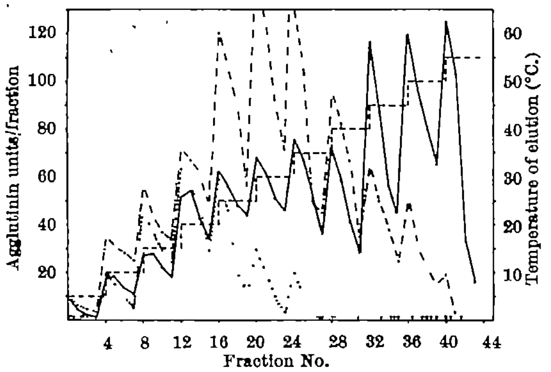

Fig. 4. Comparison of the thermal fractionation of different human anti-A sera

Landsteiner[4] established the chemical basis of the antigen–antibody reaction and showed that antibody specificity is a function of the complementarity of the physico-chemical configuration of the antibody-combining site to that of the antigenic determinant. Pauling[4] analysed the chemical forces involved in antigen and antibody interaction, and provided further evidence for the complementary configuration of the antigen- and antibody-combining sites as a basis for the stability of their union. The nature of heterogeneity in terms of the combining properties of antibody has been a subject of speculation although it is well known that the stability of the antigen–antibody complex formed by antibody molecules may vary widely within a single antibody population[5–7]. The difficulty of dealing with such antibody heterogeneity is evident in such diverse areas as physico-chemical investigations of antigen–antibody interaction[8] and serological reactions of the human blood groups[8,9].

In this work antibody heterogeneity was examined by thermal elution and exhaustive absorption. A major aspect of the work was the examination of the human A sub-groups to determine the bases for their difference in serological behaviour and the relationship of their serological properties to antibody heterogeneity.

Differences in the physico-chemical structure of the human A sub-groups have been indicated by their immunological behaviour[1] rather than by chemical methods. The absorption of an anti-A serum with A_2 cells results in a serum which is capable of reacting with A_1 cells but not with cells of the weaker A sub-groups. Antibody eluted from sensitized A_2, A_3 and A_4 cells produces stronger and higher titred agglutination with A_1 cells. Structural differences in the A sub-groups are also indicated by the fact that A isoantibodies are commonly found in individuals who have the weaker A sub-groups. In addition to indicating physico-chemical differences in the A sub-groups, these findings suggested an inter-relationship between the human A sub-groups indicative of a type of antigenic heterogeneity related to the heterogeneity of the antibody.

Assuming that the temperature required for elution of A antibodies from the A antigen is related to the degree of complementarity between the antibody-combining site and the antigenic determinant, the A antibodies requiring the highest temperature for elution would have a more complementary configuration for the A determinant than the A antibodies eluted at lower temperatures. This premise provides a basis for interpreting the experimental findings of the human A sub-groups: (1) a greater percentage of antibody was recovered from an A_2 column than from an A_1 column[3]; (2) the tested A sub-groups reacted best with antibodies eluted at higher temperatures; (3) the antibody remaining after absorption with A_2 cells reacted only with A_1 cells[1]; (4) the antibody eluted from A_3 and A_4 cells reacted best with A_1 cells[1].

The human A sub-groups apparently do not reflect different antigenic determinants for which there are a number of specific antibody configurations. If the A antigen has several different antigenic determinants, antibodies would be formed with an equally strong binding for the individual determinants. If an A sub-group represents one or more of such determinants, at least part of the antibody population could be bound equally well by any of the A sub-groups. This interpretation of multiple antigenic determinants being responsible for differences in the serological behaviour of the human A sub-groups is contra-indicated primarily by the observation that all antibodies with A specificity form more stable complexes with A_1 cells than with other A sub-groups. Consequently, there is probably a single antigenic determinant or physico-chemical configuration for the A antigen which is most 'ideally' represented on A_1 cells. The reactions of the A sub-groups with different fractions of antibody obtained by thermal elution indicated that the antibody molecules forming the most stable complexes with A_1 cells are also the same antibody molecules which form the most stable complexes with A_3 and A_4 cells. Therefore, the A antigen as represented by the more weakly reacting A sub-groups would appear to be related structures of the A antigen as found on A_1 cells. The A sub-groups may possess only minor variations of such a nature that the most specific antibody configuration for the A determinant (as represented on A_1 cells) also forms the most stable complex with all variants of the A antigen.

Anti-A hæmolytic activity and cross reactions with sheep cells in this work also provided information which could be interpreted on the basis of the configuration of the combining site of antibody responsible for these reactions. Both anti-A hæmolytic activity and sheep cell cross-reactions were associated with antibody which could be eluted at lower temperatures. Similarly these antibodies were not absorbed by the weakly reacting A_3 and A_4 cells, were partially absorbed by A_2 cells and completely absorbed by A_1 cells. These results agree with results obtained by Winstanley, Konugres and Coombs[10] and Konugres and Coombs[11] on the correlation of the A hæmolysin titre and the cross-reaction with group A pig cells of human anti-A sera. They also agree with results obtained by Owen[12] on the cross-reactions of human B isoantibodies with various animal cells.

The cross-reactions of human isoantibodies with heterologous antigens may also be interpreted on the basis of configuration of the antibody-combining sites. Assuming that the antibodies formed in response to an antigenic determinant constitute a spectrum differing in the degree of fit for the determinant, the antibody with the highest degree of fit would be expected to be the most specific and, conversely, the antibody with the least degree of fit, the least specific. The latter type would have the greatest possibility of cross-reacting with another antigenic determinant of related physico-chemical structure. The thermal elution characteristics of the cross-reacting antibodies appear to agree with this interpretation. Although gross differences in the physico-

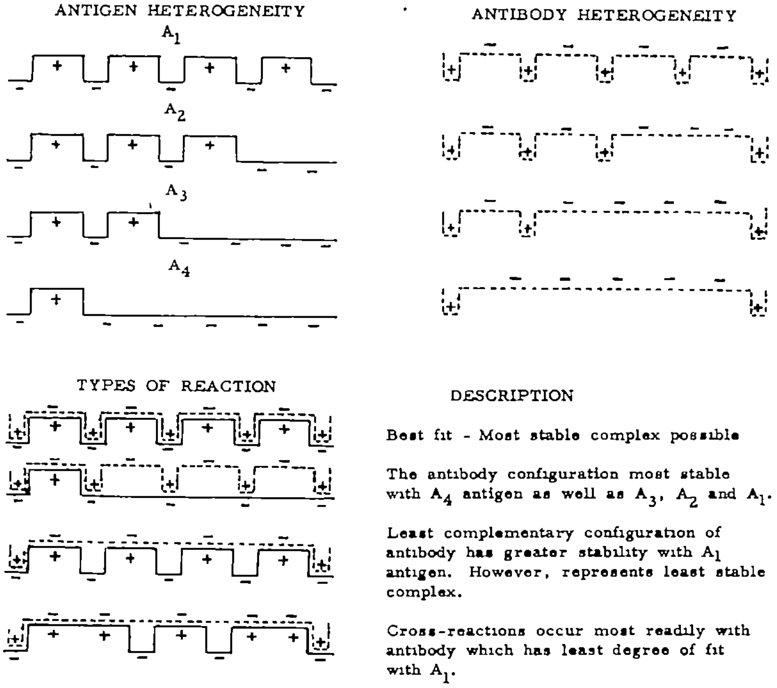
ANTIGEN HETEROGENEITY
A_1
A_2
A_3
A_4
ANTIBODY HETEROGENEITY
TYPES OF REACTION
DESCRIPTION
Best fit - Most stable complex possible
The antibody configuration most stable with A_4 antigen as well as A_3, A_2 and A_1.
Least complementary configuration of antibody has greater stability with A_1 antigen. However, represents least stable complex.
Cross-reactions occur most readily with antibody which has least degree of fit with A_1.

Fig. 5. Diagrammatic representation of the antigen–antibody configurational relationships as represented by the reactions of human anti-A isoantibodies and the human A sub-groups

chemical properties of antibody such as molecular weight and electrophoretic mobility may be associated with characteristic differences in combining properties, it is questioned whether such properties have any direct effect on antibody specificity.

Pressman and Siegel found that antibody prepared by immunization with one haptene was bound more firmly by a heterologous but similar haptene[13]. If antibody heterogeneity exists for a single antigen and if the antibodies vary in their degree of fit for this antigen, it would be expected that part of the antibody population might fit a cross-reacting antigen better than the homologous antigen. In other words, antibody molecules may be formed the structure of which would not be completely complementary for the homologous antigens. Related antigens may have physico-chemical configurations more nearly complementary for antibody variants than the homologous antigen.

The reactions involving the thermal elution fractions of human anti-A sera and the A sub-group absorbed sera, with human A sub-group cells or with sheep cells raise questions with respect to the definition of cross-reacting antigens. Cross-reacting antigens have been assumed to indicate a similarity in physico-chemical configuration. Antibody heterogeneity has been presented in this discussion as a progressively decreasing complementarity of an antibody-combining site to a basic antigen configuration. That portion of the antibody population having a less complementary configuration for the homologous antigen more readily combines with a similar physico-chemical structure found in another antigen. This prediction appears to be validated by the cross-reactions of human anti-A serum with animal cells.

The human A sub-groups may also be defined as cross-reacting antigens, having similar but not identical physico-chemical structure. However, in contrast to cross-reacting antigens, which reacted with antibody of less specific configuration, the A sub-groups reacted with antibody possessing a more specific configuration. Consequently, the A sub-groups probably represent differences in physico-chemical configuration unlike those associated with cross-reacting animal cells. Therefore, the physico-chemical differences associated with the human A sub-groups may be described as variants of an antigen to distinguish them from the physico-chemical differences associated with heterologous cross-reacting antigens. Race and Sanger[1] suggested that human A sub-groups represent segments of a continuous spectrum of antigenic variants. The isolation of serological variants similar to the human A sub-groups from a single individual's cell population[14] lends support to the idea of antigenic variants. A spectrum of variants was also suggested by Renton and Hancock[15] for the Rh_0 antigen.

The conclusion of this investigation may now be summarized. A human A isoantibody population includes a spectrum of antibodies differing in their degree of complementarity for a single human A antigenic determinant. The human A sub-groups represent segments of a continuous spectrum of antigenic variants related to one basic structure for the A antigen. All variants of the human A antigen form a more stable complex with those antibody molecules having the most complementary configuration for the A determinant. Finally, cross-reactions with heterologous antigens occur most readily with that portion of the human A isoantibody population having a less-specific configuration for the human A determinant. An attempt to indicate the salient configurational relationships suggested by the reactions of the human A isoantibodies and the human A sub-groups is presented in Fig. 5.

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ERYTHROPOIETIC ACTION OF TISSUE EXTRACTS

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SINCE the publication of the communication by Jacobson *et al.*[1] many authors have directed their attention to the kidney as the site of erythropoietin formation, and Naets[2] suggests the importance of developing an adequate method for the demonstration of erythropoietin with extracts of renal tissue.

For our experiments, tissue extracts of kidney, spleen and liver of normal pig were prepared using the technique for obtaining rennin[3]. In the case of renal extracts, only the cortical region was used. The technique was as follows: (*a*) Extract the minced tissue with saline, adding three volumes of 6 per cent sodium chloride, agitating and covering it with 1 cm. of toluol; leave for about 20 hr. at room temperature (always below 30° C.); (*b*) separate the tissue, fat and toluol by filtration through gauze and centrifugate, proceeding with the liquid thus obtained; (*c*) add concentrated acetic acid to *p*H 3·8, then merthiolate 1/5,000; later filter. After several hours, a light yellow liquid is obtained. Later purification followed two different stages:

(1) Add 39 gm. ammonium sulphate for every 100 ml. of filtered liquid. Filter and dissolve the precipitate with a 0·9 per cent saline solution. Dialyse with tap-water until the ammonium sulphate is completely separated, then add 0·9 gm. sodium chloride per 100 ml. and 1/5,000 merthiolate. This constitutes the crude rennin-type extract.

(2) The light yellow filtrate liquid is then taken to *p*H 5·5 and placed in a boiling water bath for 10 min.[4]. It is then filtered and the liquid obtained taken to *p*H 4·5 with 0·1 *M* acetic acid. As soon as this is cooled (4° C. or less) add 4 volumes of ethanol at the same temperature[5], slowly and continuously stirring; leave overnight in a refrigerator. Next day the precipitate is separated in a refrigerated centrifuge at 0° C. and then dissolved in a *p*H 8·0 buffer of 0·01 *M* phosphate. The solution thus obtained is again centrifuged at 0° C. and 14,000 r.p.m. for 15 min. to separate and discard the insoluble material. Thus the alcohol-boiled extract is obtained.

The quantity of positive biuret material[6] was determined in all extracts.

The erythropoietic action was determined by intraperitoneal injection in starved rats and then measuring their erythropoietic activity with the test devised by Hodgson *et al.*[7,8], using iron-59. The duration of starvation and the quantity of extract injected in a total of two injections in successive days is given in Table 1. 24 hr. after the second injection of the extract, 1–3 μc. of iron-59 of high specific activity incubated with normal rat plasma was given to each rat in the saphena. 3 hr. later the rats were bled through the abdominal aorta and the percentage of the dose of iron-59 was determined in the plasma, erythrocytes and liver. Volume of plasma was measured by injecting normal rat plasma labelled with iodine-131 following McFarlane's method[9].

The radioactivity was measured in a well scintillator with a γ-ray spectrometer (analyser computer, model 132, from the Nuclear Chicago), making the corresponding correction for the activity incidence of iron-59 at the peak of iodine-131.

The plasma iron was determined by the technique of Schade *et al.*[10], making a pool of the plasma with the same number of c.p.m. of iron-59 in 1 ml. of plasma.

With the results obtained the time constant (*K*) of disappearance of iron-59 from plasma and the plasma iron turnover were calculated. To determine the percentage of the dose of iron-59 in the total of the rat's erythrocytes, the volume of blood was calculated from the plasma volume and the hæmatocrit. The results in the unknown and control rats were submitted to statistical treatment of the significance between averages, with the distribution of t for $N_1 + N_2 - 2$ degrees of freedom.

The action of the rennin-type renal extract did not become significant ($P < 0·1$; $t = 2·32$ for 7 degrees

Table 1. TISSUE EXTRACTS

Exp. No.	Material injected	No. of rats	Wt. of rats (gm.)	Per cent dose of ^{59}Fe 3 hr. after injection: Total plasma	Total erythrocytes	Liver	*K* per cent hr.	Plasma iron μgm./ml.	Turnover μgm/hr.	Hr. of starvation
79	Rennin-type renal extract (equivalent to 750 U Bs.As. of rennin)	5	223	7·4	17·2 $P<0·1$	3·9	89	0·8	5 7	110
	Control 4 ml. of normal dog plasma	4	224	19·6	3·2	6·2	54	1·9	7·5	
86	Rennin-type spleen extract. Total dose . 7·5 mgm. proteins	5	181	19 5	11·1 $P<0·01$	4·9	56	2·1	10·3	79
	Alcohol-boiled kidney extract. Total dose : 7·5 mgm. proteins	6	179	16 1	9·6 $P<0 01$	7·0	62	2·1	11·0	
	Rennin-type liver extract. Total dose · 7·9 mgm. proteins	6	178	20 1	4·8 $P<0·05$	7·4	55	2·1	8·1	
	Control 0 9 per cent NaCl solution	4	176	38 7	2·3	—	31	2·3	6·0	
100	Alcohol-boiled liver extract. Total dose : 12·1 mgm. proteins	5	178	8 9	11·0 $P<0·05$	3·4	85	0·8	4·5	72
	Alcohol-boiled spleen extract. Total dose : 10·4 mgm. proteins	5	182	11·2	8·1 $P<0·01$	3·5	74	1·1	5 8	
	Control Normal plasma alcohol-boiled extract. Total dose · 9 0 mgm. proteins	5	185	22·4	3·1	4·5	51	1·4	4 9	

of freedom), in spite of the noticeable difference of the average with the control group. This might be due to great dispersion of the values (between 7·4 and 35·8 per cent) observed in this group, attributable to an exaggerated period of starvation. In this connexion, the control group injected with 4 ml. of native whole plasma, which normally raises the erythropoiesis of these starved animals, has low values of incorporation of iron-59 in the erythrocytes, similar to that of the control group injected with only 9 mgm. of proteins and with only 72 hr. of fasting. This observation seems to be confirmed by the results of the injection of renal extract boiled in alcohol, of a high statistical significance (Exp. 86) $P < 0{\cdot}01$.

The splenic and hepatic extracts show statistically significant results. The incorporation of small amounts of iron-59 in the erythrocytes, in the case of hepatic extract of Exp. 86, appreciably increases when the dose of this extract goes up to 12 mgm. of proteins (Exp. 100).

The results referring to the percentage dose of iron-59 in the plasma confirm those of the erythrocytes. As the dose of the iron-59 which remains in the plasma lessens at the end of 3 hr. after injection, so much greater is the uptake of iron-59 by the erythrocytes and greater is the time-constant of disappearance from plasma of iron-59. Thus we see in the active groups that a lower proportion of iron-59 goes to the liver—one of the storage organs.

All these results indicate an erythropoietic stimulation of the groups injected with different types of extracts when compared with the control groups. The results of plasma iron turnover, except in Exp. 86, are not a good index of erythropoiesis since the values of plasma iron were less than 2 µgm./ml. (ref. 11).

The hæmatocrit values in the experimental and control groups (48·4 and 48·7 respectively) support the idea that the erythropoietic stimulus must be ascribed directly to the extracts, and not to a secondary effect due to hæmolytic action.

In Exp. 100, both the control and the active groups had received approximately the same quantity of proteins and in Exp. 72 the controls had received several times more. Therefore, the erythropoietic stimulus cannot be ascribed to the quantity of injected proteins.

Like erythropoietin, the active substance of the different extracts showed that it was heat-stable[4,13] and precipitated at pH 4·5 with 4 volumes of ethanol[5,14]. On the other hand, the strongly active extract boiled in alcohol was proved to be free of rennin-type substances.

The substance obtained from normal pig kidney is substantiated by the results which Lagrue *et al.*[14] obtained with normal rabbit kidneys, though they failed when using pig's kidneys. Their method of extraction differed from ours in that they used successive freezing and de-freezing. We believe that our success lies principally in the stage of incubation for 20 hr. with a 6 per cent sodium chloride solution at room temperature without oxygenation.

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ROLE OF THE PITUITARY GLAND IN KETOGENESIS

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IN previous experiments an increased production of ketone bodies was found to be most closely associated with a shortage of utilizable fuel and the need for an additional source of energy[1]. The ketonæmia of rats consuming a completely fat diet persisted in animals fed sub-calorific amounts but became reduced or absent when sufficient fat was consumed to satisfy the calorific requirement.

Evidence may be drawn from several sources to account for the promotion of ketogenesis by a shortage of utilizable fuel and for the antiketogenic effect of calorie sufficiency. Ketogenesis *in vivo* is invariably accompanied and sustained by the mobilization of fat from the fat depots in the form of unesterified fatty acids. The release of unesterified fatty acids from the depots of fasting subjects is inhibited after administration of glucose[2], amino-acids[2] and fats[3], which is strongly indicative of an underlying mechanism sensitive to the availability of calorigenic substrates. Furthermore, the unesterified fatty acids released may stimulate 'latent' adenosine triphosphatase activity in the liver, making more adenosine diphosphate available for the acceptance of high-energy phosphate and allowing hepatic fatty acid oxidation to proceed at an accelerated rate[4]. Consequently, the production of acetyl-coenzyme A would exceed the capacity of the liver to oxidize it and ketogenesis would ensue. However, elevation of serum unesterified fatty acids resulting from the administration of the pituitary fraction H of Rudman *et al.* does not lead to acetonæmia in fed rabbits[5], a finding which suggests that there may be an additional hepatic effect of calorie deficiency or starvation which facilitates the conversion of mobilized unesterified fatty acids to ketone bodies in the liver. It appears that a basic mechanism could exist for the control of ketosis which does not necessarily require the active participation of hormones.

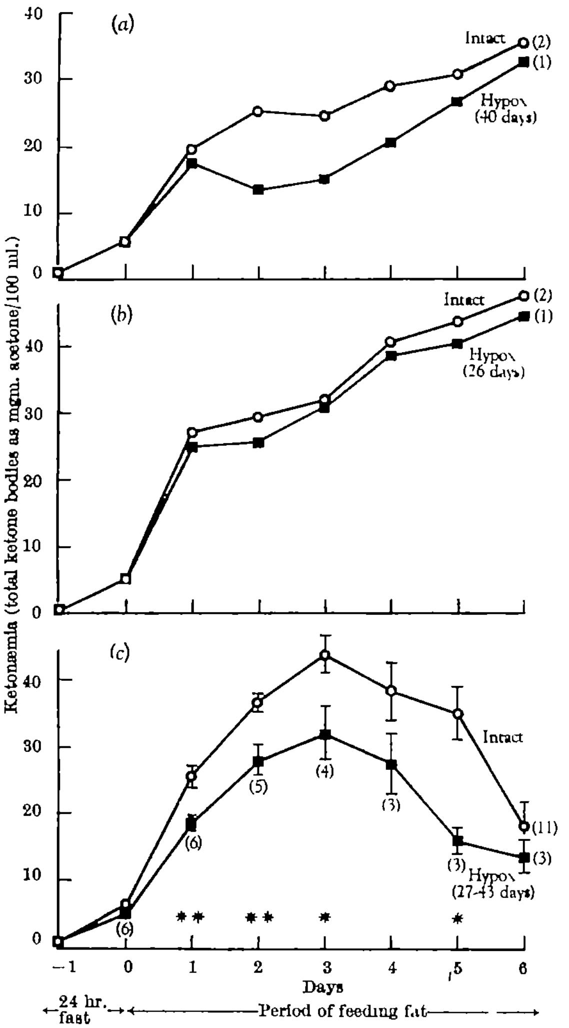

Fig. 1. Ketonæmia in hypophysectomized (hypox.) and intact rats fasted for 24 hr. and then force-fed (*a*) 2 gm., (*b*) 4 gm. and (*c*) 6 gm. butter fat/day. Average initial body-weight was 184 gm. and 187 gm. for the hypophysectomized and intact groups respectively. Temperature was held at 26–27° C. Numbers of animals are shown in parentheses. The bars indicate the *S.E.* and asterisks show where differences between operated and intact rats are significant; *, $P = 0 \cdot 05$–$0 \cdot 01$; **, $P = 0 \cdot 01$–$0 \cdot 001$

There is also much experimental work which suggests an active role for the pituitary gland in ketogenesis[6]. It is possible to envisage the secretion of the pituitary gland responding to the stimulus of calorie deficiency by promoting the mobilization of fat and ketogenesis, its effect being additive to that of the mechanism outlined here. Certain pituitary gland extracts have adipokinetic and ketogenic activity[7,8] and substances having one or both of these properties have been found in the urine of fasting rabbits[9], and in the urine of fasting humans provided a functional pituitary gland is present[10]. Removal of the pituitary gland reduces the ketonuria of phlorhizinized or pancreatectomized dogs[11]. Oastler and Anderson[12] fed hypophysectomized rats a high fat diet and found that ketonuria was abolished while ketonæmia was still present, but they did not consider their results as necessarily contradicting the hypothesis of a pituitary 'ketogenic factor'. However, the calorie intake was not controlled, a factor now considered in this laboratory to be of importance in regulating ketogenesis[1].

The experiments reported here were carried out to assess the part played by the pituitary gland in the promotion of ketosis in rats on a fat diet. A comparison was made of the ketonæmia of hypophysectomized female rats fed on an all-fat diet with that of intact controls, the calorie intake being regulated as in previous experiments[1]. Force-feeding of both groups was adopted because of the depressed appetite of hypophysectomized animals. All rats were fasted for 24 hr. and were then force-fed butter fat (2, 4 or 6 gm./day) in two equal portions given at 10 a.m. and 10 p.m. The diet contained 3 per cent salts and kaolin to reduce the incidence of diarrhœa. No results have been used where the alimentary absorption of fat was less than 98 per cent of the amount fed. Other experimental procedures have been described elsewhere[1].

The ketonæmia of the intact rats force-fed butter fat rose to approximately double the concentration found previously in intact animals consuming a corresponding amount of fat by free-feeding[1]. Qualitatively, however, the ketonæmia was similar, a reduction in ketonæmia being observed only in those rats fed 6 gm. of butter fat per day the calorie requirement of which was being satisfied from the diet (Fig. 1). These results suggest that the underlying mechanism controlling ketogenesis was the same with both methods of feeding. Force-fed intact rats were considered, therefore, to be suitable controls for force-fed hypophysectomized animals.

Comparison of hypophysectomized rats with controls showed that removal of the pituitary gland did not have any marked effect on the pattern of the ketonæmic responses of rats fed all fat diets. In hypophysectomized rats force-fed butter fat in subcalorific amounts there was an ensuing and persistent ketonæmia, similar to that of intact animals (Fig. 1*a*, *b*). In those fed 6 gm. of fat daily, thereby satisfying calorie requirements, there was a reduction of ketonæmia in the operated animals, the response again being similar to the controls (Fig. 1*c*). Nevertheless, the magnitude of the ketonæmia tended to be lower as a result of hypophysectomy, and significantly so in the case of the rats fed 6 gm. of butter fat per day. Whether this reduction in ketonæmia can be attributed to the removal of a ketogenic hormone of pituitary origin is doubtful. If such a hormone is directly involved, the smallness of the reduction suggests that it is of little physiological significance. The lesser ketonæmia of the hypophysectomized rats is more probably accounted for by their known hypothyroid condition causing a reduction in energy requirement, a situation where a diminution in ketogenesis is to be expected[1]. Confirming the hypothyroid condition of the hypophysectomized rats was their reduced requirement for metabolic fuel. This is shown by the finding that during the period of feeding fat the operated rats fed on 6 gm. fat a day gained an averge of 5·3 gm. per rat body-weight, whereas the intact controls lost 0·5 gm. per rat during the same period. The lesser ketonæmia following hypophysectomy may also result in part from reduced activity of the adrenal cortex, as an adequate level of circulating adrenal cortical hormone is necessary to permit a maximal response to many stimuli which promote fat mobilization[13]. These results are in keeping with those of other workers who have observed that hypophysectomy does not abolish either the rise in plasma unesterified fatty acids[14] or the increase in ketonæmia[6] of the fasting rat.

The failure of hypophysectomy to alter qualitatively the difference in the pattern of the ketonæmia resulting from feeding fat diets of differing calorific value to rats demonstrates that both the ketogenesis resulting from calorie deficiency, and the antiketogenic effect of feeding a sufficiency of calories in the form of fat, are not likely to be caused by variations in the hormonal secretion of the pituitary gland. Provided the secretion of a ketogenic hormone of extra-hypophyseal origin is not being stimulated, available experimental evidence suggests that one of the main sites of action of the stimulus of calorie insufficiency in promoting ketosis is to be found in the mechanism of release of unesterified fatty acids from the fat depots. In turn, the unesterified fatty acids passed into the plasma provide the principal substrate for the production of ketone bodies in the liver.

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'ADAPTATION' OF A LINE OF MOUSE BONE MARROW TO AN INCOMPATIBLE STRAIN

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TRANSPLANTATION of hæmopoietic tissue to an incompatible mouse strain following lethal whole-body irradiation usually results in the appearance of secondary radiation disease; but when fœtal hæmopoietic tissue is used in preference to adult bone marrow in the graft the disease may occur only mildly or may be avoided[1,2]. Retransplantation of bone marrow from radiation chimæras made from homografts of incompatible fœtal hæmopoietic tissue to further lethally irradiated test animals has shown a propensity for the marrow to 'take' without death ensuing from homologous disease. We have used the term adaptation without implication as to the mechanism involved at the cellular level to describe this phenomenon[3]. Koller *et al.*[4], transplanting marrow from radiation chimæras, where adult tissue had been given to effect resuscitation from the lethal effects of radiation, observed a similar phenomenon. Rubin[5] has described a related occurrence in parabiosis.

Transplantation of the adapted bone marrow to further lethally irradiated test animals of host and donor type strains has shown persistence of the phenomenon as judged by the results of genetic transplantation tests. However, as emphasized by Urso *et al.*[6], it has been necessary to exclude the possibility of reversion to host-type marrow in the primary or subsequent radiation chimæras giving a false impression of adaptation or the carrying over of a syngeneic graft with transplanted marrow. Accordingly, we have labelled the donor cells with a marker chromosome and examined cytogenetically the hæmopoietic tissues of the test animals into the second and third passage of bone marrow for its continued presence.

CBA mice were given 1,050 rads cobalt-60γ lethal irradiation. Twenty were given adult bone marrow from (*C57BL* × *T6*)F_1 hybrids and all succumbed to secondary radiation disease between the twenty-first and sixtieth days post-irradiation. In the particular line to be described, five lethally irradiated *CBA* received 1·2 × 10^7 embryo liver cells from a (*C57BL* × *T6*)F_1 litter of approximately 17 days gestation. This transplantation is considered as the initiation of the line of hæmopoietic tissue and two *CBA* were well nourished, greyed animals without evidence of diarrhœa at death 215 days later. Macroscopically, their hæmopoietic and lymphoid organs appeared normal. Bone marrow, thymus, spleen and lymph node were taken for cytogenetic examination, and passage 1 of the hæmopoietic tissue, in this case, bone marrow from the two radiation chimæras, was made to six lethally irradiated *CBA* and four lethally irradiated (950 rads) *C57BL*. Our usual dosage and method of administration of bone marrow[3] was used. Two *CBA* and two *C57BL* survived the period of secondary disease appearing greyed, well nourished and without evidence of diarrhœa after 90 days. The results of the cytogenetic investigation of the two *CBA* are shown in Table 1 as line 1*a* and 1*b*.

Table 1. CYTOGENETIC ANALYSIS OF *CBA* CHIMÆRAS

Passage	Bone-marrow *T6+*	Bone-marrow *T6−*	Spleen *T6+*	Spleen *T6−*	Thymus *T6+*	Thymus *T6−*	Lymph node *T6+*	Lymph node *T6−*
1*a*	72/1	−/1	12		—		—	
1*b*	113		10		7		11	1
2*c*	120		68		41		25	
2*d*	55	/1	16		8		14	
3*e*	16		52		—		—	
3*f*	—		49		5		5	
3*g*	53	−/1	38	−/4	—		—	

Figure to right of stroke indicates less than 40 chromosomes, that is, probably a broken cell.

The two-passage 1 *CBA* chimæras from sub-line 1*b* were killed 127 and 134 days respectively from the inception of chimærism, their tissues examined cytogenetically (Table 1, 2*c* and *d*) as before and transplantation made of bone marrow (passage 2) to eight *CBA* and seven *C57BL* lethally irradiated mice. Four *CBA* and two *C57BL* survived longer than 90 days and near the ninetieth day the *CBA* chimæras were grafted with *CBA* and *C57BL* skin on the left and right flanks respectively. One mouse died during the grafting procedure, the *C57BL* graft failed in one animal and both grafts were accepted by two. The animal with the failed *C57BL* skin graft was regrafted successfully. Transplantation of bone marrow (passage 3) was made from two-passage 2 *CBA* chimæras 166 days and 270 days respectively from the inception of chimærism to further lethally irradiated *CBA* and *C57BL* mice. Both these chimæras, the cytogenetic details of which are recorded as 3*f* and *g* in Table 1, had carried *C57BL* skin grafts for 93 days and 146 days respectively to the time of their death. The passage-2 *CBA* chimæra 3*e* from 2*d* was killed earlier (105 days) without further passage simply for confirmation of the presence of the marker chromosome.

The results show that cells carrying the marker chromosome, and those cells only, are present in this line as judged by our ability to recognize the marker in the dividing tissues examined. The marker has been followed in an unbroken line (1*b*, 2*d*, 3*e* and *f*) through three transplant generations of $(C57BL \times T6)F^1$ hæmopoietic tissue to the incompatible strain *CBA* hosts (also 1*b*, 2*c* and 3*g*) and the line will be maintained, if possible[7], at approximately six-monthly passages. In other marked lines we had previously shown the propensity for adapted marrow to take in host and donor type lethally irradiated hosts[8]; but it is felt that these serial transplantation results strengthen our hypothesis that adaptation, whether of a selective or instructive nature, does occur in recovery from or avoidance of secondary disease. Somatic mutation of the cells to enable proliferation in either host or donor type environment as an explanation is unlikely as passage of the line through donor type lethally irradiated mice abolishes their propensity to resuscitate lethally irradiated host type test animals. The similarity to the predictions of clonal selection[9] in acquired tolerance is remarkable.

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RECOVERY EFFECT OF HIGHLY POLYMERIZED HOMOLOGOUS LIVER DEOXYRIBONUCLEIC ACID INJECTED INTO LETHALLY IRRADIATED RATS: HISTOLOGICAL EXAMINATION OF THE SPLEEN AND BONE MARROW

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OUR previous work has shown that the homologous highly polymerized (native) nucleic acids from the liver and spleen of young donors may reduce mortality due to radiation[1-3]. In the course of this work on the recovery of the irradiated rats by means of nucleic acids, we showed significant thirtieth-day survival of the irradiated rats ($LD_{100/30}$) (refs. 1, 2 and 3); recovery of the metabolic functions concerning the incorporation of formate labelled with carbon-14 and phosphorus-32 in the nucleic acids of the irradiated treated recipients[4]. In addition, a recovery of the processes leading to the synthesis of the antibodies in the irradiated rats was obtained by means of native nucleic acids[5]. Our histological investigations, however, have demonstrated that the irradiated rats treated with deoxyribonucleic acid (DNA) show less-evident damage in the structure of the adrenal glands and some organs of the digestive system, that is, the structure of these organs was less altered after DNA treatment[6,7]. On the basis of these results, we are inclined to believe that the animals treated with DNA have a greater capacity for recovery from the injury caused by X-rays than the controls.

In our attempts to understand the mechanism of this recovery from injury caused by radiation, it was of interest to ascertain whether a similar recovery of the bone marrow and the spleen can be obtained after DNA treatment of the irradiated rats ($LD_{100/30}$). The present article is concerned with a histological examination of the modification of radiation-induced changes in the spleen and bone marrow of the rats exposed to a $LD_{100/30}$ of X-rays, and treated with homologous native liver DNA.

Adult albino rats, bred at our animal farm, were used. The experimental conditions and mode of treatment have already been described[1-3]. The animals treated with DNA and their respective con-

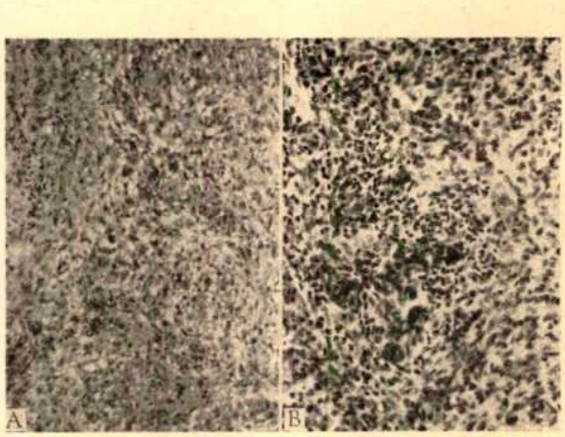

Fig. 1. Spleen of irradiated rats. *A*, untreated; *B*, DNA-treated with fairly well preserved Malpighian body

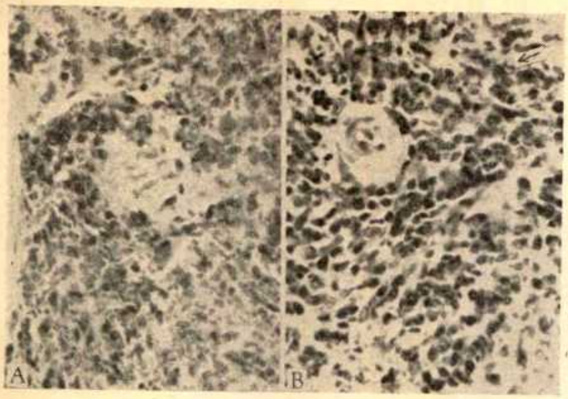

Fig. 2. Malpighian bodies of irradiated rat spleen. *A*, untreated; *B*, DNA-treated; note mitotic division

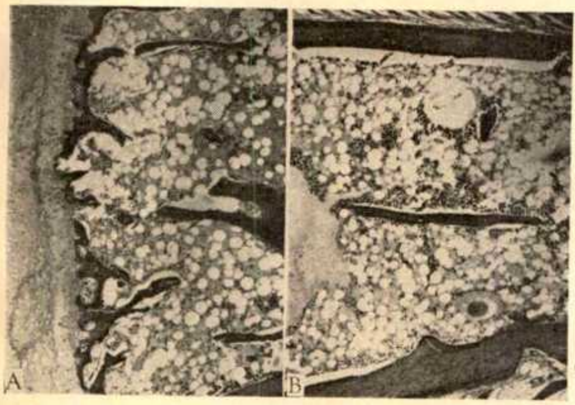

Fig. 3. Bone marrow of irradiated rats. *A*, untreated; *B*, DNA-treated

trols were killed on the fourth or ninth day after irradiation.

The microscopic specimens were fixed in 10 per cent neutral formol, Bouin and Serra fixative and 1 per cent of isotonic osmic acid. Decalcification was carried out by Ebner's method. The sections were stained with hæmatoxylin–eosine and according to Azan and Brachet.

Spleen. In the spleen of the lethally irradiated rats, as already known, only closely packed reticular cells and fibres, macrophages and some lymphocytes can be seen (Figs. 1*A* and 2*A*).

In the spleen of the animals treated with DNA the changes are, however, considerably less marked: the venous sinuses in the red pulp are less dilated and hæmorrhage is less frequent. The reticular cells are less damaged and, in addition, cells in the blast stage are found. In the cytoplasm of fixed macrophages there are many particles of pigment granules and erythrocytes in various stages of digestion. Among these cells the lymphocytes are diffusely scattered. In the spleen of the animals treated with DNA there are lymphoblasts and lymphocytes around the v. centralis in the form of small-size irregular Malpighian bodies. Some lymphoblasts in these bodies are dividing; the peripheral part of the white pulp consists mostly of altered reticular cells, macrophages and some lymphocytes (Figs. 1*B* and 2*B*).

Despite the pronounced reduction of the lymphopoietic elements in the spleen of the irradiated rats treated with DNA, the number and the structure of the lymphoblasts and lymphocytes seem to indicate a favourable recovery effect produced by the homologous liver DNA on the spleen of the irradiated animals.

Bone marrow. Injury to the bone marrow of the sternum in all the lethally irradiated rats is very great. In most of the investigated regions only the reticulocytes and fat cells were observed (Fig. 3*A*). Hyperæmia and hæmorrhage are expressed to a considerable degree. Alterations in the form, size and structure of the nuclei and cytoplasm of the reticular cells vary from cell to cell. Among these cells there is a small number of myelopoietic and erythropoietic lines formed, and occasional megakaryocytes. These forms usually appear along the compact parts of the sternum. In some areas an increased number of eosinophils is found. Closely packed, more or less altered, leucocytes are found also (Fig. 4*A*).

It has been noticed that in the bone marrow of the rats treated with DNA hyperæmia is less evident, whereas hæmorrhage is less frequent. The structure of the bone marrow gives the impression of being less damaged judging by the number and a more uniform numerical relationship of the formed elements in it (Fig. 3*B*). The number of the megakaryocytes is greater and the nucleoli are more numerous and of

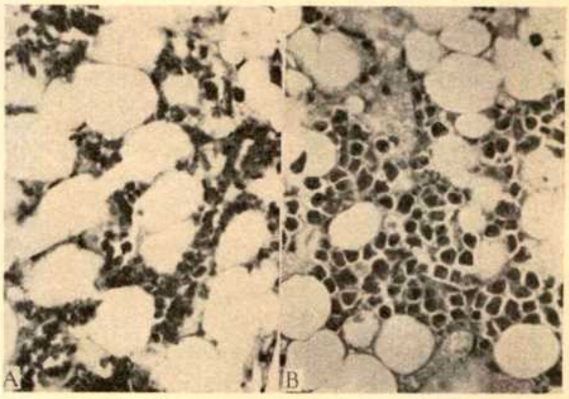

Fig. 4. Myelopoietic and erythropoietic elements in bone marrow of irradiated rats. *A*, untreated; *B*, DNA-treated

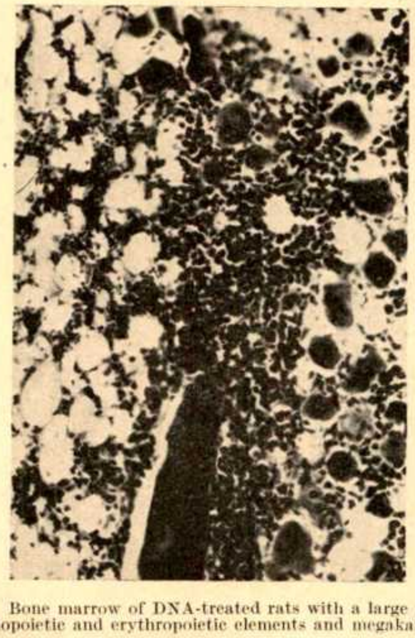

Fig. 5. Bone marrow of DNA-treated rats with a large number of myelopoietic and erythropoietic elements and megakariocytes

larger size (Figs. 4*B* and 5). The blood elements and the evolutionary forms of the blasts of the myelopoietic and erythropoietic line are distributed in groups, most frequently along the trabeculæ. The division of the cells is more numerous in the bone marrow of the rats treated with DNA. Groups of eosinophilic granulocytes in all phases of development are also found in the bone marrow of these animals.

On the basis of these preliminary results, we consider that the spleen and the bone marrow of the lethally irradiated rats treated with DNA are less damaged than the controls. The reticular cells are not so much altered and there are more lymphopoietic elements in the spleen and myelopoietic and erythropoietic elements in the bone marrow.

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ANTAGONISM OF PENTOBARBITONE ANÆSTHESIA IN HAMSTERS BY 5,5-DIPHENYLHYDANTOIN MODIFIED BY SALICYLATE

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DURING work[1] concerned with the action of salicylate[2,3] on periodontal effects of 5,5-diphenylhydantoin (phenytoin, B.P.), it was noted that some hamsters receiving phenytoin could not be satisfactorily anæsthetized with amounts of pentobarbitone adequate for the same animals before treatment. With satisfactory anæsthesia the required dental impressions could be taken during a 15-min. period without eliciting reflex movements. In contrast, in animals treated with phenytoin, impression-taking was often accompanied by alternate depression and elevation of the mandible; apparently touching the hard palate elicited a combination of linguomaxillary (mandibular) and palatomaxillary reflexes similar to that previously noted in other animals[4]. These reflex movements ceased on removing the impression tray from the mouth, but in order to complete the dental procedure unhindered additional pentobarbitone had to be administered. Nevertheless, despite such supplementation, the duration of anæsthesia appeared to be no longer than in the controls.

Further periodontal studies have now provided the opportunity to confirm our preliminary observations. Forty-eight young (40–80 gm.) male hamsters were assigned among four groups so as to give a balanced distribution of body-weights. The animals were housed singly in wire mesh cages with hay bedding and fed, as previously[1], a powdered high carbohydrate diet, the physical properties of which induce marginal gingivitis[5]. Eleven days later they were anæsthetized with a standard dose (65 mgm./kgm., intraperitoneal) of pentobarbitone sodium in order to take dental impressions. Table 1 shows the duration of 'sleeping time'[6] and the abolition of jaw reflexes in response to lightly touching the hard palate. For 65 mgm./kgm. pentobarbitone, analysis shows that the variance of group means for both parameters is not significantly different from that of individuals. Unlike our previous experience with older animals[1], in this series of young animals three required supplements of pentobarbitone for satisfactory anæsthesia. On experimental day 1, the following group treatments were started and continued as indicated in Figs. 1 and 2: (*a*) controls, sodium carbonate (intraperitoneal) and/or sodium chloride (subcutaneous) to match the pH of the phenytoin solution and provide sodium equivalent to phenytoin and/or salicylate; (*b*) phenytoin sodium 25 mgm./kgm. (intraperitoneal); (*c*) sodium salicylate 60 mgm./kgm. (subcutaneous); (*d*) both phenytoin and salicylate as in (*b* and *c*). At intervals during this regimen the hamsters were

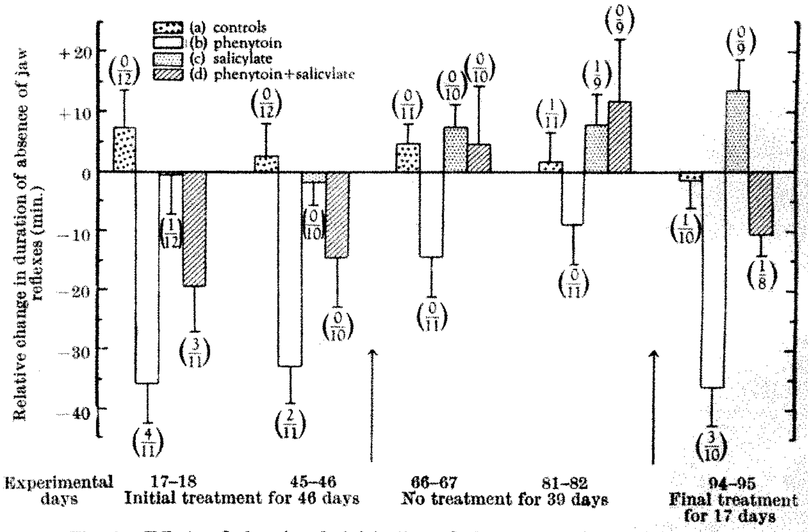

Fig. 1. Effects of chronic administration of phenytoin and salicylate on the duration of abolition of mandibular reflexes by pentobarbitone, 65 mgm./kgm. (intraperitoneal). The bars represent the means of the individual differences, calculated with reference to the duration the reflexes were absent before treatment began, each animal serving as its own control throughout the experiment. The number of animals and the proportion requiring supplements of pentobarbitone to complete dental procedures are indicated in parentheses by the fractions accompanying the measure of the standard error

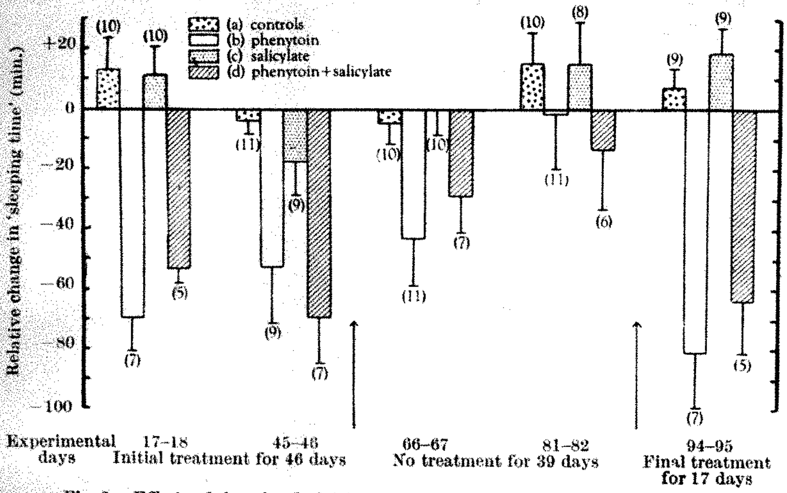

Fig. 2. Effects of chronic administration of phenytoin and salicylate on 'sleeping time' following pentobarbitone, 65 mgm./kgm. (intraperitoneal). As in Fig. 1, the bars represent means of the individual differences calculated with reference to the pre-treatment period. The number of animals is shown in parentheses accompanying the measure of the standard error

anæsthetized with pentobarbitone, 65 mgm./kgm. and tested as already described, 24–30 hr. following the previous daily treatment.

In Figs. 1 and 2 the duration of the absence of jaw reflexes and 'sleeping time' are compared with the initial values (cf. Table 1) for the same individuals before treatment. Clearly, chronic treatment with phenytoin reduced both the duration of the absence of mandibular reflexes and 'sleeping time', which is reflected by the increased number of animals requiring supplements of pentobarbitone for the completion of the dental procedure. These supplements did not increase 'sleeping time', as was the case for the control group (*a*) and for some animals in groups (*c* and *d*) before treatment (Table 1). Factorial analysis of variance for data on jaw reflexes revealed some interaction between phenytoin and salicylate, but this was not significant ($F_{1\cdot 39} = 3\cdot 43$, $0\cdot 05 < P$); furthermore salicylate treatment did not have a significant effect. Data for days 38–39, not shown in Figs. 1 and 2, confirmed these findings. In contrast, during the second treatment period (experimental days 94–95), when interaction between the two drugs was negligible, salicylate was shown to have a highly significant effect ($F_{1\cdot 33} = 8\cdot 82$, $0\cdot 01 > P$). Thus in group (*d*) salicylate counteracted the effect of phenytoin; similarly it prolonged the action of pentobarbitone in group (*c*). No interaction was found in the data for 'sleeping time', and it was clear that salicylate did not affect the action of phenytoin in group (*d*), nor that of pentobarbitone in group (*c*).

Table 1. PENTOBARBITONE ANÆSTHESIA IN HAMSTERS BEFORE TREATMENT

	(*a*) Controls*	(*b*) Phenytoin*	(*c*) Salicylate*	(*d*) Phenytoin salicylate*
Duration (min.)† jaw reflexes absent after pentobarbitone, 65 mgm./kgm.	25·7 ± 4·2 (12)	39·5 ± 4·2 (12)	27·1 ± 3·8 (12)	24·0 ± 7·5 (12)
Proportion of animals needing supplements of pentobarbitone to abolish jaw reflexes	0/12	0/12	1/12	2/12
'Sleeping time' (min.)† (i) animals receiving pentobarbitone, 65 mgm./kgm.	81·1 ± 7·7 (11)	110·9 ± 10·6 (12)	91·3 ± 12·2 (11)	95·8 ± 11·6 (9)
(ii) All animals including those receiving supplements of pentobarbitone	as (i)	as (i)	106·3 ± 18·7 (12)	117·2 ± 17·2 (11)

* Treatment subsequently administered.
† Mean ± *S.E.*, number of animals in parentheses.

In the interval between treatments (for example, experimental days 66–67 and 81–82), 65 mgm./kgm. pentobarbitone again gave satisfactory anæsthesia, but residual effects of phenytoin seemed to be present in group (*b*), as shown by the short period when jaw reflexes were absent and by the reduced 'sleeping time' on experimental days 66–67 ($0\cdot 05 > P > 0\cdot 02$). This accords with previous reports of the persistent action of a single[7], or chronic[8,9], administration of phenytoin.

Our experiments in hamsters show that phenytoin can antagonize some depressant actions of pentobarbitone, which seems paradoxical in view of the use of phenytoin and barbiturates in combination to suppress experimentally induced convulsions and treat grand mal epilepsy. In line with our findings, however, is an earlier report[10] that, in dogs, a single intravenous administration of phenytoin, 50 mgm./kgm., restored the knee jerk reflex previously depressed by pentobarbitone, 70 mgm./kgm.; conversely, phenytoin enhanced the depression of this reflex by ether. Moreover, while phenytoin suppresses the tonic phase of electroshock convulsions in rats it prolongs the clonic phase[7], and in low doses (< 100 mgm./kgm.) enhances carbon dioxide-induced spontaneous and withdrawal seizures[11]. Apart from the central nervous system, however, antagonism between phenytoin and pentobarbitone might possibly arise from adaptive increases in barbiturate-metabolizing enzymes, as recently reported[12] for a number of substances of widely differing chemical composition. Previous findings[2,3] would support the concept that phenytoin can enhance the central excitatory action of salicylate; but this is not a satisfactory explanation of our results, particularly as regards jaw reflexes on experimental days 94–95 (Fig. 1).

Since epileptics receiving phenytoin therapy may well try self-medication with salicylates, further examination of phenytoin–salicylate interaction seems desirable.

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CYTOLOGY OF COLLEMBOLA

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UNTIL 1956 the chromosome numbers of only three species of Collembola (Insecta : Apterygota) were known[1-3]. Since then, data for eighteen other species have been published[4-6]. Table 1 gives chromosome numbers for twenty additional species from Bahía Blanca. The material was fixed in Clarke's solution[7]—glacial acetic acid previously saturated with ferrous hydroxide. The gonads were dissected out and squashed in acetic hæmatoxylin.

Table 1

ARTHROPLEONA	n♀	$2n$♀	$2n$♂
Hypogastruridae			
Hypogastrurinae			
Hypogastrura manubrialis (Tullb.)	7	14	
Ceratophysella armata var. *communis* (Fols.)	7		
Brachystomellinae			
Brachystomella parvula (Schäff.)	4	8	
Probrachystomella rhodosoma Rap. (in litt.)	6		
Isotomidae			
Proisotominae			
Ballistura schötti (Dalla Torre)	7		
Proisotoma fatonei Rap.	7		
P. minuta (Tullb.)	4		
Isotominae			
Isotomina thermophila (Axels.)	5		
Isotomurus palustris (Müller)	7		
Entomobryidae			
Entomobryinae			
Entomobrya pseudodecora Rap. (in litt.)	6	12	11
E. lanuginosa olivacea Rap. (in litt.)	6	12	11
E. multifasciata (Tullb.)	6		11
E. atrocincta f. *pseudoperpulchra* Mills	6		11
Pseudosinella sexoculata Schött.	6		
Lepidocyrtinus domesticus (Nic.)	6	12	
Cyphoderinae			
Cyphoderus assimilis empodialis Rap. (in litt.)	6		
SYMPHYPLEONA			
Sminthuridae			
Sminthuridinae			
Sminthurinus mime Börner	5		
S. nunezi Rap. (in litt.)	5		
Sphaeridia pumilis (Krausb.)	5	10	
Katianna sp.	5		9
Dicyrtominae			
Dicyrtomina sp.	5		9

Hypogastrura manubrialis and *Ceratophysella armata* var. *communis* have normally n♀ = 6, but now and then females turn up with 1–3 supernumerary chromosomes.

Saure and Brummer-Korvenkontio[5] found $n = 6$ in *Isotomurus palustris* and observed oocytes with a small additional chromosome. In the two forms of this species I have studied all the oocytes had seven bivalents (Fig. 1).

Tuzet and Manier[4] have determined $2n$♂ $= 8 + X$ in 3 species of *Entomobrya*, but my findings in 4 species of this genus show $2n$♂ $= 11$ (Fig. 3). It is noteworthy that one male of *E. pseudodecora* exhibited a spermatogonial metaphase with $2n = 12$ (Fig. 4) and another with $2n = 14$; other abnormal numbers appeared also to be present in this specimen. Moreover, I have found in this species a chromosome with differential behaviour, at the very early meiotic prophases, where a heteropycnotic element shows up by the nuclear membrane.

As the diploid set of males of two species of *Katianna* and *Dicyrtomina* exhibited also one chromosome less than that of the females, one should accordingly ascribe to Collembola, or at least to the families *Entomobryidae* and *Sminthuridae*, the XX♀ : XO♂ type of sexual determination.

No evidence has been found supporting Tuzet and Manier[4] to the effect that the diploid spermatogonia originate from polyploid.

Saure and Brummer-Korvenkontio[5] observed chromosome bridges in polyploid anaphases of young individuals of *Tomocerus vulgaris*. In most species I have studied, these so-called bridges are a regular feature of mitotic and second spermatocyte anaphases as a result of the distal ends of the longest chromosomes remaining in contact when the other chromosomes have already moved apart (Fig. 2).

Available results show a decrease of the basic chromosome number parallel to phylogenetic advance to reach a striking constancy within each of the more evolved families. It is not possible to advance as yet a sound opinion on the mechanism of this decrease because the nature of the material at hand and the limitations of the technique prevent a satisfactory analysis of the caryotypes.

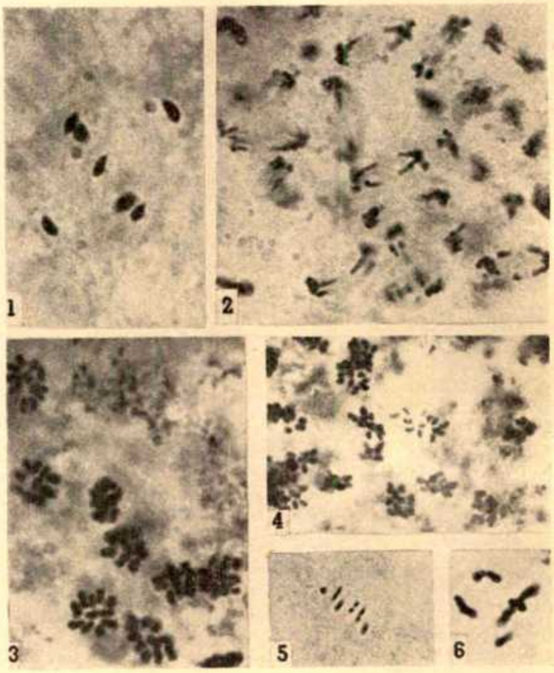

Fig. 1. Photomicrographs of chromosome behaviour in Collembola from temporary acetic hæmatoxylin squashes. All the plates have been pressed to spread out the chromosomes. (1) *Isotomurus palustris*, oocyte late diakinesis, $n = 7$ (× *c.* 1320); (2) *Hypogastrura manubrialis*, second spermatocyte anaphases (× *c.* 870); (3) *Entomobrya pseudodecora*, spermatogonial metaphases, $2n = 11$ (× *c.* 1320); (4) *E. pseudodecora*, same gonad as 3, $2n = 12$ (× *c.* 660); (5) *E. lanuginosa olivacea*, oocyte metaphase, $n = 6$ (× *c.* 600); (6) *Brachystomella parvula*, oocyte diakinesis, $n = 4$ (× *c.* 1320)

Collembola support Mather's[8] views on the connexion between a life-cycle with several generations per annum and low chromosome numbers (see also ref. 9).

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UPTAKE OF AMINO-ACIDS BY THE FUNGUS *BOTRYTIS FABAE*

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THE active accumulation of amino-acids has been intensively studied in bacteria[1–3] and animal cells[4,5]. Active transport of amino-acids in yeasts has been described[6,7]; but in the fungi generally information on the mechanism of action and specificity of the uptake process is slight. A study of this transport process in the fungi, with particular reference to the plant parasitic fungus *Botrytis fabae*, has now been made and is the subject of this report.

Botrytis fabae was grown in liquid culture 20° C. with constant shaking. The density of the spore inoculum greatly affected the diameter of the mycelial pellets and it was found most convenient to inoculate heavily to induce the growth of small pellets. The spheres were gathered by filtration, washed well with distilled water and then suspended in a 10^{-4} *M* solution of the amino-acid the uptake of which was being measured. The uptake of amino-acid from the suspending liquid was measured over periods of up to 40 min. by determining the amino-acid remaining in aliquots of the supernatant by a ninhydrin assay[8]. Recovery experiments were carried out by washing the pellets after incubation in the amino-acid, extracting in 70 per cent ethanol and assaying the ninhydrin-positive material in the alcoholic extract. The identity of the recovered amino-acid was checked by two-dimensional paper chromatography in butanol/acetic acid/water (4 : 1 : 5) and saturated phenol–water systems.

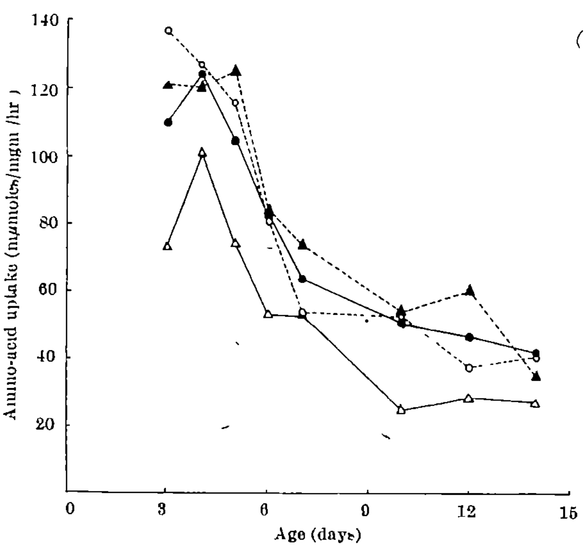

Fig 1 Change with age in rate of uptake of amino-acids by mycelium of *Botrytis fabae*
○, L-Glutamic acid; ▲, L-lysine, ●, L-methionine, △, L-tyrosine

It was found that the age of the fungus preparation greatly affected its activity (Fig. 1) and 4–5-day-old cultures were generally used in order to gain near maximal activity. The nitrogen content of the growth medium also profoundly affected the rate of amino-acid accumulation. When grown on a normal nitrogen-level medium (1 gm. nitrogen per litre as peptone) a high level of endogenous amino-acid was produced in the cells and the resulting outward diffusion of amino-acids masked any uptake. Growth at 0·1 gm. nitrogen per litre overcame this problem. Uptakes of amino-acid were found to be proportional to the dry weight of mycelium taken, which enabled comparisons of uptake rates of different amino-acids to be made (Table 1).

Table 1. UPTAKE OF AMINO-ACIDS BY *Botrytis fabae* FROM 10^{-4} *M* SOLUTION AT *p*H 5

Amino-acid	Uptake of L form (mμ*M*/mgm./hr.)	Uptake of D form (mμ*M*/mgm./hr.)
Glycine	135	—
Lysine	126	130
Glutamine	125	—
Valine	123	105
Serine	118	121
Methionine	116	107
Threonine	116	110
Glutamic acid	108	100
Isoleucine	106	45
Phenylalanine	104	140
Leucine	100	88
Histidine	100	129
Citrulline	100	94
Aspartic acid	97	—
γNH_2butyric acid	96	—
Alanine	96	150
Tyrosine	64	—
Di-OH phenylalanine	63	—
Tryptophan	38	45 (DL)

The most interesting feature of the results in Table 1 is the small difference in rates of uptake between different amino-acids, unlike the rates found in bacteria[2], and the lack of any marked stereoisomer specificity. Tyrosine, dihydroxyphenylalanine and tryptophan gave consistently low results. The rates of uptake appeared independent of the amino-acid recovery, which varied from 35 to 100 per cent for different amino-acids. Amino-acids may be concentrated up to a cellular concentration approaching 10^{-2}–10^{-4} *M* external concentration, with no marked differences between amino-acids.

Similar experiments, using a limited number of amino-acids, were carried out on a range of fungi (Table 2). In general, it appears that although the absolute rate of uptake of each amino-acid varies

Table 2. RATES OF UPTAKE OF FIVE DIFFERENT AMINO-ACIDS FROM 10^{-4} *M* SOLUTION BY A RANGE OF FUNGI

Fungus	Rate of amino-acid uptake in mμ*M*/mgm./hr. Phenyl-alanine	Glutamic acid	Lysine	Leucine	Meth-ionine
Botrytis fabae	112	89	108	115	105
Aspergillus niger	18·8	21·7	—	—	25·6
Pythium ultimum	4·0	7·0	10·7	12·6	12·4
Phytophthora palmivora	Any uptake masked by outward diffusion				
Fusarium culmorum	190	182	182	190	210
Cladosporium herbarum	26·3	25·2	42·3	51·6	48·5
Helminthosporium gramineum	10·4	4·1	20·3	9·4	13·2
Alternaria solani	40·5	28·1	39·8	50·7	49·1

widely in different fungi the ratio of rate of uptake of one amino-acid compared with another tends to be the same in different fungi, suggesting a common means of uptake.

The specificity of the accumulatory process was investigated by measuring the uptake of a labelled 10^{-4} *M* amino-acid labelled with carbon-14 in the presence of other unlabelled amino-acids at 10^{-3} *M*. The results of such an experiment with L-histidine given in Table 3 are typical. It is found that there is competition for accumulation between all the amino-acids tested, suggesting a common uptake process of perhaps varying substrate affinity. However, some degree of specificity is suggested by the greater effect of L-arginine and L-lysine on L-histidine accumulation and the similar more effective competition between L-glutamic acid and L-aspartic acid, L-alanine and L-valine.

The accumulation of an L-amino-acid is unaffected by the presence of its corresponding keto acid or by peptides, and only slightly by short straight-chain aliphatic amines. When *N* substituted derivatives of glycine were used as competitors of L-histidine uptake it was found that *N* acetyl glycine, hippuric acid, sarcosine, *N* : *N* dimethyl glycine and betaine were completely inactive, as was glycine hydroxamic acid.

Table 3. INHIBITION BY 10^{-3} *M* AMINO-ACIDS OF UPTAKE OF 10^{-4} *M* L-HISTIDINE

Competing amino-acid	Inhibition of uptake of L-histidine (per cent)
L-Glutamic acid	50
L-Phenylalanine	63
L-Valine	43
L-Arginine	83
L-Leucine	50
L-Lysine	84
L-Citrulline	44
L-Isoleucine	42
DL-Ornithine	75
γ-Aminobutyric	27
L-Methionine	65
L-Alanine	56
β-Alanine	10
L-Tryptophan	17
L-Glutamine	48
L-Serine	53
Glycine	31
L-Threonine	35
L-Aspartic acid	56
L-Asparagine	66
L-Proline	38

It is concluded that this accumulatory process is specific for α-amino-acids in which the carboxyl and amino-groups are unsubstituted. The competition between amino-acids for a transport system may help to explain results where high concentrations of added amino-acids have increased plant resistance to fungus attack[9,10]. Such added amino-acids may compete for uptake with an amino-acid which is essential for fungal growth.

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MODIFICATION OF METABOLISM AND DISTRIBUTION OF LIPIDS BY ETHYL CHLOROPHENOXYISOBUTYRATE

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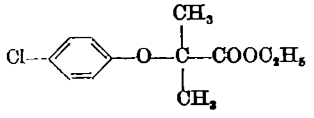
IN a screening test in rats on a normal diet it was found that a series of aryloxyisobutyric acids and related compounds showed marked activity in reducing total lipid and cholesterol concentration in blood and liver[1]. The compound, which combined maximum activity with minimum toxicity, was ethyl α-(4-chlorophenoxy)isobutyrate (I). The corresponding free acid, to which (I) is metabolized[1,2], was found to be equally active:



(I)

(I) was effective at levels of 0·15–0·3 per cent in the diet, or at 100–200 mgm./kgm. daily by oral or subcutaneous administration. At these doses no interference with growth of the animals occurred. Although active in both intact and gonadectomized rats, (I) enhanced the reduction of blood cholesterol induced by subcutaneously implanted œstradiol or testosterone. *In vitro* conversion of acetate labelled with carbon-14 into cholesterol by liver slices from rats treated with (I) was reduced by 30–50 per cent compared with controls[2]. In contrast to 2-(4-biphenylyl)-Δ^4-hexenoic acid[3], and similar branched-chain acids, which have been used clinically for the reduction of serum cholesterol, (I) caused no inhibition of the acute hyperlipæmia and hypercholesterolæmia induced in rats by intravenous administration of the surface-active polymer 'Triton *WR*-1339'.

The effects of (I) were also examined in other species. In the dog intravenous doses up to 100 mgm./kgm. did not alter cardiac or respiratory function, nor have any choleretic action. In the rabbit there was no significant inhibition of the aortic atheroma resulting from cholesterol feeding. The rise in serum cholesterol induced by atherogenic diets in the monkey and chicken was increased by treatment with (I). In the monkey, aortic atheroma was exacerbated in proportion to the rise in serum cholesterol, whereas in the chicken[4] there was no alteration of the degree of aortic or coronary atherosclerosis. Renal hypertrophy which was noted in the chicken was found to be due to inhibition by (I) of the tubular secretion of uric acid. In other species uric acid excretion was increased by (I), without renal hypertrophy.

The mode of action of (I) in the rat became clearer when it was found that it potentiated the metabolic and other actions of exogenous steroids, and that its activity in reducing the serum cholesterol varied in phase with a variation in serum cholesterol in control animals (Table 1).

Table 1. Seasonal Variation of Serum Cholesterol in Control Rats and in the Response to Treatment with (I)

Mid-point of observations	Control rats* No. of expts.	Control rats* Mean serum cholesterol (mgm./100 ml.)	*S.E.*	(I)-treated rats† Mean serum cholesterol (mgm./100 ml.)	(I)-treated rats† Percentage reduction of serum cholesterol
January	10	77 8	1·95	62	20
February–March	9	71·5‡	1·7	38	47
April	13	80·5‡	1·65	56	30
June	9	76·2	1·7	51	33
August	14	70·0‡	1·65	35	50
October	9	70·2	1·65	47	32
November–December	5	81‡	1·9	68	15

* Groups of 4–12 ovariectomized 130–160 gm. rats in each experiment.

† Treatment with 0 25 per cent (I) in diet for two weeks. Average values interpolated from results of 14 experiments.

‡ Mean significantly ($P < 0·05$) different from preceding value in column.

The seasonal variation of cholesterol in control rats was similar in amplitude and frequency to a variation in adrenal weight and adrenal ascorbic acid content[5], and was also correlated with a variation in morphological indices of adrenal and thyroid function[6]. As the periods of maximum activity of (I) corresponded with those of maximum thyroid and adrenocortical function, it seemed that (I) might be acting by potentiating the metabolic effects of adrenal steroids, the secretion and metabolic fate of which are known to be influenced by thyroxine. (I) was therefore tested in combination with exogenous steroids. Androsterone, on oral administration with (I), proved the most effective in reducing both liver and serum lipids at times of the year when (I) alone was relatively ineffective[7]. The results of a typical experiment are shown in Table 2, giving the average values found in groups of three male rats treated for 11 days. When administered orally, androsterone alone was devoid of significant action, nor did it increase the action of (I) at times when this compound alone was showing maximum activity.

Table 2. Effects of Treatment with (I) and Androsterone in the Rat

Group	Body-wt. (gm.) Initial	Body-wt. (gm.) Final	Serum cholesterol (mgm./100 ml.)	Liver composition (per cent fresh wt.) Water	Protein	Total lipid	Phospholipid	Cholesterol
Control	128	164	60	70·3	19·0	10·0	3·2	0 235
Treated*	133	157	34	70·3	22 9	6·8	3 5	0·200

* Administration of (I) (0·25 per cent) and androsterone (0·01 per cent) in the diet for 11 days.

Despite its marked effects on lipids, androsterone administered in combination with (I) showed as little androgenic activity as when administered orally alone. In castrated male rats (170 gm.) treatment with 0·5 mgm. adrosterone subcutaneously daily for 9 days increased ventral prostate weight by 81 mgm./100 gm. body-weight. At a dose of 2 mgm. androsterone orally, with or without 50 mgm. of (I) per rat daily, the increase in weight of the prostate was 11 mgm./100 gm. body-weight. The possible reasons for this dissociation of action are discussed elsewhere[10].

The discrepancy of the lipid responses in the rat and monkey to treatment with (I) alone may depend on differences in adrenocortical function. It is known that the ratio of hydrocortisone to corticosterone secreted by adrenals of monkey and man differs markedly from that of the rat and it is possible that this may be associated with differing levels of androsterone. In man the rate of secretion of adrenal steroids, their state of oxidation, and the amount of androsterone formed are largely dependent on thyroid function[8,9]. It has been gratifying to find that the combination of (I) with androsterone reduces serum cholesterol and triglycerides, without apparent effect on thyroid function, in the monkey[10], and in man[11], as in the rat. The results of these studies suggest that androsterone may function primarily as a regulator of metabolism rather than as an androgen.

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PTYCTODONTID FISHES AND THE ANCESTRY OF HOLOCEPHALI

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DR. T. ØRVIG has recently[1] described, as *Ctenurella gladbachensis*, n.g., n.sp., a most interesting fossil fish from the Oberer Plattenkalk (top-most Middle Devonian) of the Bergisch Gladbach area east of Cologne. This is clearly a member of the group of placoderm fishes known as Ptyctodontida, and *Ctenurella* shows a number of features of very great significance to work on comparative anatomy and phylogeny. The deep, strongly built palatoquadrate has three large articulation-areas which imply extremely firm attachment to the cranium, and the new material adds much to descriptions of this element in *Rhynchodus*[2]. Ørvig's account has very greatly strengthened the case for regarding the Ptyctodontida as ancestors of the Holocephali, with their autostylic suspensorium. The rostral structures described by Ørvig are equally significant.

The post-cranial skeleton also shows new features of great interest. Hitherto the only Ptyctodontida in which substantial remains are known have been *Rhamphodontus eximius* Jaekel (syn. *Rhynchodus tetrodon* Jaekel) from the Upper Devonian of Wildungen[2,3] and *Rhamphodopsis* Watson[3-8] from the Middle Old Red Sandstone of Scotland. Both genera are characterized by well-developed spinalia representing at least part of the pectoral fin-structures[9]; and *Rhamphodopsis* has a large median spine, inserted in a slot in the median dorsal, which was presumed to form a 'cutwater' in front of an anterior dorsal fin, a basal element of which is also preserved. *Ctenurella* has a large mobile pectoral fin, but the spinale is apparently completely absent, and the anterior dorsal fin-skeleton is well developed, with no spine (though a low crista is present on the median dorsal). The pectoral fin is reconstructed as a tribasal fin on what seems very reasonable evidence. The pelvic fins are remarkable in that they appear to show sexual dimorphism (as seems likely also in the case of *Rhamphodopsis* Watson[4] where the evidence has been less complete). The presumed males, with long basipterygia, have also a small 'spine' anterior to the pelvic fin, identified by Ørvig as homologous with the tenaculum of Holocephali. It is interesting that a small 'spine' also precedes the main structures of the posterior dorsal fin.

This new material clearly adds very greatly to the probability that Ptyctodontida include the ancestors of Holocephali, as long ago suggested in refs. 5–8 and 10. There are two matters of considerable interest in Ørvig's account that call for immediate comment.

The first concerns the interpretation of the skull-roof and cheek, which Ørvig found very puzzling. It would seem that the difficulties may arise from the incompleteness of his material and from errors in the identification of the bones. Watson's reconstruction of *Rhamphodopsis*, with a few modifications and some corrections in homology as compared with more normal Arthrodira, can be confirmed on additional material collected by me, by Dr. R. Denison and by Mr. R. Miles, which is now under active investigation. I have already (ref. 6) ventured a re-interpretation of Watson's reconstruction (in which the hyoid arch was shown in front of the orbit) by adding a post-marginal and by re-interpreting Watson's 'suborbital' and 'bone *X*' as marginal and suborbital respectively. The new specimens confirm this structural pattern, which is shown diagrammatically in text-figure 1*a*. The structure of *Rhamphodopsis* has been reviewed in refs. 5 and 8, and Ørvig[1] gives yet another version.

The homologies used by several authors for the same element in *Rhamphodopsis* are shown in Table 1.

Table 1

Rhamphodopsis Watson 1938	 Stensio 1948	 Ørvig 1960	 Westoll 1945, 1962	*Ctenurella* Ørvig, 1960 *A*	 *B*
MB	*N*	*Nu*	*Nu*	—	—
ExB	*Pan*	*M*	*PNu*	*Pan*	*M*
C	*C*	*Cl*	*C*	*CNu*	*Pan*
PrO	*PrO*	*C.Pro*	*PrO*	*C.Pro*	*C.PrO*
'*Y*'	*Pp*	*Cm*	*PP*	—	*CNu*
PtO	*Cl*	*Pto*	*PtO*	*Pto*	*Pto*
—	—	—	*PM*	*M*	—
SO	*Pto + M*	*So*	*M*	*SO*	*SO*
'*X*'	*SO*	*X*	*SO*	—	—

In Table 1 the abbreviation-letters are as given in the original works, and indicate the use of well-established arthrodiran bone names: *N* or *Nu* (nuchale) ≡ *MB* (median basal); *PNu* = *Pan* (paranuchale) ≡ *ExB* (external basal), *C* = centrale; *PrO* = *Pro* (pre-orbitale); *PP* (post-pineale) = '*Y*' = *Pp*; *PtO* = *Pto* (post-orbitale); *M* = marginale; *Pm* = post-marginale; *SO* (sub-orbitale). In the text these names are anglicized. Stensio's 'lateral central' (*Cl*) and '*Pto* + *M*' and Ørvig's 'centro-nuchal' (*CNu*), 'centro-preorbital' (*C.Pro*), 'lateral central' (*Cl*) and 'median central' (*Cm*) represent versions of the usual view of the Swedish school that differences in bone-patterns frequently involve redistribution and differential fusion of a number of separate elements rather than the results of differential growth.

In brief justification of some of the homologies implied by the terminology for *Rhamphodopsis* used in the present account, it should first be pointed out that the bone called 'suborbital' (*SO*) by Watson and by Ørvig is suturally united to more dorsal elements, a condition not known in other arthrodires, and that the true suborbital is always associated with the outside of that part of the palatoquadrate that supports the posterior supragnathal, the homologue of the 'upper tooth-plate' of ptyctodonts. Furthermore, the backward movement or enlargement of the orbit so that the marginal enters the orbital margin between *PtO* and *SO* is exactly paralleled in many euarthrodiran genera from the Upper Devonian of Wildungen[11], where there is no doubt of the homologies (for example, *Pachyosteus*, *Rhinosteus*, *Enseosteus*, *Brachydirus*, etc.). In the skull-roof, a post-pineal fontanelle in the dermal bones is found in several Wildungen genera (for example, *Belosteus*, *Trematosteus*), and may be partly filled by a median post-pineal bone, comparable directly with Watson's bone '*Y*'. 'Scissors-and-paste' homologies are unnecessary.

Ctenurella, as interpreted by Ørvig, shows some remarkable anatomical inconsistencies as compared with its nearest relatives and with other arthrodires. The most obvious are: (*a*) that the bone identified as paranuchal, which normally would be expected to articulate with a condylar trochlea on the anterior dorso-lateral, is shown with a posterior process lying far admesial to that trochlea; (*b*) that the articulation with the trochlea is said to be on the posterior surface of the bone called marginal, in both *Ctenurella* and *Rhamphodopsis*, a condition quite unknown in normal arthrodires. It seems clear that a drastic re-interpretation is necessary, and fortunately *Rhamphodopsis* allows this to be done.

Ørvig thought that the posterior process of his 'paranuchal' may have passed beneath the median dorsal and prevented excessive downward movement of the head. Examination of his Pl 26, Figs. 1–2 suggests that there is a flanged area at the back of this bone. Two possibilities may be considered:

(*A*) The best specimen figured by Ørvig (Pl. 26, Figs. 1–2) is clearly somewhat damaged and incomplete. If Ørvig's 'paranuchal' is correctly identified, then its posterior process in life should have adjoined the trochlea on the anterior-dorso-lateral. Com-

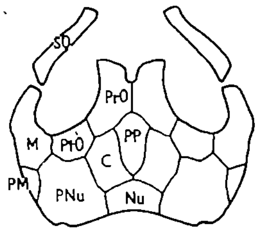

Fig. 1. *Rhamphodopsis trispinatus*, flattened skull-roof modified after Watson from new material

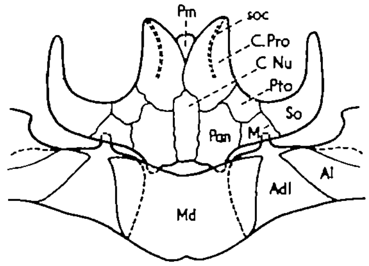

Fig 2. *Ctenurella gladbachensis*, redrawn from Ørvig, that author's interpretation

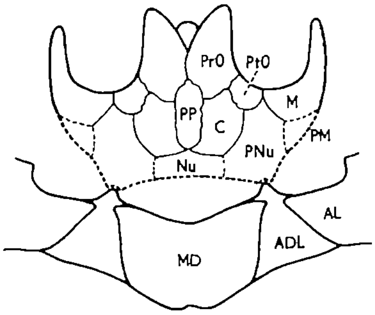

Fig. 3. Same as Fig. 2, re-interpreted and restored where necessary (broken lines)

parison with the diagram of *Rhamphodopsis* would then suggest the equivalences shown under *Ctenurella*, *A* in Table 1.

In other words, the bone taken by Ørvig as a median 'centro-nuchal' is not median; two important median elements are not preserved in the figured fossil material, and the articulation with the body-armour could be adjusted to this. Such an arrangement, however, is found on trial to require a very broad set of median elements, and if Ørvig's recognition of the median line between the bones he labels '*C.PrO*' is correct, leads to a number of serious inconsistencies of shape. Furthermore it requires the homologues of the postorbital and postmarginal to meet so as to separate the *PNu* and *M*; while perhaps not impossible, this would be very unusual. A possible point in favour of this view, however, is that Ørvig's 'centro-nuchal' becomes the normal paired central, on which sensory-canal grooves would in fact be expected to occur. On the whole, however, this interpretation leads to more difficulties.

(*B*) The second, and certainly the more acceptable, comparison is of the simplest and most direct kind, in that every element in *Ctenurella* has a one-to-one equivalence to one of *Rhamphodopsis*, the major difference being the absence or loss of the nuchal element in the former The homologies are then those shown under *Ctenurella*, *B*. in Table 1 and Fig. 3.

It was noted above that the posterior process of Ørvig's 'paranuchal' seems to have flanges developed, which could well represent overlap-surfaces for a median nuchal (*Nu*) and for an admesial extension of the existing 'marginal', which on this comparison, and on the evidence of its articulation with *ADL*, must be a paranuchal. The centrale of *Rhamphodopsis* (according to our material) has a precisely similar posterior process and flanged surface. The simplest interpretation is that the posterior margin of the skull-roof of Ørvig's best specimen has been damaged and that *Nu* and part of the *PNu* have been lost. There would then be no difference from the pattern seen in *Rhamphodopsis*. The alternative, that *PNu* has suffered true reduction and that *Nu* was not developed, is of course possible; but *Nu* has important functions in the arthrodiran occipital region and its failure to develop is *a priori* unlikely.

It seems rather likely that more complete material of this most remarkable fossil will demonstrate the presence of a true *Nu* and of a more extensive true *PNu*.

It is important to make these points since there is now every reason to believe that *Ctenurella* and *Rhamphodopsis* are closely related members of the Ptyctodontida, a highly distinctive sub-group of the Arthrodira (cf. classification used by Westoll[6]), derived almost certainly from primitive Euarthodira with well-developed spinalia. *Rhamphodopsis* has a large spinale, while *Ctenurella* has a mobile tribasal pectoral fin and has no spinale. In the terminology of Stensiö[8], one is merosomactidial and spinothoracic, the other holosomactidial and aspinothoracic. Stensiö[8,12], has used such morphological differences to set up a new classification and evolutionary configuration for placoderms that is opposed by many other considerations (cf. refs. 9 and 13) The importance of *Ctenurella* for me is that it seems to give striking evidence of the rapid development of one condition from the other in a single specialized group. In every reasonably well-defined lineage the evidence points firmly to the spinothoracic condition being earlier. Mr. R. S. Miles and I hope to present further convincing proof that the sequence *Coccosteus*–*Plourdosteus*–*Dinichthys* is such a narrowly defined lineage, in which the spinale is rapidly reduced, and Miles[14] has described the Lower Devonian *Coccosteus angustus* Traquair, and has shown how clearly it bridges the gap between *Coccosteus* and Arctolepida.

Ctenurella thus appears to demonstrate a rapid development in its fin structures, from a *Rhamphodopsis*-like condition to one highly suitable as an ancestor for the Holocephali. Its structure is also of great importance in assessing the interpretation of the paired fins of placoderms recently made by Prof. Stensio[8] and used by him as a major factor in a new classification of these forms. There now seems to be a very strong case for regarding the mobile pectoral fins of several groups of placoderms as independent phylogenetic derivatives from an essentially arctolepid condition, in which these fins are represented largely, or even in some cases wholly, by the bone-jacketed spinalia (cf. Westoll[6,9]).

Since the foregoing was written I have visited Stockholm and Dr. Ørvig has kindly shown me material of *Ctenurella* and a new reconstruction of that genus soon to be published. Some of the points already noted are clarified, and others raised, by this new work Briefly, Dr. Ørvig now agrees that the

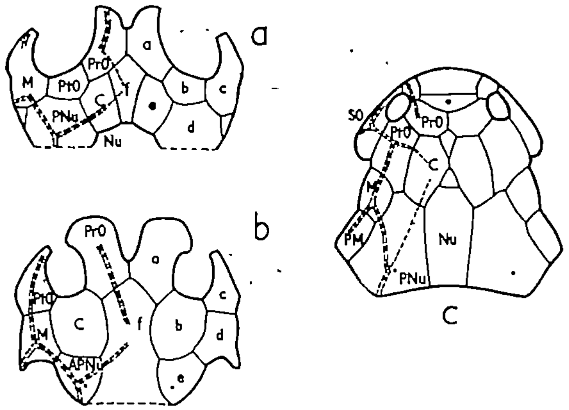

Fig. 4. Homœomorphy in orbital regions of some Arthrodira. *a*, *Rhamphodopsis*, posterior part cut off along broken line; *b*, the petalichthyid *Lunaspis*, posterior part cut off along broken line; *c*, an arctolepid, semi-diagrammatic. In *a* and *b* the right-hand side has topologically similar bones similarly labelled, *a–f*; true homologies, independently determined on left; and latero-sensory system indicated on left by double row (deep canals) or single row (pit-lines) of broken line. In *c*, the position of median (? anamestic) bones, occasionally and separately present, is shown in front of *Nu*, behind *P*. *PPNu* omitted in *b*

bone previously labelled 'marginal' by him is the paranuchal, and his new reconstruction avoids the more obvious anatomical inconsistencies of the first. Only in one specimen does he find a median bone of the skull-roof behind his "*ONu*", and there is no indication of a post-marginal bone (which may not be present in all specimens of *Rhamphodopsis*).

He also demonstrates that the latero-sensory lines of the head are largely enclosed in canals in the dermal bones, opening by pores. An important feature is the meeting of the supraorbital and "posterior pit-line" canals in a median X-shaped anastomosis at the centre of his "*O.Nu*" (my *PP*). This arrangement is paralleled in *Rhamphodopsis*, where, however, only surface grooves represent the "posterior pit-line" (as may also be the case in some *Ctenurella*). There can no longer be any residual doubt that the bone-pattern in the two genera is exactly comparable, with the exception that *Nu* and *PM* (my notation) may be lost in *Ctenurella*.

Dr. Ørvig compares this pattern with that of petalichthyid arthrodires, mainly on the basis of the median X-junction of latero-sensory lines. In order to do so he must 'suppress' the structurally very important posterior part of the petalichthyid skull. If this is done, there is a most remarkable resemblance in the pattern of the dermal bones in the two groups, so long as the mere topographic relations to one another and to the orbit are concerned (cf. right-hand side of Fig. 4*a*, *b*). The only real difference is that bone *a* meets *c* in ptyctodonts and not in petalichthyids, where they are separated by the junction of *b* and *f*. Such alternative junctions are common in euarthrodires, and are explicable by slight differences in proportional growth of bones. There is in fact an almost perfect topological correspondence between the bones labelled *a–b*; yet it can easily be shown to do violence to every other principle of comparative anatomy, and represents a very instructive example of convergent homœomorphy.

This is made clear by the true homologies as determined by other factors (cf. left-hand side of Fig. 4). *PNu* in the ptyctodonts is obviously correctly so named in comparison with normal euarthrodires, since it is concerned in the dermal cranio-thoracic articulation, and shows the characteristic Y-shaped latero-sensory lines; in euarthrodires it also transmits the endolymphatic duct. In petalichthyids the bone is represented by two elements, *APNu* and *PPNu*. The former shows the characteristic relations to the endocranium, latero-sensory lines and endolymphatic duct of *PNu* of Euarthrodira; *PPNu* (and the long posterior part of *Nu*) may best be understood as reflexions of the great relative elongation of the occipito-spinal region in petalichthyids, *PPNu* being a special development for the more remote exoskeletal cranio-thoracic articulation. The elements *M* are clearly homologous because of their latero-sensory lines. The petalichthyid *PtO* is recognized as such by its relations to *M* and the latero-sensory-lines; its position lateral to the orbit is paralleled in *Homosteus*. Though *C* in petalichthyids does not receive the 'central latero-sensory line' from *PtO* as in euarthrodires, its true nature is revealed by its relation to a similar endocranial region in both; again comparison with *Homosteus* is instructive. In the ptyctodonts *PtO* and *C* have been compared above with other well-known euarthrodires. Thus the bones lying lateral and postero-lateral to the orbits in ptyctodonts and petalichthyids are not severally homologous in spite of their remarkable superficial similarity. It should be noted that a very similar pattern is seen in such typical euarthrodires as *Coccosteus*, and that the bone lying lateral to the orbit in those forms is the suborbital *SO*, not homologous with the positionally corresponding bone in either petalichthyids or ptyctodonts (Fig. 4*c*). This 'topographical' similarity has caused great confusion in the past, and is due to the fact that the orbit has relatively 'migrated' in different directions; in ptyctodonts it has, so to speak, pushed back between *SO* and *PtO* and indented *M*, so that the last is now moulded to the orbit, and in petalichthyids it has moved postero-medially to meet *C* (as in *Homosteus*), separating *PtO* and *PrO* and causing the latter to be so moulded.

The only remaining resemblance between petalichthyid and ptyctodont skull-patterns is in fact the X-shaped median junction of latero-sensory lines. In primitive Euarthrodira the supraorbital canal has a short and variable posterior extent. If a 'fontanelle' in the dermal roof existed between the paired *PrO* and *C* elements the supraorbital lines and posterior pit-lines could easily continue into it, and a bone developing in such a position (PP in ptyctodonts) could 'capture' these portions. The conditions in ptyctodonts and petalichthyids thus have almost certainly been acquired independently.

Dr. Ørvig has also further prepared the cranial fragments described as *Epipetalichthys minor* Gross, and I am now convinced (against my previous opinion) that it is probably a ptyctodont. I appreciate his discussion of these matters with me.

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POTASSIUM–ARGON AGES ON ROCKS FROM EASTERN GREENLAND

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THE geological expeditions to the northern and eastern sides of Greenland over the past thirty years have developed a body of information in an area of profound geological complexity. With the development of quantitative methods of geochronometry in the past few years it appeared that the geological history in eastern Greenland might be placed on an absolute basis so that correlation from North American to Scandinavia and thus to Britain would be possible. As a preliminary step in this direction, the available petrographic collections of the Danish East Greenland Expeditions at the University of Basle were surveyed, and the most suitable samples selected for analysis by the potassium–argon method of geochronometry. In virtually all cases of igneous or metamorphic rocks, this method gives a reliable minimum absolute age. If the rock or mineral being analysed has remained a closed mineral system subsequent to its formation, the calculated isotopic age will be the actual time of formation.

The laboratory techniques are described elsewhere[1], and yield an analytical error of the isotopic age of less than 3 per cent. The location of the samples is given in Fig. 1. The analytical results are given in Table 1.

The detailed description of the geological studies carried on by the East Greenland Expedition under the sponsorship of the Danish Government are published in a special series, which now runs to more than 100 volumes. A more comprehensive discussion of the samples and dates reported here with complete petrographic descriptions of the rock types will be published in one of the above series[2].

The geological history in north-eastern Greenland is long and complex. The oldest observable rocks constitute a complex metamorphic plutonic basement of unknown age which may have undergone multiple cycles of metamorphism prior to its stabilization on the platform on which a thick pile of clastic sediments and basaltic sills, dykes and flows were deposited. This latter sequence, called the "Groenlandian" by Koch, includes an earlier (Thule group) series and later (Hagen Fjord group in north-east Greenland, Eleonore Bay group with Tillite group at top in central East Greenland) series which are separated by the Carolinidian orogeny in north-east Greenland. All these events happened in Pre-Cambrian times since Cambrian and Ordovician sedimentary rocks were deposited on top of the Groenlandian strata with distinct unconformity. In the geosyncline of central East Greenland, deposition ceased in Middle Ordovician time followed by a great orogeny (Caledonian). Deposition recommenced in the early Middle Devonian and continued intermittently through the rest of the Palæozoic, interrupted locally by folding, late orogenic granite intrusion and volcanism. Finally, after some Mesozoic deposition and Tertiary plateau basalts, a series of superficial Cainozoic disturbances occurred in northern Greenland.

The samples for this survey were grouped according to the problem. An attempt was made to determine the age of the ancient basement, the time of the deposition of the Thule group, the Carolinidian mountain building, the end of the main Caledonian orogony and duration of related post-orogenic events. The attempt was only successful in part but is reported for the interest of other workers in North Atlantic geology and as a guide to further more adequate collection of material when the opportunity presents itself.

Sample *GAA*-135 from the basement exposed on the western edge of the fold belt in the Scoresby Sound area was selected since it showed no petrographic evidence of having been affected by the Caledonian metamorphism which affected most of eastern Greenland and was at least 20 km. from the

Table 1. Isotopic Ages Grouped by Structural Period

Sample No.	Rock type	Mineral	Isotopic age (m.y.)	K (per cent)	^{40}Ar s c.c./gm.	Location
I. Pre-Cambrian basement						
GAA-135	Alkali granite	Biotite	1,900 ± 50	6·74	$6·98 \times 10^{-4}$	70° 20′ N./29° 47′ W.
HN-89	Migmatite	Biotite	490 ± 12	4·80	$1·07 \times 10^{-4}$	75° 00′ N./21° 50′ W.
II. Pre-Palæozoic supracrustal rocks						
BNW-44	Quartz-dolerite	Whole rock	375 ± 10	0·48	$7·98 \times 10^{-6}$	77° 10′ N./24° 00′ W.
GW-59	Phyllite	Whole rock	410 ± 10	4·23	$7·74 \times 10^{-5}$	73° 10′ N./28° 30′ W.
JH-560	Phyllite	Whole rock	425 ± 10	5·02	$9·53 \times 10^{-5}$	73° 11′ N./28° 35′ W
III. 'Caledonian' infrastructural rocks (Pre-Middle Devonian)						
JH-105*B*	Schist	Phlogopite	400 ± 10	7·95	$1·44 \times 10^{-4}$	73° 20′ N./26° 35′ W.
HN-202*A*	Pegmatite	Biotite	405 ± 10	5·14	$9·34 \times 10^{-5}$	79° 20′ N./19° 40′ W.
IV. Late orogenic intrusives						
HN-7*b*	Pegmatite	Muscovite	395 ± 10	8 90	$1·57 \times 10^{-4}$	75° 41′ N./22° 15′ W
KF-11-1 180-182	Granite	Biotite + muscovite	395 ± 15	7·87	$1·37 \times 10^{-4}$	73° 15′ N./22° 10′ W.

nearest aureole. The potassium–argon date of 1,900 ± 50 m.y. demonstrates the great antiquity of this basement. Since there is no guarantee, until more extensive geochronometric investigations are made, that this rock was not mildly affected by the Caledonian event or by deep burial for a prolonged period, the actual time of the last metamorphism of the basement complex could be much older than 1,900 m.y.

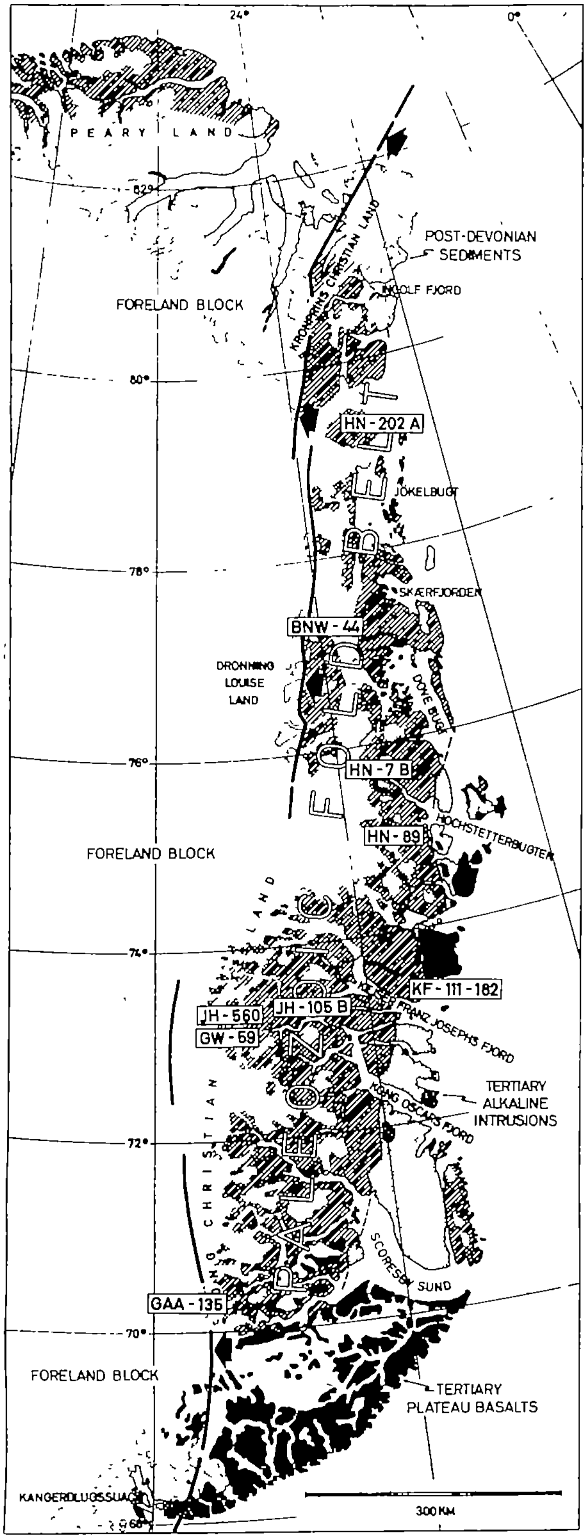
24°
0°
PEARY LAND
82°
POST-DEVONIAN SEDIMENTS
KRONPRINS CHRISTIAN LAND
INGOLF FJORD
FORELAND BLOCK
80°
HN-202 A
JÖKELBUGT
78°
SKÆRFJORDEN
BNW-44
DRONNING LOUISE LAND
DOVE BUGT
76°
HN-7 B
HN-89
HOCHSTETTERBUGTEN
FORELAND BLOCK
74°
KF-111-182
JH-560
JH-105 B
GW-59
FRANZ JOSEPHS FJORD
TERTIARY ALKALINE INTRUSIONS
KONG OSCARS FJORD
KONG CHRISTIAN LAND
72°
SCORESBY SUND
GAA-135
70°
FORELAND BLOCK
TERTIARY PLATEAU BASALTS
KANGERDLUGSSUAQ
68°
300 KM

Fig. 1. Sketch map of eastern Greenland showing sample locations

It is significant that the isotopic age came out 1,900 m.y., since the biotites in the Karelian and Svecofennian belts in Scandinavia and north-eastern U.S.S.R. all give 1,650–1,780 m.y. Thus it may be concluded that: (1) this basement in eastern Greenland formed earlier than the time of the Karelian and Svecofennian orogenies; (2) that the basement in this area was not affected by these major tectonic events of north-eastern Europe.

Sample *HN*-89 is a polygenetic migmatite from the old basement that was affected to some extent by Caledonian orogeny. The isotopic age of 490 m.y. indicates that all the accumulated argon was not released during this Silurian event.

Three attempts were made to identify the time of deposition of the Groenlandian Pre-Cambrian supracrustal rocks. Samples *GW*-59 and *JH*-560 are phyllites which may have been generated during a Pre-Cambrian metamorphism or the Carolinidian orogeny. Whole rock analyses can give correct ages for phyllite. The isotopic ages on these samples fall in the range of time of the Caledonian orogeny. Thus, it is not possible from the isotopic ages to identify the time of formation since the Caledonian event could have produced a temperature increase with consequent loss of the argon yet without causing the phyllite to be re-crystallized. *BNW*-44 was a basalt boulder of presumed Thule age, but it also appears to have been outgassed in Caledonian time.

More definitive results were obtained from samples *JH*-105*B* and *HN*-202*A*. *HN*-202*A* represents a late-tectonic pegmatite which intruded the latest shearing pattern in the coast area of Lambert Land. The emplacement of this pegmatite dates the relaxation of Caledonian activity in north-east Greenland. Since there was no further metamorphic activity in this area and no proximate igneous intrusions, the isotopic age of 405 ± 10 is probably an absolute age and represents the end of the main Caledonian orogeny. *JH*-105*B* is geologically from an early state of the main orogeny derived from one of the earliest upwarpings of the infrastructure in central East Greenland. The age, in conjunction with that of *HN*-202*A*, suggests either that the total length of the major orogeny was only about 10 m.y. or, more likely, that the schist containing *JH*-105*B* was sufficiently warm until the end of the main orogeny that it could not retain argon.

The post-orogenic but pre-clastic deposition granite pegmatite *HN*-7*b* gives an age of 395 ± 10 m.y., while a pre-mid Middle Devonian granite which cuts lowermost Middle Devonian clastics gives 395 ± 15 m.y. The analytical uncertainties in these results are sufficiently great so that they could be considered 20 m.y. apart without further data.

It is interesting to see how these results relate to the absolute geological time-scale[3] and the Caledonian of Britain, where extensive analyses have been made[4]

First, deposition ceased in the geosyncline of eastern Greenland in the Middle Ordovician. Based on a number of excellent measurements on rocks of Trenton Time (basal Upper Ordovician) and lower Upper Carodocian[4], it appears that deposition in eastern Greenland ceased about 440 m.y. ago. The beginning of the Silurian is estimated as 425 m.y. and the end, 405 m.y.[4]. Thus it appears from the above dates that in eastern Greenland the climax of the main orogeny occurred just at the Silurian–Devonian boundary or slightly into the Lower Devonian. This leaves almost 40 m.y. for the Caledonian orogeny to take place, but from data in Nova Scotia and Britain, as well as northern Greenland, where Silurian sedimentary rocks are involved in the orogeny, the actual time interval may be much shorter, possible 10–20 m.y.

Finally, it is noted that the oldest post-tectonic granites in east Greenland occur in the middle or late part of the Lower Devonian. This is precisely the time of the early post-tectonic granites in Britain and Ireland[4].

With the superb exposures and the excellence of the geological investigations which are already completed, the region of eastern Greenland presents an unusual challenge for geochronometric study.

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ANOMALOUS LORENZ PARAMETER IN SPECIMENS OF HIGH-PURITY COPPER AT LOW TEMPERATURES

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FROM investigations of the low-temperature transport properties of high-purity copper, annealed as well as cold-drawn, Powell, Roder and Hall[1] have shown an unusual behaviour of the (electronic) Lorenz parameter L. For the annealed specimen, they have given the value of L, extrapolated to 0° K., as $1·9 \times 10^{-8}$ and, for the 26 per cent drawn specimen, the value $2·1 \times 10^{-8}$ W.-ohm-deg.$^{-2}$. On the other hand, for a copper specimen of the same nominal purity (99·999 per cent), obtained from the same source (Central Research Laboratories, American Smelting and Refining Co.), White and Tainsh[2] derive a Lorenz parameter which, at the lowest temperatures, does not deviate more than 2 per cent from the normal Sommerfeld value ($L_n = 2·45 \times 10^{-8}$).

Attention is directed to the facts that, for the Boulder annealed specimen and the Sydney specimen, respectively . (1) the residual electrical resistivity is 1·01 and $0·87 \times 10^{-9}$ ohm-cm. ; (2) the impurity thermal resistivity coefficient B (the electronic thermal resistivity due to impurities and imperfections, at temperature T° K., being given by B/T) is 0·059 and 0·035 cm.-deg.^{2}/W. ; (3) the maximum value of thermal conductivity is 135 and 190 W./cm.-deg. These suggest that the Boulder annealed specimen contains some contamination compared with the Sydney specimen.

A close scrutiny of the experimental points (as distinguished from the extrapolated curves), in the case of the Boulder annealed specimen (ref. 1, Fig. 8) shows that the trend of the L-T curve between 20° and 8° K. is such as to attain the normal value L_n at temperatures close to 0° K. However, at about 8° K., the curve starts flattening ; and, below 5° K., it shows a tendency to fall with temperature. Similarly, in the case of the cold-drawn specimen, the L-T curve between 30° and 10° K. suggests that L would rise to the value L_n at temperatures close to 0° K. Actually, L presents a maximum (of about 2·16) at 9° K., falling to (what looks a minimum) 2·09 at 4° K. These authors mention that the L-T curves for very dilute alloys containing silver also gave similar results[3], either flattening out or bending over significantly For the annealed high-purity copper sample, they give $1·9 \times 10^{-8}$ as the extrapolated value of L at 0° K., unless the curve changes its behaviour again below about 4° K.

It appears to me that this change in behaviour could indeed be the case if the contamination referred to here is, for example, iron. For, in the case of some alloys of silver with small amounts of a transitional metal, manganese, in random solid solution, it has been found[4] that the electronic Lorenz parameter L_e, at liquid helium temperatures: (1) dipped below the normal Sommerfeld value L_n ; (2) attained a minimum ; (3) rose to the value L_n at the lowest temperatures, below about 1° K. This fall in L_e has been interpreted as follows : the transition of these alloys to antiferro/ferro-magnetism[5] removes the spin degeneracy of the ground-state of the impurity (manganese) ion. The scattering of the conduction electrons by these impurity centres, each of which can occupy one or the other of the energy states, could be inelastic. Since inelastic scattering makes a much larger contribution to thermal resistivity than to the electrical resistivity, the electronic Lorenz parameter dips below L_n. The approach of L_e towards L_n at temperatures close to 0° K is due to the cessation of this inelastic scattering.

Fig. 1 gives a plot of the electronic Lorenz parameters in the above-mentioned cases, with respect to temperature. Curves 1, 2 and 3 refer to the three silver–manganese alloy specimens (containing respec-

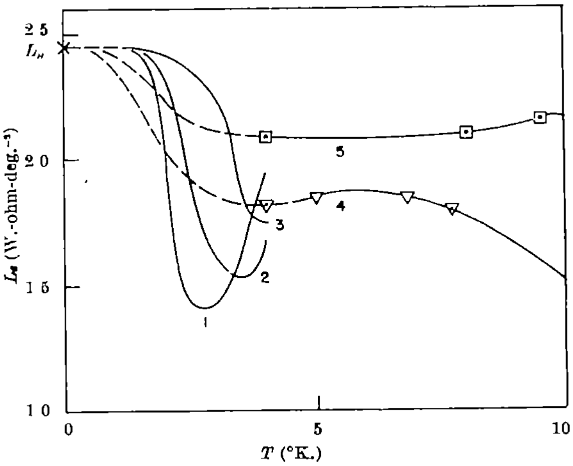

Fig 1. Anomalous (electronic) Lorenz parameter (W.-ohm-deg.$^{-2}$) against temperature (°K.). Curves 1, 2 and 3 are for the dilute silver–manganese alloys, in the order of increasing manganese content. Curves 4 and 5 are, respectively, for the annealed and the 26 per cent drawn specimens of high-purity copper

tively 0·14, 0·32 and 0·55 atoms per cent of manganese in random solid solution). Curves 4 and 5 are for the high-purity copper specimens (annealed and cold-drawn, respectively) reported in ref. 1. The dashed lines at the lowest temperatures are my extrapolations, presuming that L_e would attain the value L_n at 0° K., owing to the cessation of the inelastic scattering of the conduction electrons.

In cases 1, 2 and 3, there is not much surprise in their departure from the requirements of the law of Wiedemann–Franz–Lorenz (namely, that in the 'impurity scattering' region, L_e should have the value L_n and L_e should be independent of temperature), since the electrical and thermal conductivities are themselves anomalous. In cases 4 and 5, the electrical and thermal conductivities do not show noticeable anomalous behaviour, at any rate, down to 4° K. Plots of wT against T^3, however, show an anomalous rise above rectilinearity, at temperatures below about 14° K. in the case of the 26 per cent drawn specimen, and a less marked rise below about 10° K. in the annealed specimen. The temperatures at which these wT–T^3 curves have their maximum rise above the rectilinearity are found broadly to correspond to the temperatures at which L exhibits the minimum value (w being the thermal resistivity).

After a series of experiments to determine the effects of minute amounts of various elements in copper, it has been concluded by investigators[6] at Ottawa that anomalous thermo-electric powers and electrical resistivity minima can ultimately be traced to small amounts of iron contamination in solid solution. In fact, they find large anomalous thermo-electric powers even in cases where the amount of iron is so small (around about 0·0001 per cent) that the residual electrical resistivity does not present a noticeable anomaly. This is because the size of the electrical resistance minimum depends on the total (not the relative) iron concentration.

It is thus possible that the Boulder high-purity copper specimens (and their dilute alloys with silver) contain a small contamination of iron which does not show up vividly in the residual electrical resistivity measurements down to 4° K., but is sufficient to affect the Lorenz parameter in the temperature region where the electrical conductivity is limited primarily by the impurity scattering. The Sydney specimen has apparently too small (if at all) an iron content even to affect the Lorenz parameter. This view is supported by the disparity between the Sydney specimen and the Boulder annealed specimen as regards the residual electrical resistivity, the impurity thermal resistivity coefficient B and the maximum value of thermal conductivity.

The depression of L below L_n would be a measure of what fraction inelastic scattering of the conduction electrons constitutes, out of the total impurity scattering (which includes elastic scattering from chemical as well as from physical imperfections). In the Boulder 26 per cent drawn specimen, cold-work has introduced a considerable number of physical imperfections in the form of lattice defects. The interaction of the conduction electrons with the magnetic impurity centres would now constitute a smaller fraction (than in the case of the annealed specimen) of the total impurity/imperfection scattering. Thus, for the cold-drawn specimen (as can be seen from curve 5, Fig. 1), the Lorenz parameter is less depressed below the value L_n, just as the large anomalous (negative) thermo-electric power is considerably reduced[1,6–8] on cold-working.

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RADIAL DISTRIBUTION OF THE RANDOM CLOSE PACKING OF EQUAL SPHERES

RANDOMLY packed equal spheres can provide a useful model for simple liquids. In previous experiments[1] with steel balls packing densities were found to be between 0·60(1) and 0·63(7). The arrangement corresponding to the upper limit 0·63(7) is referred to as random close packing and probably it is related to the configuration of the atoms in liquids such as those of the rare gases. Bernal and Mason[2] have used a special marking technique to obtain the physical co-ordination of random close packing and found it to be somewhat more than 8·5. They also refer to the fact that the fractional difference between the densities of regular close packing and random close packing is nearly the same as the percentage increase in volume which occurs in the melting of argon.

To extend the available data concerning the random packing of equal spheres, and in particular to determine the radial distribution in random close packing, a method was devised for determining the positions of a reasonably large number of randomly packed ⅛-in. steel balls. A standard optical comparator was modified so as to be suitable for making measurements of the three cartesian co-ordinates over a space approximately 5 × 5 × 10 cm. About 4,000 balls were poured into a cylindrical container 4·5 cm in diameter and 15 cm. long, the walls of which were dimpled as previously described[1], and which could be split open lengthwise. The balls were shaken down to give random close packing[1] and then after the container was warmed to about 65° C., molten paraffin wax was poured in and the whole allowed to cool. One side of the container was taken off and the outside layers of balls and wax were removed. Under the comparator the co-ordinates of the balls in a roughly spherical cluster were determined. The top surface of each ball in turn was cleared of wax and then polished. The image of the filament of a small incandescent lamp reflected in the top surface of the ball was used as a reference for making the settings. After the measurement the surrounding wax was scraped away and the ball was removed. The readings were reproducible within ±0·03 mm. in the vertical direction and to better than ±0·01 mm. in the two horizontal directions. Such precision is adequate for the purpose since it means that the balls

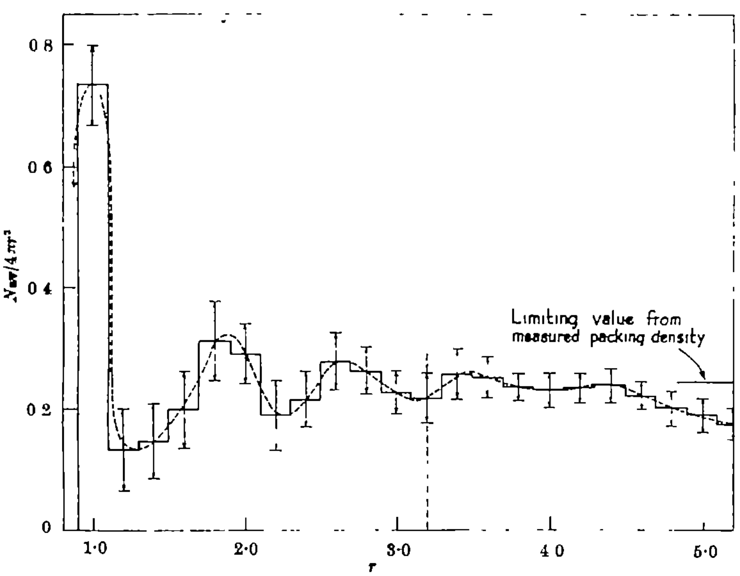

Fig. 1. Histogram of $N_{av}/4\pi r^2$ versus r. N_{av} is average number of spheres in intervals of 0 2 of sphere diameter averages with standard deviations for 25 centres. r, Distance from centre in units of sphere diameter

are located to better than 1 per cent of their diameter whereas the increment used for the radial distribution was 20 per cent of the diameter.

A preliminary set of measurements was made on a cluster of about 200 balls in order to develop both the technique of making the measurements and the computational methods required for the results. A final set of measurements was made for a cluster of approximately 1,000 balls.

The computations were carried out with the aid of the IBM 650 of the University of Toronto Computation Centre. An arbitrarily selected ball near the middle of the cluster was used as origin, and the distances to all the other balls were found. The radial distribution was then determined for intervals of one-fifth of the ball diameter. The distributions were computed first for a group of five origins and later for a group of twenty-five origins. The averages and standard deviations at each interval were then calculated for the group. The fact that the deviations were approximately the same for both the five and twenty-five centre groups suggests that they are characteristic of the random packing.

A histogram showing the results for the group of twenty-five centres in the 1,000 ball cluster is given in Fig. 1. The plot is made for the average number N_{av} per interval divided by $4\pi r^2$ so that the distribution function will become constant for large r. For the 1,000 ball cluster and twenty-five centres the distribution cannot be taken beyond 3·2 diameters without having this distance fall outside the cluster in some direction for at least one of the centres. However, reasonable significance appears to be maintained well beyond the 3·2 limits, although by 4·6 the expected falling off of the function is quite marked. The distribution function appears to be consistent with the previously measured packing density of 0·63(7), as indicated by the value of $N_{av}/4\pi r^2$ for large r corresponding to the density of 0·63(7), which is shown by the horizontal line at the right of the graph.

The first peak in the distribution function, although shown for the interval 0·9–1·1, corresponds in fact to the interval 1·0–1·1 since the balls are essentially rigid. The number of nearest neighbours determined from the average number of balls in this first interval is 9·3 ± 0·8, which is consistent with the estimate of the co-ordination number made by Bernal and Mason and mentioned above.

From neutron diffraction experiments Henshaw[3–5] has determined the radial distributions of the atoms in liquid helium, neon and argon. Henshaw estimates the number of nearest neighbours as 8·5–9·7 for helium, depending on the method of analysis, 8·8 for neon and 8·0–8·5 for argon, although the value by X-rays is rather higher. The figure of 9·3 ± 0·8 obtained from random close packing of balls can be considered in agreement with the experimental results for the liquefied rare gases.

The relative positions of the peaks in the radial distributions for liquid helium, neon and argon were taken from Henshaw's graphs. They are compared in Table 1 with the relative positions of the peaks found for the random close packing of balls.

Table 1. RELATIVE POSITION OF PEAKS IN THE RADIAL DISTRIBUTION

	Second	Third	Fourth
By neutron diffraction			
Liquid Helium	1·8(7)	2·6(6)	3·5(8)
Neon	1 8(5)	2 7(7)	3·5(7)
Argon	1·8(1)	2·6(4)	3 4(4)
Random close packing of balls	1 8(3)	2 6(4)	3·4(5)

In each case the radial distances of the second, third and fourth peaks are expressed in terms of the distance to the first peak. The agreement is surprisingly good; certainly it is well within the expected errors.

As mentioned by Bernal and Mason, the fractional change of volume on the melting of argon is the same as the fractional difference between the densities of regular close packing and random close packing. From the data given by A. C. Hollis Hallett[6] the ratio of solid and liquid densities at the triple points for neon, argon, krypton and xenon are 1·15(8), 1·15(2), 1·15(8), 1·14(8) respectively, whereas the ratio of the regular to random packing densities of balls was found to be 1·16(3). Again the results agree within experimental error.

I acknowledge the assistance of Mr. A. M. Charlesworth in making the measurements and in carrying out the computations.

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Dr. Scott and Mr. Charlesworth are to be congratulated on carrying out the careful and tedious measurements of co-ordinates of random close-packed spheres. We are in a position to realize this, as one of us has recently carried out a similar set of measurements with a thousand ballbearings of larger size

(¼ in.), prepared in the manner already described[1] and using a mechanical three-point contact device for measurement.

Although this is an apparent duplication, the object of our investigation was different. We were not concerned so much with radial distribution function as with the neighbourhood number distribution function for which rough measurements had been obtained in previous work[1]. However, computation based on our results so far as they go $(r \ngtr 2r_0)$ are in close agreement with Dr Scott's results. We have now exchanged our co-ordinate data and it would be interesting to see how far, if at all, they are statistically distinguishable from each other. Dr. Scott will be able to carry out radial distribution function calculations on our sample and we will be able to carry out neighbourhood distribution function measurements on his.

We attach particular value to the latter because we believe that, from the point of view of a physical theory of liquids, the distribution of numbers of neighbours is a more important criterion than the shape of the radial distribution curve. Dr. Furth has already made use of this idea for deriving a very interesting and radically new statistical-mechanical theory of liquids which should be published as soon as the measurements become available.

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DYNAMIC YIELD STRESS OF 'PERSPEX'

In a recent communication Roberts[1] presented values of the "dynamic yield stress" in compression for 'Perspex' (poly(methyl methacrylate)); the stresses were calculated, by the theory of Hertz, from estimates of the maximum contact area arising on impact between a metal sphere and a plane 'Perspex' surface. Using the same theory and observed times of contact, the dynamic modulus of 'Perspex' was also calculated.

The experimental technique was based on the fact that electrostatic charge is generated on the surface of the insulator as the strain develops in it; charge-sharing between the sphere and the surface produced observable potential changes on the sphere. The residual charge on the 'Perspex' was revealed by lycopodium powder attracted to the surface and the area of contact was assumed to be that covered by the powder.

It is clear from the data of Ely[2] and of Hoff[3] that Roberts's values of yield stress are much too low and that their apparent dependence on strain-rate is much too great.

Values of compressive yield stress obtained at low rates of strain $\dot{\varepsilon}$ by various workers are given in Table 1. Some measurements we have made recently are also given for comparison.

Table 1. Effect of Strain-Rate and Temperature on the Compressive Yield Stress of Poly(methyl Methacrylate)

Temp. (°C.)	Compressive yield stress (dyne/cm.$^2 \times 10^{-8}$) $\dot{\varepsilon}$, Strain-rate—sec^{-1} (approx.)						
	(*a*)	(*b*)	(*c*)	(*b*)	(*d*)	(*d*)	(*e*)
$\dot{\varepsilon}$	$1 \cdot 6 \times 10^{-4}$	$3 \cdot 2 \times 10^{-4}$	1×10^{-2}	3×10^{-1}	4×10^{5}	6×10^{5}	10^{6}
0	12	18	17·7	22			
20	9	—	15	—	3·2	6·8	17
50	5·6	6·3	10	12·5			

(*a*) Hoff (ref. 3), (*b*) Ely (ref. 2), (*c*) present authors, (*d*) Roberts (ref. 1), (*e*) Hunter and Davies (see ref. 1).

The stresses determined at low rates are nearly all greater than Roberts's, irrespective of temperature; and in Fig. 1 it can be seen how improbably large is the dependence on rate he has calculated for his yield stress. We also show in Fig. 1 some yield stresses determined in tension at similar rates of strain; these give a somewhat lower dependence on rate of yield stress than the compressive work; but the magnitudes both of stress values and of their rate dependence are vastly different from Roberts's values. We suppose, incidentally, that the tensile yield stress, defined at the peak (mathematical maximum) of the load versus extension curve, is not necessarily a material constant; a peak in the load may merely be an indication of mechanical instability preceding the formation of a neck; analogous effects were observed by Hoff[3] when he investigated the compression of hollow 'Perspex' cylinders; buckling of the cylinders was accompanied by 'peaking' of the load. In our own measurements the slenderness ratio (height/diameter) of the solid cylinders was made small (~ 0·5) to eliminate buckling; no load peak occurred but plastic deformation at constant (true) stress was observed.

Roberts chose to extrapolate his yield stresses on a linear scale to short times (higher strain-rates) and attached significance to the fact that an unpublished value of Hunter and Davies lay on this line; it is certain that this value was a brittle failure stress,

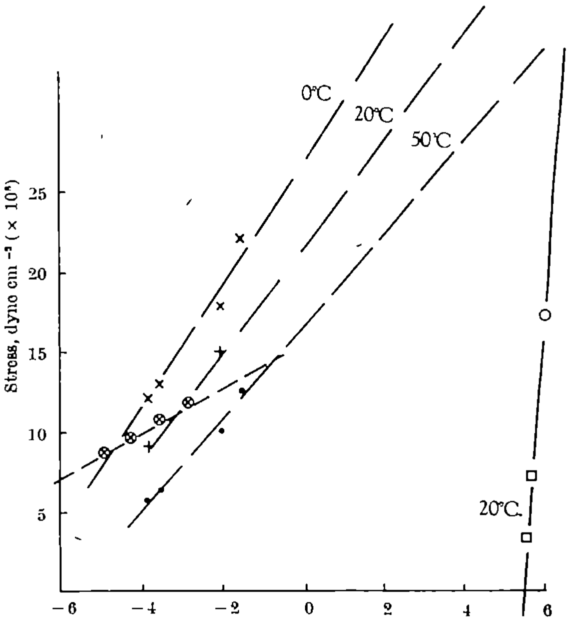

Fig. 1 Effect of strain-rate on the compressive yield stress of poly(methyl methacrylate). ○, Hunter and Davis (ref. 1), □, Roberts (ref 1). ⊗, Tensile yield, 20° C. (present authors), ×, + and ●, from Table 1

as was that of Kolsky[4], well below the yield point. We would estimate from the trend of the various compressive results that the yield point at 20° C. is near 40 × 10^{8} dynes/cm.$^{-2}$ at a strain-rate of 10^{5} sec.$^{-1}$; at − 50° C. we found the yield stress to be 35 × 10^{8} dynes/cm.$^{-2}$ for $\dot{\varepsilon} \simeq 10^{-2}$.

The discrepancies which exist between the various measurements we have quoted and those of Roberts can probably be explained by a critical examination of his theoretical and practical assumptions. We have not attempted an exhaustive inquiry, but one point requires immediate comment. Charge generation at an interface may occur simply by separation of two bodies; it is not necessary for extensive or permanent shear deformation, which is involved in compressive yielding, to occur. Roberts, however, has assumed without justification that yielding has taken place throughout a considerable volume near the area of contact between the steel sphere and the 'Perspex'.

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THE first point made by Sandiford and Vincent concerns the magnitude of the stresses compared. The yield stress which I have attempted to estimate[1] is the stress giving rise to slight initial yielding. A convenient mathematical description of this yield stress is given by the Guest–Mohr hypothesis[2]. This hypothesis was tentatively adopted as a starting point as it has been useful with metals although it is fully realized that there are many criticisms which may be brought against its use. In what follows the above yield stress will be referred to as the initial yield stress.

The compressive yield stresses of Hoff[3] and Ely[4] appear to correspond to maximum load points[3] and may be termed yield strengths[5] as distinct from initial yield stresses. It seems reasonable to assume that these quantities are not identical, the yield strength being greater than the initial yield stress.

Unfortunately neither Hoff nor Ely give actual stress–strain curves from which initial yield stresses might be estimated. So far as is known the only result available is that of Kolsky[6], whose curve suggests an initial yield of approximately 1·2 × 10^{8} dynes/cm.^{2}, assuming that the purely elastic behaviour of 'Perspex' is Hookean. This result was taken to indicate that the estimated yield stresses[1] were possibly of the correct order.

Kolsky's[6] dynamic curve does not, however, show a very distinct linear region, but an inspection of his experimental points might suggest a value of 2·7 × 10^{8} dynes/cm.^{2}. This seemed rather low, so later information was sought leading to Hunter's[7] stress–strain curve, which is linear up to an initial yield of the order of 16·9 × 10^{8} dynes/cm.^{2}.

A second point concerns the linear extrapolation and plot used in comparing the results for various rates[1]. This was merely the simplest empirical presentation. The broken line was supposed to indicate this very limited significance. It is probable that the functions between rate and (1) initial yield, and (2) yield strength are not identical.

Thirdly, the question concerning charge generation at an interface. In the present case it is possible that charge generation on separation is restricted to charge being induced on the steel ball since it is near the charged polymer surface. The charge generated at the interface is associated with the discharge of the charge induced on the ball during its approach to the polymer surface and possibly the additional process of charge transfer usually occurring when a conductor and non-conductor are in contact[8]. Both these processes may occur most efficiently where really good contact is made, and it was assumed, in view of the results obtained[1], that good contact is associated with initial yielding. The charge transfer aspect is being considered in more detail at the moment so that the above is only a tentative view. Problems associated with physical contact of surfaces are also involved.

A final point is concerned with the yielding which has occurred in the volume near the area of contact. In view of the above it was assumed that the stresses throughout a volume near the area of contact exceed an initial yield stress but probably do not exceed the yield strength of the material since no permanent indentation is easily discernible.

By its very nature the original preliminary communication[1] posed more problems than it solved and discussion at this stage is valuable.

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ACCURACY IN THERMOGRAVIMETRIC ANALYSIS

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VARIOUS factors limiting the accuracy obtainable in thermogravimetric analysis have recently been discussed[1,2]

The main sources of error appear to be due to: (1) air buoyancy and convection effects, (2) measurement of temperature; (3) atmosphere in the furnace; (4) heating-rate; (5) heat of the reaction studied.

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* From June 1962 at the Research Laboratories, Mineral Division, C S I.R O , Melbourne, Australia

These sources of error can result in considerable inaccuracies in the records, of the temperature and the weight loss (or gain) functions, obtained in the course of thermogravimetric investigations. These properties are generally interrelated and thus cannot be considered separately, although they may vary in importance with instruments of differing design. Full consideration must be given to all these factors in the operation of thermobalances.

Accurate thermogravimetric analysis requires that a correction be applied for the buoyancy of the sample holder and its support. This buoyancy effect appears as a progressively changing non-linear weight function which is related to the heating-rate.

Air buoyancy introduces an appreciable error if the density of the object being weighed differs greatly from that of the weights used to counterpoise it. The importance of such a correction is often not realized, and its application is neglected. Large errors may thus be introduced into the terminal loss in weight at the completion of reactions, and the overall shape of the weight-loss curve may be affected resulting in the misinterpretation of reaction kinetics[3].

The spurious gains in weight observed may be attributed to three main causes: (*a*) a decrease in air buoyancy with increasing temperature; (*b*) increasing convection with increasing temperature; (*c*) effect of heat on the balance mechanism.

However, further effects may be superimposed on the above three, namely, (*d*) thermal turbulence in the hot-zone of the furnace; (*e*) random fluctuations in the recording mechanisms; (*f*) furnace induction effects; (*g*) electrostatic effects; (*h*) the environment of the thermobalance.

All these factors result in an extremely complex non-linear buoyancy function in the temperature-range : Ambient to 1,500° C., which is subject to the heating-rate employed.

A further complication is introduced by the effect of a dynamically altering load, in the course of a reaction involving weight loss (or gain) of the sample. The effective load is compound, consisting of the crucible with support assembly and a sample capable of sudden weight changes. Large changes will modify the buoyancy function.

Considerable thermal turbulence can occur in the hot-zone of the furnace owing to convection currents. Expansion of the air in the hot-zone produces changes in volume which exert a pressure on the column of air in the furnace, and hence on the crucible and its support. This pressure is proportional to the heating-rate and the effective volume occupied by the crucible and support. Non-linearity in the heating-rate and turbulent heat-flow conditions produce fluctuating pressures which tend to modify the buoyancy function in an asymmetric manner, and hence affect the weight record.

The volume of the crucible, its shape and load have a considerable effect on the streaming of turbulent air or other gas. This also applies to the supporting assembly. Under turbulent conditions, therefore, it is highly desirable that the crucible and its support be streamlined so as to decrease interactions. Furthermore, the control of the heating-rate should be as linear as possible, in order to minimize thermal fluctuations in the hot-zone.

The present discussion refers to observations made during the operation of a Stanton thermobalance (model *HT–D*), under various conditions.

An automatic electric weight-loading servomechanism enables the instrument to follow a change of weight up to 0·1 gm. ± 0·05 mgm., in a manner unlimited by the effective width of the rectilinear chart, or the range of a single beam deflexion. This arrangement allows the full weight-range to be covered in ten increments of 10 mgm., each of which represents the full width of the recording chart. The maximum rate of change in weight per minute that can be recorded is 40 mgm., and when correctly adjusted, the instrument will record such variations with a reproducibility better than ± 0·05 mgm.

The sensitivity of the instrument is such that it will tend to record accurately all the phenomena described here as they occur in the hot-zone of the furnace.

The true loss in weight characteristic of a sample is difficult to evaluate in the presence of these large, spurious, non-linear contributions to the weight record. It is therefore necessary to minimize them so far as possible in order that the fine detail of the thermogravimetric curves may be resolved.

Good design in the Stanton thermobalance permits factors (*c*), (*f*) and (*g*) to be neglected. No appreciable contributions from these have been observed.

Factor (*h*) is within the control of the operator, and adequate precautions should be taken to ensure a suitable environment for the thermobalance.

The main modifications of the weight function result from (*a*), (*b*) and (*d*), which are subject to the hot-zone parameters of the furnace. In the *HT–D* model, the hot-zone is cylindrical (1·5 in. in diameter and 1·5 in. high), and is located at the mid-position of the furnace, which is mounted vertically, being closed at the top by means of a ceramic plug filled with powdered alumina (Fig. 1).

Under these conditions, the maximum decrease in the air buoyancy occurs in the region: Ambient to 50° C., and results in a rapid relative increase in

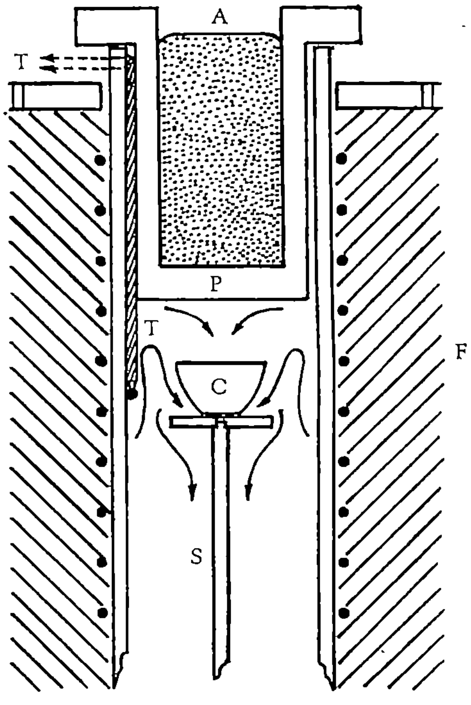

Fig. 1. The hot-zone of a standard thermobalance furnace. *A*, alumina powder; *P*, refractory ceramic plug; *T*, thermocouple; *F*, furnace wall; *C*, crucible; *S*, support assembly

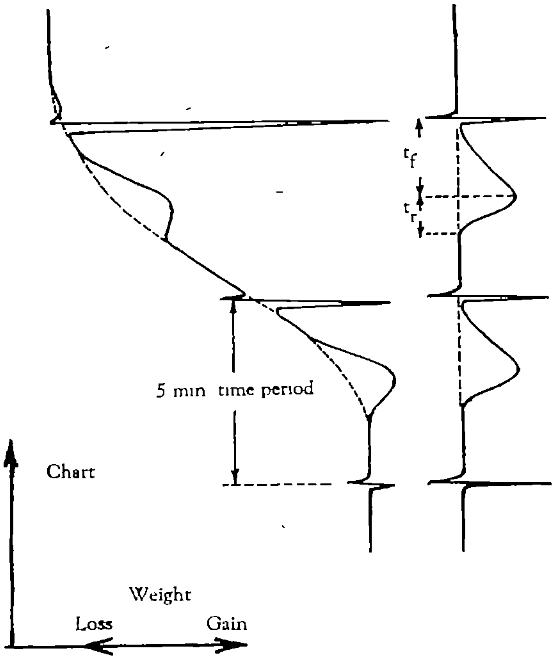

Fig. 2. A thermogravimetric record (diagrammatic), showing the effect of thermal pulsing in the furnace. t_r, rise time; t_f, fall time

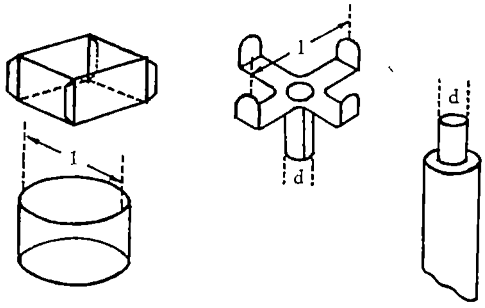

Fig. 3. Recommended crucible and support assembly. $l \simeq 0{\cdot}5 - 0{\cdot}75$ cm.

weight (a shift of original weight 'zero' position) when the furnace is first switched on.

In the enclosed hot-zone of the furnace, transference of heat occurs mainly by natural convection and radiation, with the latter predominating at elevated temperatures, that is, above 400° C. The buoyancy curve shows extreme susceptibility to thermal fluctuations in the region: Ambient to 400° C. The rapidly changing resistance of platinum furnace windings with temperature makes a full continuous control of input power impossible, so that to advance the temperature and maintain a desired heating-rate, increases must be made incrementally according to a pre-set programme. The periodic application of relatively large power increments to the furnace windings aggravates the thermal turbulence in the hot-zone and produces thermal pulsing effects. This is particularly noticeable in the range quoted here, where appreciable transference of heat occurs by convection. The weight record then depicts a series of periodic pulses which assume a 'saw-tooth' appearance and define a positive weight contribution (Fig. 2).

It has been claimed[1] that the magnitude of the various buoyancy effects can be considerably reduced by appropriate venting at the top of the furnace. This amounts to critical damping of the hot-zone volume, and in general, may introduce further non-linearity in the buoyancy curve. The response to venting will vary with the hot-zone parameters and the heating-rate, and appears to be advantageous where gas streaming techniques are utilized and the maintenance of linear non-turbulent flow rates is desired.

In view of the situation, various types of crucible assemblies were investigated in order to minimize the interactions discussed above.

A vast improvement was obtained with the assembly shown in Fig. 3, for which data are presented in Table 1. It is possible to utilize alumina or platinum crucibles in this arrangement. Crucible parameters may also be varied without detrimental results, if the following general conditions are observed:

Table 1. RESULTS OBTAINED WITH A PLATINUM BOAT CRUCIBLE (Fig. 3); DIMENSIONS: 0·7 CM. SQUARE × 0·5 CM. DEEP, WEIGHING 1·6 GM.

(a) *Buoyancy correction*:

Temperature-range	Heating-rate	Increase in weight (mgm.)
Ambient (plus)	1–3° C. per min.	+0·4 ± 0·1* (gain in 5 min.)
Ambient–350° C.	,,	+0·3 ± 0·05
350° C.–1,400° C.	,,	+0·2 ± 0·05

* The initial deviation may be neglected, since it occurs so quickly and only amounts to a change of relative 'zero' position on the weight record.

The total significant buoyancy correction over the range Ambient to 1,400° C. is then +0·5 ± 0·1 mgm. for a sample weight of 100 mgm.

(b) *Spurious effects*.

Effect	Temperature-range	Contribution (mgm.)	Time of effect. (min.)	
			Rise time	Fall time
(i) Thermal pulsing ± 5° C.	Ambient–400° C.	+0·2	1	5
	400° C.–1,400° C.	+0·1	2	7·5
(ii) Change of heating-rate: 1° per min. to 40° per min.	100° C.–400° C.	+0·2 ± 0·1	1	5
	400° C.–1,000° C.	+0·1 ± 0·05	2	10
(iii) Noise	Ambient–1,400° C.	± 0·05 mgm. Superimposed on pulsing.		

(1) That symmetrical crucibles of minimal volume are used, together with support assemblies which are as small and as streamlined as possible.

(2) Well-controlled, linear heating-rates of the order 1°–2° per min. (or less) should be used.

(3) The use of small quantities of sample material, with crucible to sample weight ratios of the order 20 : 1.

(4) Initial trial buoyancy determinations should be made with the crucible, at each relevant heating-rate. Comparison of the buoyancy effects obtained for the empty crucible with those for the crucible filled with inert material.

(5) The maintenance of constancy in experimental technique. All experimental conditions and precautions should be reported and the technique described.

(6) Consideration should be given to the nature of any reaction to be studied.

The information provided should allow appreciable control over the various factors governing the buoyancy effects.

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LETTERS TO THE EDITOR

RADIOPHYSICS

Very Low Frequency Noise at Brisbane

MEASUREMENTS of the broad-band component of ionospherics in the 5 kc./s. region, 'very low frequency noise', have been reported by Aarons, Ellis and Dowden[1-3].

This type of noise has now been recorded at Brisbane using equipment consisting of a loop of 1,440 sq. metres effective cross-section oriented for north–south directivity, a high-gain audio-amplifier and an asymmetric integrator of the type described by Ellis[2]. This method of recording makes use of the period between impulsive disturbances whether atmospheric or man-made to measure the wanted signal, and the ability of the apparatus used in Brisbane to do this was enhanced by carefully adjusted 'limiting' at several stages in the amplifier. Limiting occurred for an input signal of 0·9 μV. at 4·6 kc./s. corresponding to a noise-field of at least 0·09 μV./mc./s.$^{-1/2}$ for a source in the direction of maximum sensitivity.

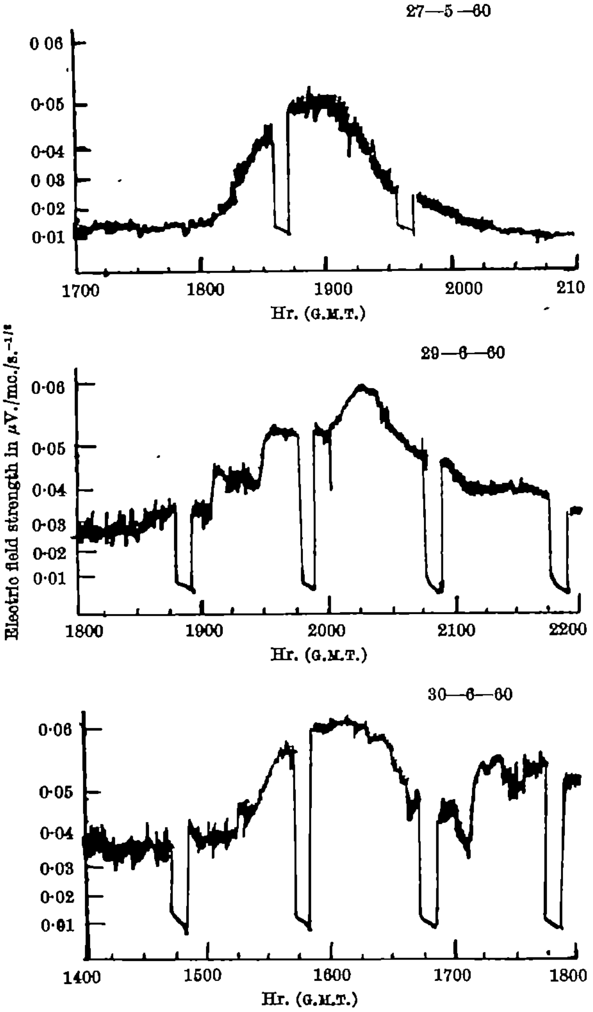

Fig. 1. Very low frequency noise at 4·6 kc./s. at Brisbane for a band-width of 800 c./s.

Unambiguous records of noise-bursts were obtained on three occasions during the winter months of 1960, each occurrence being coincident with a magnetic disturbance. These noise-bursts were recorded also at Camden and Hobart by Prof. G. R. A. Ellis (private communication), and close agreement in the time variation within the bursts was found for these observing points. The maximum noise-signal recorded at Brisbane corresponds to a field-strength of approximately 0·05 μV./mc./s.$^{-1/2}$ for a band-width of 800 c./s. to 6 db. points (Fig. 1). This is about 10 db. less than the measurement made at Camden.

No definite broad-band component could be distinguished during magnetic disturbances other than the three mentioned, although bursts were recorded at Hobart and Camden during many of them. The records made at Brisbane on these occasions were greatly disturbed by atmospherics, the time between large impulses received at Brisbane being negligible.

Brisbane at 27·5° S., 149° E. and 35·7° S. geomagnetic is believed to be the lowest latitude location at which very low frequency noise has so far been recorded.

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ASTROPHYSICS

Cosmic Magnetic Fields

MAGNETIC energy appears to be as widespread as matter itself and perhaps of comparable basic significance in cosmology. Its origin poses one of the outstanding problems in astrophysics.

The magnetic fields of the Sun and its planets and of other stars may be explained in terms of dynamo action[1] on the galactic field. The latter appears to result from a similar winding up of a much weaker intergalactic field[2]. However far this process may be effective we must eventually face the problem of explaining the original 'seed' field.

In the 'explosion' theory of the universe the difficulty may be insurmountable. Going backwards in time the intergalactic field must increase as R^{-2}, where R is the radius of the sphere of matter. The magnetic pressure and energy density will increase as R^{-4} and the total magnetic energy as R^{-1}. Before R is reduced sufficiently these quantities may become prohibitively high.

In the 'steady-state' theory[3] an intergalactic field, if present, must always have been present and we need only explain its maintenance. There are objections to the hypothesis of maintenance by turbulent motion of the gas, even if the particles created are neutrons rather than hydrogen atoms[4]. The statistical accumulation of momentum in limited regions would appear to be strongly inhibited by any significant intergalactic field. It is the object of the present communication to suggest an alternative origin of such a field resulting from neutron creation—a hypothesis which already has several attractive features[4].

If some or all of the particles created are neutrons then one would anticipate a slight excess of neutrons to appear with their spin axis and magnetic axis aligned with the intergalactic magnetic field. The reason is that this satisfies the condition of minimum energy, and even though the energy difference is minute it may be significant. Aligned neutrons will remain aligned for their brief lifetime and, on decay, non-conservation of parity will ensure an excess of electrons ejected along the field lines. These constitute newly created electric current and newly created magnetic field.

In a first approximation the resulting magnetic field is force free (see, for example, ref. 5), having the form curl $B = \alpha B$, where α is a scalar function of space co-ordinates. Simple force-free fields show a periodic variation in the direction of B, rather like a series of onion skins of thickness $2\pi/\alpha$. Since curl $B = 4\pi j$, where j is the current density, the 'wave-length' of a force-free field is $\lambda = B/2j$.

In a second approximation the field is not force-free. The decay electrons, whether aligned or not, comprise a gas at a temperature of about 2×10^9 °K. which is trapped within a magnetic onion skin. The gas expands the skin and thereby transfers momentum to the proton gas by the medium of the Lorentz force $j' \times B$ due to a current j' flowing across the field lines. The protons are accelerated to a velocity of about 3×10^8 cm. sec.$^{-1}$, at which time they have their share of the energy of neutron decay.

If the intergalactic density[4] is 10^{-6} protons cm.$^{-3}$, then the magnetic field strength needed to explain the galactic field in terms of condensation is less than 10^{-8} gauss and the corresponding magnetic energy density less than 10^{-5} the gas energy density. Thus only a small proportion of the neutron decay energy is needed to create the field.

The field wave-length λ is found by identifying the above proton recession velocity of 3×10^8 cm. sec.$^{-1}$ with the observed recession velocity $3\lambda H$, where H is Hubble's constant. This gives λ as about 30 mpc., a dimension already suggested[6] as a likely size of the regions from which families of galaxies are formed. The significance of this dimension is now enhanced by the concept of regions within which the gas may condense but which are magnetically isolated from similar external regions.

The importance of the magnetic field in the partition of energy between the proton and electron gases is now evident because the collision distance of the β-decay electrons is about 300 mpc., an order of magnitude larger than the condensation regions. The field may also be important in causing hydromagnetic instability which may involve variations in space and time of the rate of creation of aligned neutrons.

It is of interest to compare the aligned-neutron hypothesis with a possible mechanism for the creation of magnetic fields in some cosmic radio sources. There can be little doubt that the field in the Crab Nebula was created long after the supernova explosion and that the process may be continuing to-day at a rate corresponding to about 10^4 times the Sun's power output[7]. The field is remarkably ordered and seems to have a force-free form[5]. The only suitable source of energy (already invoked to explain the light emission[5,8]) seems the decay of unstable isotopes, probably silicon-32 and argon-39, formed during the explosion and having half-lives of 200–300 years. These nuclei, with magnetic moments greatly increased by orbiting electrons, tend to align themselves in the existing field and to strengthen that field as they suffer β-decay. A difficulty is met, however, in the very long relaxation time of unaligned nuclei and some alignment mechanism must be sought.

The creation of magnetic field from radioactive energy, either by aligned nuclei or by a 'spinning' process[7], would also explain the vast concentrations of magnetic energy in the radio galaxies[9] and why these galaxies appear to be exploding[10].

In conclusion, a laboratory experiment is suggested using the principle of aligned radioactive ions. With a field perpendicular to two insulated metal plates, electric charge is developed on one plate and a high-voltage generator is provided.

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Star Formation in Elliptical Galaxies and Intense Radio Sources

G. R. BURBIDGE has recently reviewed the problem of the energy sources required to give the non-thermal radio emission from intense extragalactic sources[1]. The intrinsically strongest sources appear to be mostly associated with giant elliptical galaxies, and to be radiating at power-levels up to 10^7 times that of our own Galaxy. Burbidge argues convincingly that the high-energy particles responsible for the radio emission must be accelerated in supernova explosions. A typical strong radio source would require the explosion of 10^7 or 10^8 supernovæ in a period of 10^7 or 10^8 years. This is a considerably greater supernova-rate than normally occurs in a galaxy.

Burbidge suggests that a Type I supernova chain-reaction can provide the necessary explosive frequency. He requires an unusually close-packed cluster of old stars, not much more massive than the Sun, to be detonated by the blast waves from their exploding neighbours. This requires that a significant fraction of the explosive energy of a supernova be emitted into space in a small fraction of a second, that when incident on a stellar surface the energy can propagate into the interior as an intense shock, and that it will commonly encounter a degenerate core which can be exploded (since non-degenerate matter is resistant to thermonuclear excursions[2]). The conditions are singly and collectively implausible.

However, because of the force of Burbidge's arguments concerning the energy sources, we should see if periods of intense supernova activity can arise in some other way. Let us consider the process of star formation in a giant elliptical galaxy.

From the virial theorem we can write the condition for a cloud of gas to become gravitationally unstable as:

$$2U + \mathfrak{M} + \Omega < 0$$

where U is the internal thermal energy, $\mathfrak{M}$ is the magnetic energy inside the cloud, and Ω is the gravitational potential energy of the cloud. We neglect turbulent energy, rotational energy, and external pressure. For a spherical cloud of solar composition, the above relation may be written as:

$$M^{2/3} > 17{\cdot}8\, T n_{\mathrm{H}}{}^{-1/3} + 1{\cdot}11 \times 10^{15} H^2 n_{\mathrm{H}}{}^{-4/3}$$

where M is the mass of the cloud in solar units, T is the temperature, H is the magnetic field in gauss, and n_{H} is the number density of hydrogen atoms.

As an illustration that may not be inappropriate for a giant elliptical galaxy with small gas content, suppose $T = 100°$ K., $H = 10^{-6}$ gauss, and $n_{\mathrm{H}} = 0{\cdot}01$ cm.$^{-3}$. Then, for gravitational instability, $M > 3 \times 10^8 M_{\odot}$. The significance of this result lies in the large mass of gas required for gravitational instability, and the fact that the magnetic energy term contributes overwhelmingly to the right-hand side of the above expression.

I have recently discussed the conditions for star formation in our own Galaxy[3]. The probable sequence of events is as follows: A gas cloud becomes gravitationally unstable and collapses with fragmentation until the fragments become optically thick. There follows a period of Kelvin–Helmholtz contraction, to be followed by a further period of gravitational collapse when hydrogen molecules become dissociated and hydrogen and helium atoms become ionized. However, in the present instance, fragmentation is not possible, owing to the fact that the gravitational and magnetic energies increase in proportion during the collapse, and the virial theorem cannot be satisfied for a smaller fragment. For such a large cloud, the collapse will not stop short of the ionization stage[4], but will continue until rotational instability produces a flattened disk.

The subsequent formation of stars can be presumed to take place by subcondensations occurring along the lines of force in the disk, either due to gravitational flattening or owing to magnetic friction opposing differential rotation. There is no reason for supposing that this should take more than a few million years. We have no way of knowing what the luminosity function of the new stars may be; stars of small mass may well be prevented from formation by the strong magnetic field.

The more massive stars contract quickly and all spend about 3×10^6 years on the main sequence[5]. Subsequent evolution should be rapid and should lead invariably to Type II supernova explosions[3]. Thus, it appears likely that star formation in giant elliptical galaxies will lead invariably to a period of intense supernova activity and hence to the formation of a strong radio source.

There are some interesting consequences of this analysis. The magnetic field is greatly strengthened and pinched at the place where star formation occurs. Ordinarily the energetic particles from the supernovæ will escape from the constriction and fill the confines of the galactic magnetic field. However, if there is a twist in the magnetic field which will lead to the formation of magnetic mirrors on either side of the constriction, then the particles should produce visible synchrotron radiation with three regions of greatest intensity. This seems to be the situation in the luminous jet of the strong radio source $M87$ (refs. 6 and 7). The large energy density in the particles should lead to a rapid expansion and short lifetime for such jets.

The magnetic field in the galaxy may be part of a general intergalactic field, since it is difficult to understand its formation if it is not primordial[8]. When the galactic field is filled with energetic particles, a rapid expansion will follow that will propagate out into the intergalatic region. Thus two magnetic mirrors will travel outwards. The energetic particles will tend to concentrate near the mirrors. This may account for the double nature of many strong radio sources. If this is the case, such double sources should have interesting intensity and polarization properties.

The history of both star formation and magnetic field configuration can be a very complicated function of galactic age. Hence one should be very cautious in drawing cosmological conclusions from the observed number-intensity relation of the intense radio sources.

I thank Wolfgang Priester and Harlan J. Smith for discussions which led to the development of these ideas.

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CAMERON[1] considers that the mechanism which I have proposed to explain radio emission from elliptical galaxies[2] is implausible. Since I have been looking into this question rather carefully, I do not agree; but the discussion of this problem is of great complexity and nothing can be gained by making general assertions. Let us concentrate therefore on a discussion of the mechanism of energy generation put forward in Cameron's communication with the view of deciding whether it provides a reasonable alternative to the mechanism proposed by me.

It should first be emphasized that this idea, that stars form and evolve rapidly to the supernova stage giving rise to radio emission in external galaxies, was first put forward by Shklovsky[3] and is discussed in my communication. However, if we are to explain the appearance of large sources of radio emission centred on seemingly normal elliptical galaxies, the comparatively short time-scale for radio emission which is indicated by the synchrotron mechanism and for other reasons described in my paper means that we must choose between the following two hypotheses:

(1) That the elliptical galaxies which we see now as sources of radio emission condensed into stars a very short time ($\leqslant 10^8$ years) ago and that some stars have

evolved and exploded, giving the energetic particles responsible for the radio emission.

(2) That some stars have very recently condensed, evolved, and exploded in elliptical galaxies which are old in evolutionary terms with ages $\sim 10^{10}$ years or greater.

The first hypothesis, which demands that some elliptical galaxies are exceedingly young, appears to be completely at variance with the observational evidence on the stellar populations and the structures of such galaxies. That an age of only about 10^9 years may be compatible with the observations in some cases (though not necessarily in those elliptical galaxies which are radio sources) was mentioned by me in one of the summarizing lectures at the Santa Barbara Conference on Extragalactic Astronomy[4] Although some astronomers have mentioned this possibility, it is not generally accepted, and certainly a much shorter time-scale would appear to be out of the question.

It seems best to adopt the second hypothesis at present since most astronomers still accept the conventional view that elliptical galaxies in general have ages $\sim 10^{10}$ years or greater. Let us consider the criteria developed by Cameron as applied to this model. He has written down the condition for gravitational instability, and having neglected the turbulent energy, rotational energy, and external pressure, he has put in values $T = 100°$ K., $H = 10^{-6}$ gauss, $n_{\mathrm{H}} = 0{\cdot}01$ cm.$^{-3}$ for the gas in order to obtain the critical mass for contraction.

However, Osterbrock[5] has shown, from the profile of the [O II]λ 3,727 doublet, that the turbulent velocity of the gas in the centres of some elliptical galaxies is of the order of 400 km./sec. There is also evidence for considerable rotation in the gas in some cases. These high turbulent velocities are comparable to the values of the random velocities of the stars in similar galaxies[6,7]. Thus, contrary to what has been assumed by Cameron, the turbulent velocity in the gas cannot be neglected in deriving the condition for gravitational instability. In fact, this term dominates. For an assumed turbulent velocity of 400 km./sec., the turbulent energy is of the order of 10^5 times the internal energy of the gas at 100° K., and of the order of 100 times the magnetic energy if $H = 10^{-6}$ gauss. The criterion for gravitational instability in a turbulent medium was derived by Chandrasekhar[8] and the above result follows immediately from his work. Thus the critical mass is very much greater than the value of $3 \times 10^8 M_{\odot}$ obtained by Cameron. It may be added that even this low value for the critical mass is much larger than the total amount of gas known to be present in an elliptical galaxy (Osterbrock obtains values ranging from 6×10^3 to $12 \times 10^6 M_{\odot}$ depending on the assumed degree of ionization in the case he discussed in detail).

Finally, we must consider the effect of the stars already present in the elliptical on the condensation of new stars. The radius of the sphere containing a critical mass of $3 \times 10^8 M_{\odot}$ as derived by Cameron is about 10^4 parsecs. This is of the same order as the radius of the elliptical so far as its stellar population is concerned. Thus we may ask whether this approach of applying the virial theorem for the gas alone to a gas mass which co-exists with a stellar galaxy is valid. Even if the conditions were such that the critical mass could be reduced so that it was initially contained within a radius of 1,000 parsecs (and in view of the general dynamical conditions described earlier this appears to be exceedingly difficult), it is not clear that further contraction and fragmentation into stars can go on unimpeded. The problem is that of considering further contraction of a gas cloud in a deep potential well, which is continuously being stirred by the hundreds of millions of stars which lie within it, and are moving with velocities of several hundred kilometres a second.

This discussion has brought out some of the difficulties associated with postulating that the formation and rapid evolution of stars in an elliptical galaxy will provide a source of radio emission. This is one of the reasons why I attempted to develop the model described in my earlier communication[2].

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PHYSICS

Hydrodynamic Lubrication of 'Perspex'

In recent experimental investigations of the lubrication at heavily stressed contacts, particular attention has been paid to measurements of the thickness of the lubricant film which, between gear teeth, is typically about 1μ. Using steel cylinders nominally in line or point contact the thickness of the film has been deduced from measurements of electrical capacity[1,2] or from determination of the penetration through the gap of a well-collimated and carefully aligned beam of X-rays[3]. The purpose of this communication is to direct attention to the advantages of direct observation of the contact using transparent specimens when the thickness of the lubricant film can be deduced from the examination of white light fringes; these techniques are illustrated by a description of some preliminary observations of a point contact between crossed cylinders[3] of 'Perspex'.

The lower cylinder, *C* (Fig. 1), of 2-in. diameter was rotated with surface speed 21 cm./s., while the upper cylinder, *B*, of 1-in. diameter rotated at less than 1 cm./s. and was loaded against cylinder *C*; this arrangement created an elliptical area of contact with the resolved velocity, *V*, effectively along the major axis of the ellipse. The polished surfaces were flooded with a plain mineral oil having viscosity 1·5 poise at 25° C., and the contact area, *A*, was viewed by the microscope, *M*, through the 'Perspex' rider, *R*, which was designed to reduce distortion. The source of white light was at *S*.

Fig. 2 is an impression of the contact area at a load of 2·1 kgm. (mean pressure 490 kgm./cm.^{2}); unfortunately, intense reflexion from the air bubble at recess prevents observation of fringes close to this edge, but over the rest of the contact the coloured fringes can be interpreted to give values of film thickness. The order of colours given by Newton[4], for white light fringes formed by reflexion, is used for identification; since the refractive index of the

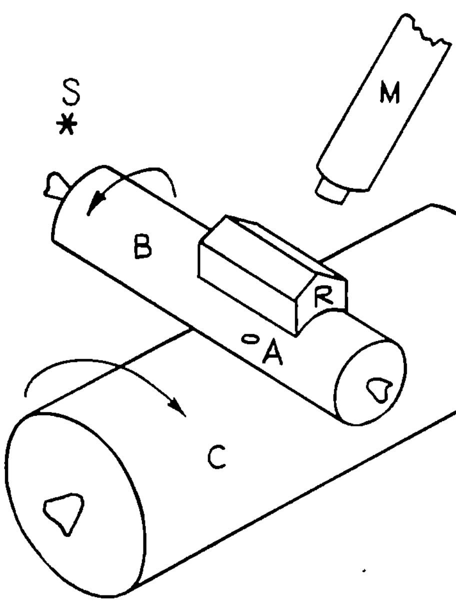

Fig. 1. Optical arrangement for viewing contact *A*

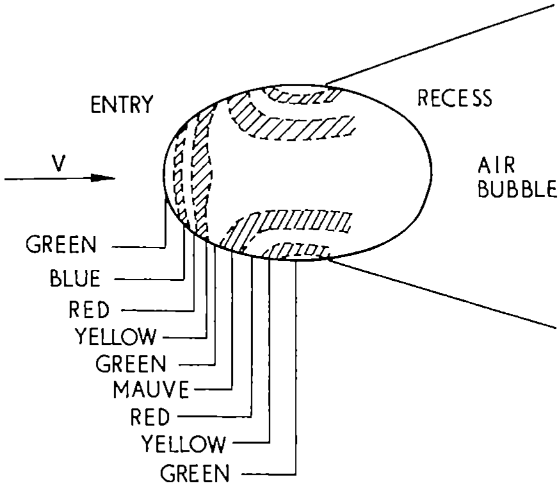

Fig. 2. Distribution of white light fringes in the contact

oil used is very close to that of glass, the film thickness for each colour can then be taken directly from Newton's table of values for glass. The effect of pressure on the density of the oil, and hence on its refractive index, is negligible in this experiment. After making a small correction for the angle of observation, in Fig. 2 the thickness of the oil film at the centre of the contact is then $4{\cdot}8 \times 10^{-5}$ cm., at entry $6{\cdot}6 \times 10^{-5}$ cm., and at the sides $2{\cdot}8 \times 10^{-5}$ cm. It is apparent that if monochromatic fringes were used not only would the absolute thicknesses be unobtainable but also the variations of thickness would be visible in far less detail.

Confirmation that the lubrication is indeed by a fluid film is obtained in two ways; first, from the fact that the fringes move steadily through the contact as the speed of cylinder *C* is varied, and secondly, from the extremely low value of the coefficient of friction, which is about 0·005. This value of the friction in a concentrated contact is in itself surprising only while the film thickness is unknown; in fact, simple calculations in respect of Fig. 2, assuming the friction is that of either a parallel-plate viscometer or a tilting-pad bearing, give coefficients of this order. There is a marked contrast between this behaviour of 'Perspex' and that of steel cylinders, where the higher pressures cause the viscosity of the oil to increase exponentially and the friction, therefore, to be very much higher[3]. Further results of experiments on the lubrication of 'Perspex' will be published elsewhere.

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Epitaxial Growth of Silicon by Vacuum Evaporation

Single crystal layers of silicon up to 50μ thick have been deposited on to a heated single crystal silicon substrate. Reflexion electron diffraction of the deposited layer showed Kikuchi lines indicating a high order of crystalline perfection. *PN* junctions with a maximum reverse breakdown of voltage in excess of 300 V. have been made. The conventional bevelling and staining techniques[1] showed a sharp junction between the deposited layer and the substrate. Maximum resistivities of 10 Ω-cm. for *P*-type and 3 Ω-cm. for *N*-type layers have been obtained.

The silicon was evaporated in a specially designed electron bombardment furnace which could be stabilized either for a constant power input or for operation at a constant evaporation temperature. The evaporations were performed in a vacuum of 10^{-5}–10^{-6} mm. of mercury. The layers were deposited uniformly over a 2-cm. diameter slice of single crystal silicon with a < 111 > orientation heated by radiation from a tungsten filament and held 5 cm. from the evaporating silicon. The maximum rate of deposition was 3 μ/min. It was possible to control the thickness of the deposited layer to within 1 per cent of its nominal value. A limited control of the resistivities was obtained by the choice of suitably doped evaporation material.

The temperature of the evaporating silicon was maintained between 1,600° C. and 1,800° C. according to the rate of evaporation wanted. The minimum temperature of the substrate for good single crystal layers was critical within a range of 1,100°–1,250° C., the exact value depending on the rate of deposition. The higher temperature was required for the faster rate of deposition.

To reduce the risk of contamination especially of the evaporating silicon: (1) the silicon was evaporated by focusing the electron beam into an area about 2 mm. in diameter in the centre of a 2-cm. diameter silicon rod which was held in a water-cooled copper pedestal; (2) both the evaporating silicon and the heated substrate were shielded[2] from direct line of sight of the tungsten cathode of the electron gun; (3) for evaporation at constant temperature a photoelectric feed-back loop could be used instead of a thermocouple: the light from the heated spot was reflected by a mirror of vacuum-deposited silicon into a photomultiplier the output of which controlled the bombardment power.

In spite of the high mobility of silicon at substrate temperatures of the order of 1,000° C., it was found possible to mask the deposited layer by means of a metal mask placed 2 mm. away from the substrate. The edge of a deposited layer 30μ thick reached full thickness in a distance of less than 10μ as measured by a Talysurf profile measuring machine.

The results obtained by this method of producing epitaxial films compare with those obtained by the hydrogen reduction of silicon halides[3], a method at present in general use by the semiconductor industry. In addition vacuum deposition seems to offer certain advantages over the chemical method such as cleanliness, the use of movable masking, the making of ohmic contacts and the possibility of the fabrication of complete devices in vacuum.

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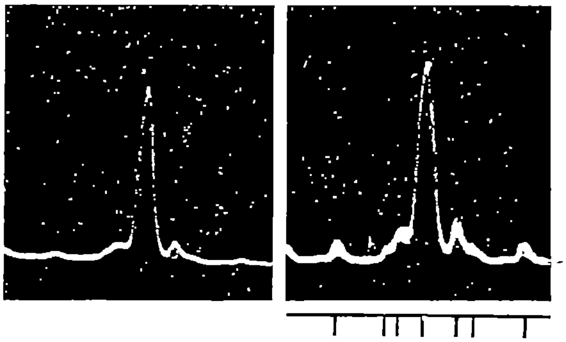
Anomalous Spectral Intensity Modification in a Hollow-Cathode Discharge

Hollow-cathode and high-frequency discharges are the light sources commonly used in optical spectral analysis of isotopic composition. Both these light sources have been used with an oscillating Fabry–Perot spectrometer in a determination of isotope abundance in mercury[1-3]. The hollow-cathode lamp appeared to exhibit anomalous variations in the spectral intensity of the odd isotope components, the magnitude of the variation depending on the discharge current strength, so a high-frequency discharge lamp was used exclusively in this work. Similar anomalous spectral intensity modifications have been reported by Schuler and Keystone[4], and by Murakawa[5], who used Fabry–Perot rings with photographic detection and consequently with less accuracy in spectral intensity measurement. The intensity variations have been further examined with an oscillating Fabry–Perot spectrometer with photomultiplier detection and oscilloscope recording.

The demountable hollow-cathode tube used consisted of a 'Pyrex' glass sleeve fitting into a 'Duralumin' base through an O-ring seal. The actual cathode was a hollow cylinder of diameter 1·4 cm. constructed of pure aluminium and fitting snugly into the 'Duralumin' base which was finned on the outside to facilitate cooling with liquid nitrogen. The nickel anode was also cylindrical in shape, being 2·5 cm. in diameter and 4 cm. long. Before use the electrodes were thoroughly outgassed.

A small blob of mercury inside the hollow cathode was sufficient to produce an intense discharge. Helium at a pressure of 1 mm. mercury was used as the carrier gas and was purified by continuous circulation through heated copper oxide and a liquid-nitrogen cooled charcoal trap. The stability of the high-voltage supply was tested by examining the spectral profile of several orders of λ5461 of a mercury-198 lamp, displayed both on an oscilloscope and on a pen-recorder function plotter. Spectral intensity variations were < 0·5 per cent.

On the oscilloscope trace spectral intensity variations in the light output of the hollow-cathode discharge were observed. The variations increase with discharge current, and at large discharge currents the anomalous intensity modifications of the odd isotope components become considerable. Fig. 1 shows two spectral profiles of λ5461 of mercury I for discharge currents of 200 m.amp. and 500 m.amp. respectively. The effective resolving power is 300,000, and mercury-199 *A*,*C* and mercury-201 *a*, *b*, *f*, *h* components are resolved. The interferometer scanning frequency is 7 c./s. corresponding to 20 m.sec. for one complete spectral profile. At a discharge current of 500 m.amp. the odd isotope components have increased in relative intensity by a factor of two while the central component, consisting mainly of the even isotope lines, has increased by only 20 per cent. The increase in half width and the change in shape of the main line can be explained by a doubling in intensity of the central odd isotope components, mercury-199 *B* being the strongest of these. Smaller magnitude rapid fluctuations in the intensity of the odd isotope components are also revealed by the 500 m.amp. spectral trace.



200 m.amp. 500 m.amp.

Fig. 1. λ 5461 mercury I; resolving power 300,000

Such anomalous intensity modification requires that the hollow-cathode discharge should be used with caution as a light source for the spectral analysis of isotopic composition and, at any event, limits the strength of the discharge current which can safely be used. On the other hand, the high-frequency discharge is more stable and controllable and increasing the discharge current to increase intensity does not affect stability nor lead to anomalous intensity changes so a greater precision in spectral intensity measurement is obtainable.

Use of an oscillating Fabry–Perot spectrometer enables the behaviour of a source under different discharge conditions to be easily and quickly examined to determine optimum operating conditions as well as permitting continuous monitoring of the spectral profile so that any anomalous behaviour is immediately detected.

I thank Prof. S. Tolansky for his advice during the course of this work.

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RADIATION CHEMISTRY

Photochemistry of Cellulose in the Far Ultra-violet

It is known that hydrogen, carbon monoxide and carbon dioxide are evolved, and aldehyde and carboxyl groups formed[1], during the photolysis of cellulose with short-wave ultra-violet radiation in the absence of oxygen; but the complications produced by the presence of oxygen and water vapour are not fully understood. We have found that temperature markedly affects the nature of the results obtained in atmospheres of dry oxygen and dry nitrogen, and have obtained evidence which suggests that the type of reactions involved are different in oxygen-containing atmospheres in the presence and absence of water vapour. In our experiments we have exposed undyed bleached cotton yarn to the radiations from a low-pressure mercury-vapour lamp emitting mainly at 2537 Å. The yarn was contained in the inner compartment of a brass cell fitted with a quartz window. The cell was maintained at a constant temperature by circulating a suitable heating (or cooling) medium through the outer compartment. During the experiment a stream of gas heated to the required temperature was passed through the cell. The degradation of the cotton was assessed by measurements of tensile strength, fluidity (poise^{-1}) in cuprammonium[2], reducing power[3], and carboxyl content[4]. Various attempts[5] have been made to convert fluidity into degree of polymerization but the accuracy of these relations is doubtful where the cellulose has been subjected to breakdown of largely unknown character. Accordingly, the rise in fluidity of the cotton produced by irradiation has been used as a quantitative measure of the extent of chemical action. A large rise in fluidity corresponds to considerable breakdown of the cellulose molecule.

The effect of temperature on the degradation of cotton cellulose exposed in dry oxygen and dry nitrogen to 2537 Å. radiation is shown in Fig. 1. The degradation of the cellulose increases markedly as the temperature rises when oxygen is present; but in its absence the degradation seems to decrease at temperatures above 50° C. It is possible that in the absence of oxygen recombination of the free radicals produced by photolysis of the cellulose molecules is favoured at the higher temperatures. The results in oxygen presumably indicate the increasing importance of secondary thermal reactions as the temperature rises. Similar results to those shown in Fig. 1 were also obtained using the reducing power of the cellulose as the parameter of degradation. The carboxyl content of the cellulose increased steadily with rise in temperature on exposure in oxygen, but in nitrogen the value was largely independent of temperature.

The rate of degradation of cotton cellulose was also studied in air at 0 and 100 per cent relative humidity at temperatures of 15°–70° C. At all these temperatures the loss in tensile strength of the cotton yarn was always significantly greater in the absence of water vapour, the difference being greatest for short times of exposure. The reducing power of the cellulose was slightly greater in dry air than humid air for short times of exposure, but after longer periods the changes were greater in humid air. The changes in cuprammonium fluidity were greater for exposure in humid air than dry air, although for short periods of exposure the difference was negligible. As a consequence of the differences obtained using different parameters of degradation, the relation between rise in fluidity and loss in tensile strength of the irradiated yarn, as shown in Fig. 2, is different for exposures in dry and moist air. The comparable relation for dyed and undyed cotton yarn degraded by exposure to sunlight in dry and moist air[6], and also that for cotton yarn degraded by acid hydrolysis[7], are both represented by a straight line joining the origin with a point corresponding to a fluidity rise of 25 and a loss in strength of 50 per cent. Consequently, exposure of cotton yarn to 2537 Å. radiation in dry air produces a large loss in strength for quite a small change in fluidity (and hence in degree of polymerization). This seems to indicate highly localized degradation of the cellulose near the end of the chain.

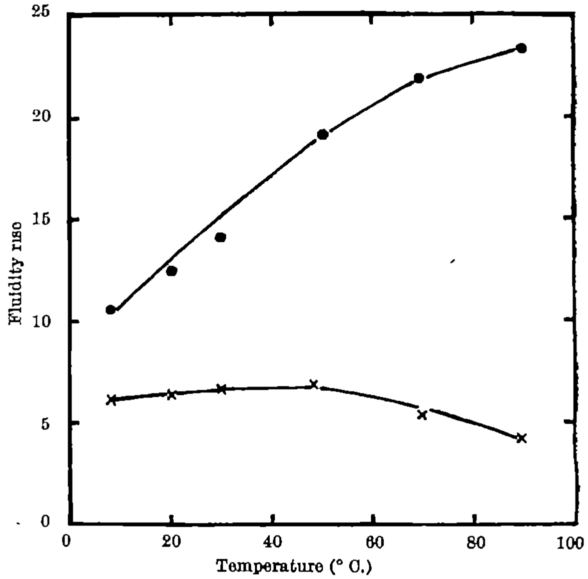

Fig. 1. Effect of temperature on degradation of undyed cotton exposed to low-pressure mercury-vapour lamp in dry oxygen (●) and dry nitrogen (×) for 20 hr.

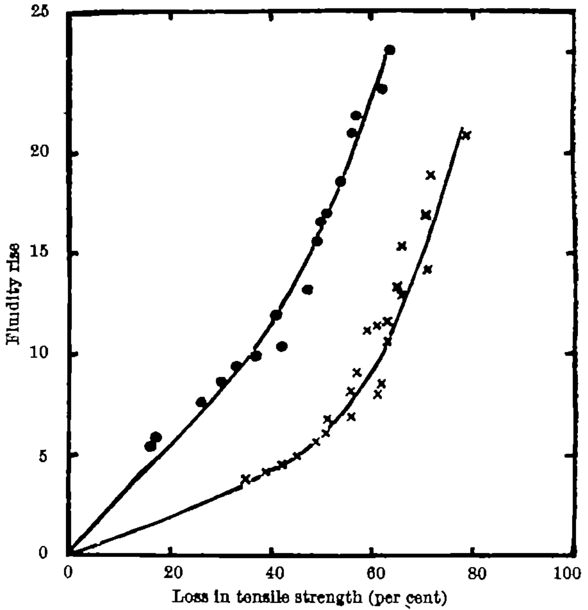

Fig. 2. Undyed cotton exposed to low-pressure mercury-vapour lamp in air at 0 per cent relative humidity (×) and 100 per cent relative humidity (●)

In the presence of moisture the degradation is not so localized, and it seems likely that the course of the reactions is significantly different. Launer and Wilson[8] have suggested that the effect of water vapour is the result of its fundamental chemical combination with cellulose. However, we have also found that the loss in tensile strength of nylon yarn after exposure to 2537 Å. radiation is markedly greater in dry air than in humid air. It is probable that the role of water vapour in these photochemical reactions is more complex than has hitherto been realized.

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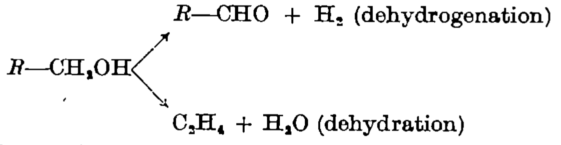
Radiation Effects on Zinc Oxide Catalyst in Dehydrogenation of Ethyl Alcohol

IT is well known that the activity of a solid catalyst may be affected by ionizing radiations, and the effects of nuclear radiations on heterogeneous catalysts have been investigated by various workers[1,2]. Recent work has shown the changes, due to irradiation, which are produced in the catalytic behaviour of some oxides[3]. These effects are due to the fact that catalytic activity is strongly dependent on the structure and energy condition of the material, and this structure may be modified under irradiation[4].

Consequently, the catalytic activity may be increased or decreased by the presence of impurities, nuclear radiations, or by illumination. Nuclear radiations may create new energetic-levels which shift the position of the Fermi-level in the solid catalyst and influence the paths for a given reaction.

In this case, I have examined the well-known reaction of the dissociation of ethyl alcohol, which proceeds along two paths:



$$R\text{—CH}_2\text{OH} \begin{cases} \nearrow R\text{—CHO} + \text{H}_2 \text{ (dehydrogenation)} \\ \searrow \text{C}_2\text{H}_4 + \text{H}_2\text{O} \text{ (dehydration)} \end{cases}$$

The particular reaction path taken is determined at the first stage of reaction, the adsorption of the alcohol molecule, and depends on the particular bond broken in adsorption: the O—H or the C—OH bond; this in turn depends on the nature of the catalyst. With zinc oxide catalysts, both reactions occur. In this case, the relative activity of the catalyst for dehydration or dehydrogenation, in the framework of the electronic theory of catalysis, depends on the position of the Fermi-level. It has been reported by Wolkenstein[5] that the lowering of the Fermi-level retards dehydrogenation and accelerates dehydration, and since Kon, Moore and Taylor[6] had shown that irradiation effects are relatively stable in refractory oxides, zinc oxide was chosen as the catalyst in the present investigation. Factors which affect the position of the Fermi-level are: acceptor impurity, donor impurity, nuclear radiation, etc.

This has been confirmed experimentally, using donor impurities, by Roginsky *et al.*[7]. This communication reports the results of irradiation effects on the catalytic behaviour of zinc oxide, prepared by ignition of zinc carbonate at 450° C. in air for 3 hr. The resulting coarse powder was pressed into pellets, and these were then crushed in order to have granules in the size range of 20–28 mesh. The amount of catalyst used in each test was 0·4 gm. The surface area was 23·7 m.^{2}/gm.

Several samples were irradiated in the Chalk River reactor and the maximum flux for catalyst sample II was $2 \cdot 3 \times 10^{13}$ n/cm.^{2}/sec. and for catalyst sample III, $3 \cdot 9 \times 10^{13}$ n/cm.^{2}/sec. (Sample I is an unirradiated sample.) The reaction has been examined in a flow system made of quartz. The specific radioactivity present in catalyst sample II was 20 mc./gm. and in catalyst sample III, 52·4 mc./gm.

Fig. 1 shows the reaction-rate at a fixed temperature of 390° C. for normal and irradiated catalysts. Fig. 2 compares the proportion of hydrogen and ethylene evolved for normal and irradiated catalysts, and it is evident that the irradiation significantly affects the reaction paths and that the effect increases with increasing irradiation time or specific radioactivity of the solid catalyst. For the temperature-range 330°–390° C. the reaction of dehydrogenation predominates in zinc oxide[8]. According to Wolkenstein[5] and Garner[9], the dehydrogenation of alcohol is an acceptor or n-type reaction; in other words, this is a reaction accelerated by the presence of free electrons and it proceeds more rapidly the higher is the Fermi-level (other conditions being equal). In contrast, the dehydration is a donor or p-type reaction.

It is evident that a higher Fermi-level is obtained in the irradiated catalysts compared with the non-irradiated ones, and this leads to an increase in the

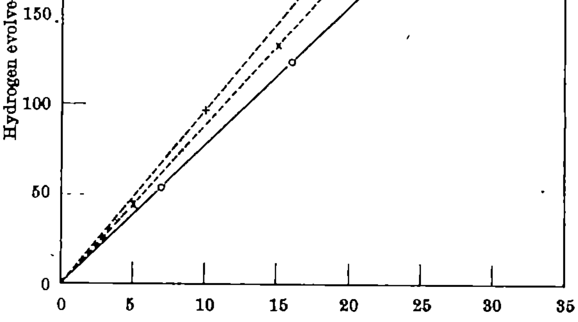


Fig. 1. Reaction-rates for different catalysts. Ethyl alcohol feed, 0 204 ml./min. I (○), Normal catalyst; II (×), radioactive, 20 mc./gm.; III (+), radioactive, 52·4 mc./gm.

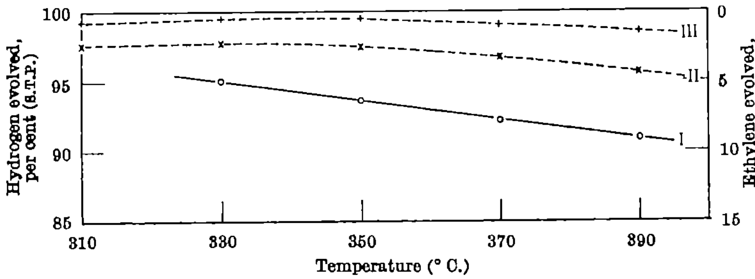


Fig. 2. Behaviour of zinc oxide catalysts. Ethyl alcohol feed, 0·204 ml./min. catalyst, zinc oxide type *A*, 0·4 gm. 20–28 mesh. I (○), Normal catalyst; II (×), radioactive, 20 mc./gm.; III (+), radioactive, 52·4 mc./gm.

hydrogen/ethylene ratio in the reaction products, which is a function of the radioactivity of the catalyst. Work is in progress to investigate the catalytic behaviour of different catalysts (*p*-type semiconductors) to see if it is possible by irradiation to introduce any Fermi-level shifting which may increase the dehydration reaction under similar reaction conditions.

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*p*H Effects in the Radiolysis of Aquo-Organic Systems

The experimental data given in our recent communication[1] on this subject refer to the systems (I) 0·1 *M* HCOONa—0·01 *M* $\mathrm{Na_2{}^{14}CO_3}$; (II) 0·1 *M* $\mathrm{H^{14}COONa}$—0·01 *M* $\mathrm{Na_2CO_3}$. The solutions were prepared with $\mathrm{NaHCO_3}$ and were adjusted to *p*H 11 with sodium hydroxide prior to irradiation.

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CHEMISTRY

Structure and Retention Time in the Gas-liquid Chromatography of Unsaturated Fatty Acids on Polyester Substrates

It is generally accepted that plots of $\log_{10}$ retention time in the gas-liquid chromatography of the longer chain normal saturated fatty acid methyl esters against the number of carbon atoms in the fatty acid chain give a straight line with both non-polar and polar substrates. On the other hand, various simple plots of similar values obtained from unsaturated fatty acid esters with polar substrates, correlating chain length or number of double bonds, yield surprisingly little information[1–4]. In the course of gas-liquid chromatographic examination of the methyl esters of the fatty acids of marine oils the retention-time data of a number of identified unsaturated fatty acids examined by different workers[1,4–9], all using polyester substrates, were plotted on semilog paper against the number of carbon atoms in the fatty acid chain. Despite the fact that a number of differing polyester substrates were used, the resulting plots showed a surprising overall similarity. This suggested that the key to the relation of the plotted points lay in the structural position of the methylene-interrupted double bond systems in the fatty acid chains in the case of the polyethylenic fatty acids, and in the position of the isolated double bonds in the monoethylenic fatty acids, rather than in the type of polyester.

The following hypothesis is therefore advanced. In plots of $\log_{10}$ retention time of ethylenically unsaturated fatty acids versus number of carbon atoms, where polyester phases are used, parallel straight lines can be drawn between those points corresponding to fatty acids of the same number of methylene-interrupted double bonds, or of single double bonds, when the number of carbon atoms beyond the double bond farthest removed from the carboxyl group, including the terminal methyl group, is the same.

A corollary to this hypothesis is that the lines are parallel to that derived from certain of the commonly occurring monoethylenic fatty acids, namely, octadec-9-enoic, eicos-11-enoic, and docos-13-enoic, and not necessarily parallel to the plot of the saturated fatty acids. Since two or more of these are normally present in most natural lipids, the basis for plotting other materials is readily available. The general observation may also be made that the precise nature of the polyester is relatively unimportant (cf. ref. 6), facilitating comparison of data from various authors.

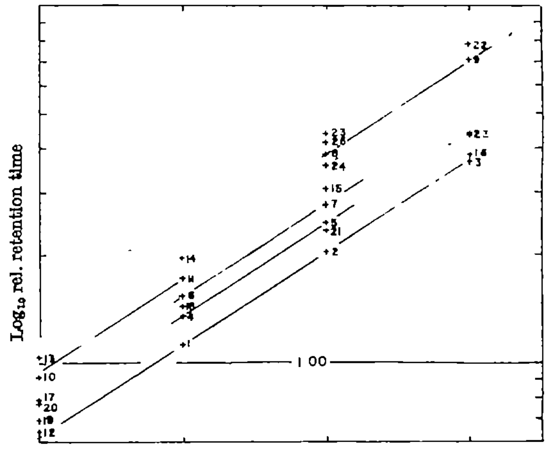

Fig. 1. Point plot of relative retention time data from Table 1, with parallel lines correlating fatty acid structure

Table 1. FATTY ACID STRUCTURES AND RETENTION TIMES USED TO OBTAIN GRAPHIC LINEAR RELATIONSHIPS

Group	Graph point*	Retention time, relative to stearic acid, used by Author	Retention time, relative to stearic acid, used by Other authors†	Fatty acid structure‡ Short-hand designation	Fatty acid structure‡ Double bond positions	End carbon chain§
I	1	1·12	1·12	18:1	9	8
	2	2·02	2·02	20:1	11	8
	3	3·60	*ECL*22·4 (7)	22:1	13	8
II	4	1·34	1·34	18:2	9, 12	5
	5	2·45	2·45	20:2	11, 14	5
III	6	1·53	*ECL*19·5 (7)	18:3	6,9,12	5
	7	2·76	2 76	20:3	8, 11, 14	5
IV	8	3·85	3 85	20·5	5, 8, 11, 14, 17	2
	9	7 00	7 00	22:5	7, 10, 13, 16, 19	2
V	10	0·904	0 904	16:3	7, 10, 13	2
	11	1·72	1·72	18.3	9, 12, 15	2
VI	12	0 634	0 634	16:1	9	6
	10	0 904	0 904	16:3	6, 9, 12	3
	13	1 04	1·04 (1)	16:4	6, 9, 12, 15	1
	14	1·97	1·97	18:4	6, 9, 12, 15	2
	15	3·04	3 04	20:4	5, 8, 11, 14	5
VII	16	3 80	*ECL*22·6 (10)	22 2	5, 13	8
VIII	17	0 785	0 785	16:2	6, 9	6
	17	0 785	0 785	16:2	9, 12	3
	18	1 43	1 43	18.2	6, 9	8
IX	19	0·695	0 68 (1)	16 2	6, 9	6
	20	0·770	0 74 (1)	16.2	9,12	3
	18	1·43	1·43	18:2	6, 9	8
	18	1 42	1·42 (11)	18:2	11, 14	3
X	18	1·43	1 43	18.2	6, 9	8
	21	2 32	2 32	20 2	8, 11	8
XI	15	3·04	3·04	20:4	5, 8, 11, 14	5
	22	7 75	7 75	22.6	4, 7, 10, 13, 16, 19	2
XII	23	4 32	*ECL*23 0 (10)	22:2	(13, 16) (10,13)	8 or 5
	6	1·54	1 54	(18:3)	(6, 9 ,12)	5
	24	3·51	3 51	20:(4)	(8, 11, 14, 17)	2
	25	4·11	4 11	20:(6)	(2, 5, 8, 11, 14, 17)	2
	23	4·38	4 38	(22 2)	(10, 13)(13,16)	5 or 8

* Points falling very close together may be assigned only one point and number.

† Data of Farquhar *et al.*[5], except as indicated by author references thus ( ).

‡ Parentheses indicate hypothetical structures.

§ No. of carbon atoms after ultimate double bond including methyl group.

The data from Farquhar *et al.*[5], obtained on ethylene glycol-adipate polyester at 197° C., provide the most comprehensive test of this hypothesis, although similar data are available in part from other authors. Certain supplemental points were also plotted, using an equivalent chain-length system[7] (*ECL*) based on the plotted monoethylenic fatty acid lines. These are indicated in Table 1 in the reference author column, and are subject to some reservations as to accuracy as a result of the transfer. Of the various groups of fatty acids listed in Table 1, groups I–IV represent accurate correlation of plotted data with the hypothesis (Fig. 1). The two points of group V do not agree so well, but the retention times for the sixteen carbon fatty acids are possibly not as satisfactory as others since the retention times are shorter and the separation of isomers inadequate (cf. refs. 1, 2, 5). In group VI are listed plotted points which should not fall on any established line since either the number of double bonds or the end carbon chains do not match. These in fact do not fall on any line (see, however, group XII for a possible tetraenoate correlation) although the sixteen carbon chain data are subject to reservations. The validity of the necessity of the methylene-interrupted system in polyethylenic fatty acids is shown in group VII, where the plot of a diene not possessing this structure[10] falls remote from any diene line. In group VIII are listed three fatty acids, the plotted points of which fall on a straight line, although they should not. However, in addition to the doubts affecting the sixteen carbon chain acids, the plotting of any number of points will inevitably give some falling on a straight line through chance. With a revised plot, based on transferred data, as given in group IX, these three points are no longer linearly related, but in both plots there is reasonable agreement between hexadeca-9,12-dienoic acid and octadeca-11,14-dienoic acid[11]. The important negative correlation of data with theory is given in group X, where two points which should be fitted to a line on the basis of the given structures cannot be related in any way. However, comparison of octadecadienoic retention times[11] suggests that the relative retention time of octadeca-6,9-dienoic acid should be approximately 1·30, correlating with point 21. The eicosatetraenoic and docosahexaenoic acids, group XI, are unique, unless the approximate correlation of the latter with the unknowns (group XII) with relative retention times 4·11 and 4·38 could indicate the presence of an eicosahexaenoic acid of the same type, although this is improbable.

The validity of any structural determination depends on the number of bits of information pointing to that particular structure. Thus if the retention time is known, and other data indicate a twenty-two carbon diene acid[10], the assumption of the structure of the acid as docosa-10,13-dienoic acid (group XII) seems indicated by the plot coincidence with a line based on that diene structure type. However, owing to possible error in transferring the data a docosa-13,16-dienoic structure is also reasonable if the structure assigned to point 21 is correct. The identities suggested for the other unknowns of group XII are, however, highly speculative, since in two cases not even the chain length is known, but these are put forward as materials which could be used under controlled conditions to check the hypothesis advanced as the basis of this submission.

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Separation of Low-boiling Hydrocarbons by Gas Chromatography using Packed Capillary Columns

BY modifying the method described by Desty *et al.*[1] for drawing glass capillaries, we have succeeded in making packed columns having an inner diameter comparable with that of capillary columns, which will be referred to here as 'packed capillaries'. A capillary packed with an adsorbant is particularly suited for

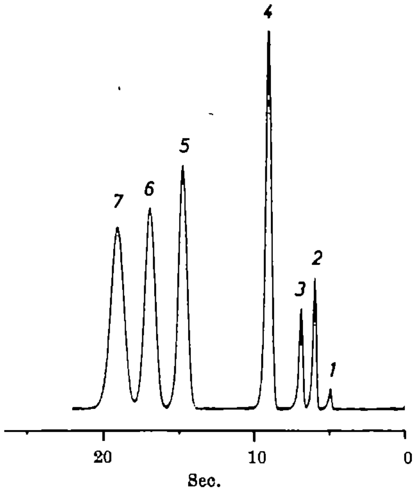

Fig. 1. Separation of (1) methane, (2) ethane, (3) ethylene, (4) propane, (5) propylene, (6) isobutane, (7) normal butane, in 20 sec. Col. length, 2 m.; temperature, 90° C.; carrier gas, hydrogen; inlet pressure, 2·0 atm.; flow-rate, 4 65 ml./min. (room temperature); sample size (syringe), 0 05 ml.; by-pass ratio, 1 : 700. Flame ionization detector with electrometer amplifier and high-speed ultra-violet galvanometer recorder

separating low-boiling hydrocarbons at room temperature, an operation requiring very low temperatures when carried out with a conventional capillary column. Filling the capillary with an adsorbant is performed thus: a carefully cleaned steel wire of 1 mm. diameter is pushed through a glass tube having an inner diameter of 2·2 mm. and the empty space between the wire and the tube wall filled with the adsorbant. Then placing the tube in a horizontal position and removing the wire carefully the glass is drawn to a capillary of 0·3 mm. inner diameter, using Desty's method. The adsorbant used in our experiments was alumina produced by calcining for 9 hr. at 400° C. hydragillite (CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG., Muttenz, Switzerland) [γ-$Al(OH)_3$], having a grain size of 0·10–0·15 mm. Afterwards the column was flushed for 24 hr. at 120° C. by a stream of dry hydrogen, in order to remove traces of water which might have been adsorbed by the alumina during the filling and drawing operation.

Fig. 1 shows the chromatogram of a high-speed analysis of a hydrocarbon mixture up to *n*-butane, obtained at a temperature of 90° C. with a packed capillary column 2 m. long. In addition, a flame ionization detector and an electrometer amplifier ('DC 60', Atlas-Weke AG., Bremen) combined with a high-speed ultra-violet galvanometer recorder ('Luminoscript', Hartmann und Braun AG., Frankfurt am Main) were used for registration. Owing to the high polarity of this alumina a complete separation of ethane–ethylene and propane–propylene in the order given could be obtained. Polarity, given as the ratio of the net retention volumes of propylene and propane, may be increased by lowering the temperature of the column. However, running in the same direction, retention times and duration of the analysis are increased, also.

Besides changes in temperature, de-activation with water is suitable for setting the activity (retention times) and the polarity at a chosen level[2]. The simplest method for impregnating the solid support with water is to pre-saturate the carrier gas with water vapour. A certain partial pressure of water vapour may be produced by passing the carrier gas through salt hydrates or through a layer of cotton-wool soaked with saturated aqueous solutions. Due to the relatively small quantity of alumina involved, equlibrium is reached within a few hours. The degree of de-activation attainable by applying a certain partial pressure of water vapour increases with decreasing column temperature. This method provides, by a suitable selection of retention volumes and polarity, a satisfactory separation of substances with very similar boiling points in an optimal time. To illustrate this, a mixture of 15 saturated and unsaturated low-boiling hydrocarbons was analysed (Fig. 2), with a packed capillary column, 10 m. long, de-activated by water released from crystalline sodium sulphate at room temperature (column temperature, 80° C.).

The packed capillary columns are suitable for high linear flow-rates like the conventional capillary columns, but they show the separation characteristics of the packed columns (4 mm. inner diameter) with active support[3] thus making possible the separation of low-boiling hydrocarbons even at elevated temperatures. Although such capillaries reveal an unevenly distributed packing, they have a good column performance. In Table 1 values of height equivalent to a theoretical plate (H.E.T.P.) are given for various flow-rates.

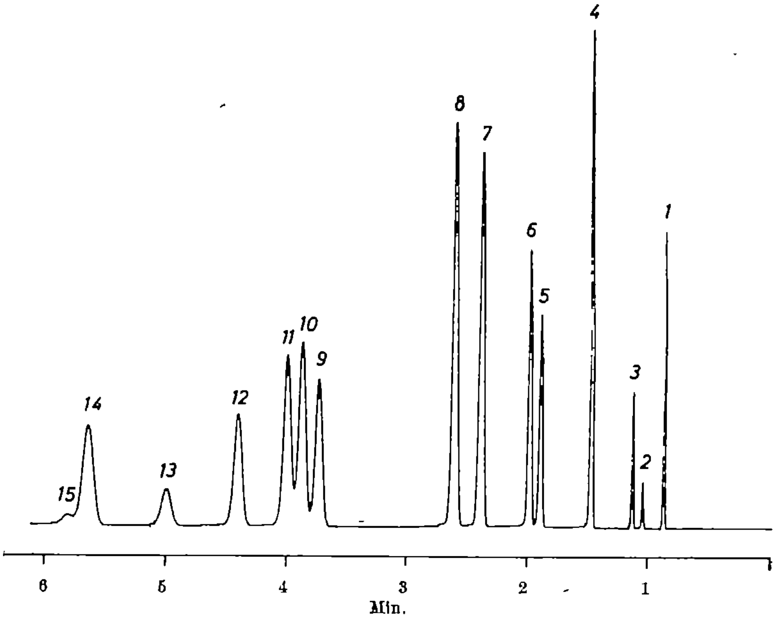

Fig. 2. Separation of (1) methane. (2) ethane, (3) ethylene, (4) propane, (5) propylene, (6) acetylene, (7) isobutane, (8) *n*-butane, (9) butene-1, (10) *trans*-butene-2, (11) isobutene, (12) *cis*-butene-2, (13) iso-pentane, (14) *n*-pentane, (15) butadiene. Col. length, 10 m.; temperature, 80° C.; deactivation, moistening of the carrier gas with crystalline sodium sulphate; carrier gas, hydrogen; inlet pressure, 4·4 atm.; flow-rate, 2·5 ml./min. (room temperature); sample size (syringe), 0·10 ml.; by-pass ratio, 1 : 600. Flame ionization detector with electrometer amplifier and 2 5 mV. compensation recorder

Table 1

Linear flow-rate (cm./sec.)	H.E.T.P. (mm.) Ethylene	H.E.T.P. (mm.) n-Butane
20	0·28	0·52
30	0·35	0·70
40	0·53	1·08

The optimal H.E.T.P. values are about 0·5 mm. for butane and 0·3 mm. for ethylene. As in conventional capillary columns H.E.T.P. values increase with increasing retention volumes. The good resolution may probably be explained by the fact that only a few grains of alumina can be located side by side, thus leaving but a few free passages to the flow of gas. The combination of narrow gas passages and empty spaces between the grains allows a quick mass transfer in the gaseous phase which, in the case of adsorption chromatography practised here, provides a short duration of analysis.

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BIOCHEMISTRY

Essential Fatty Acids

Previous work[1] on the relationship between chemical structure and biological activity of fatty acids was based on the theory that either the 6-term. (that is, a double bond between the 6th and 7th carbon-atom counted from the terminal methyl group) or the 9-term. double bonds or both were fundamental for essential fatty acid activity. By means of a new quantitative bio-assay[1] a number of fatty acids were tested and the results were in favour of the theory that both the 6-term. and the 9-term. positions are essential.

Three new essential fatty acids were found: 6,9,12-octadecatrienoic acid (= γ-linolenic acid = 18 : 6,9,12-term.), 10,13-nonadecadienoic acid (19 : 6,9-term.) and 11,14-eicosadienoic acid (20 : 6,9-term.). These acids appeared to have divergent potencies. Consequently, the 6,9-term. structure is not an exclusive condition, and sub-hypotheses (on the influence of the presence of other double bonds and of the chain-length) were put forward.

In a later investigation[2] it was shown that fractions of C_{22}-fatty acids from human brain glycero-phosphatides were active. According to Klenk[3] these fatty acids have the 6,9-term. configuration.

The work on the relationship between chemical structure and essential fatty acid potency has now been continued. The previous observations with regard to the activities of 18 : 3,6,9-term., 18 : 6,9,12-term., 20 : 6,9-term. and 20 : 6,9,12,15-term. were confirmed.

In addition two new essential fatty acids were found, namely, 7,10,13-nonadecatrienoic acid (19 : 6, 9,12-term.) and 8,11,14-eicosatrienoic acid (20 : 6, 9, 12-term.). The activity of the former was low, but significant, while the C_{20}-acid appeared to have about the same potency as linoleic acid (18 : 6,9-term.).

The results obtained to date are summarized in Table 1. The data in this table regarding previous bio-assays have already been published elsewhere[1].

Table 1. Bio-Potencies of Essential Fatty Acids

Fatty acids tested: Chain-length	Fatty acids tested: Location of double bond (terminal numbering)	Bio-potencies (U./gm.)*: Previous bio-assays	Present bio-assays: I	Present bio-assays: II	Present bio-assays: III	Present bio-assays: IV	Average
18	6, 9		arbitrary				100
18	3, 6, 9	9		8			9
18	6, 9, 12	100	117		121	122	115
19	6, 9	9					9
19	6, 9, 12				6		6
20	6, 9	43			52	42	46
20	6, 9, 12				121	83	102
20	6, 9, 12, 15	130	166		139	120	139
22	?, 6, 9, ?, ?, ?	20–50					20–50

* 1 U. = the bio-potency of 10 mgm. linoleic acid.

In all the experiments the rat-assay with restricted drinking-water was applied.

The nine fatty acids tested appear to have divergent potencies. Ordinary linolenic acid (18 : 3,6,9-term.) and the two C_{19}-acids (diene and triene) have very low potencies. Linoleic acid (18 : 6,9-term.), γ-linolenic acid (18 : 6,9,12-term.) and eicosatrienoic acid (20 : 6,9,12-term.) are about equally potent (about 100 U./gm.).

Arachidonic acid is synthesized *in vivo* from linoleic acid. The intermediary steps are not exactly known. It can be accepted that the intermediates must have the same or higher bio-potencies than linoleic acid (100 U./gm.). Therefore, 18 : 3,6,9-term., 19 : 6,9-term. and 20 : 6,9-term. cannot be intermediates. On the other hand the high potencies found for 18 : 6,9,12-term. and 20 : 6,9,12-term. are in agreement with the theory of Mead and Howton[4], who proposed the pathway:

linoleate (18 : 6,9-term.) → γ-linolenate (18 : 6, 9, 12-term.) → 8,11,14-eicosatrienoate (20 : 6,9,12-term.) → arachidonate (20 : 6,9,12,15-term.).

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Release of Peroxidase from Eosinophil Granules *in vitro*

The eosinophil is a phagocytic cell[1], and following the ingestion by eosinophils of sensitized red cells many of the eosinophil granules disappear[2]. Histochemical studies recently have shown that eosinophil granules contain significant amounts of the enzyme peroxidase[3]. The conditions necessary for lysis of the granules and release of peroxidase were investigated in the present work, using preparations of eosinophils isolated from the peritoneal cavity of the rat.

Eosinophils together with other peritoneal cavity cells were collected by a method previously described[4]. Rats were used the peritoneal cavities of which had been repeatedly irrigated with physiological saline solution. This treatment is known to increase the yield of eosinophils and to decrease greatly the mast cells. Peritoneal cavity cells collected from 10 rats were added to 5 ml. heat-inactivated rat serum and the cells were homogenized at 0° C. in a 'Teflon'-tissue grinder. The homogenate was centrifuged at 250*g* in a refrigerated centrifuge held at 0° C. This centrifugal force was sufficient to deposit most of the cell debris, but the eosinophil granules remained suspended in solution. The supernatant solution containing the granules was centrifuged at 1,200*g* to deposit the eosinophil granules and the granules were washed once in a large volume of ice-cold physiological saline solution. The granules appeared intact when stained films of the deposit were examined with the light microscope. Only an occasional mast cell granule was present in these preparations.

In some experiments intact eosinophils were used. The eosinophils were separated from the other peritoneal cavity cells by centrifugation at 0° C. in a column of 42 per cent bovine albumin in physiological saline solution. Bovine albumin was obtained as a powder from the Commonwealth Serum Laboratories, Parkville, Victoria. The peritoneal cavity cells were mixed with 2·5 ml. of the albumin solution at 0° C. and layered above a 2·5 ml. column of 42 per cent albumin solution at 0° C. in a centrifuge tube. Eosinophils and mast cells have a higher specific gravity than other cells obtained from the peritoneal cavity, and following centrifugation at 1,200*g* the eosinophils and mast cells pass through the albumin solution and may be collected at the bottom of the tube. The other cells remain suspended in the albumin or pass to the top of the column. The deposited cells were collected and washed three times with physiological saline solution. Eosinophils constituted approximately 80 per cent of the cells present, the remainder being mononuclear cells together with occasional mast cells and red cells. When necessary contaminating red cells were removed by allowing the cells to sediment in physiological saline solution. The white cells were found to clump together and settle to the bottom of the tube, leaving the red cells suspended in the solution.

Peroxidase was measured quantitatively by the method of Jermyn and Thomas[5]. The optical density of the final solution containing the enzyme was measured at 400 mμ in a direct-reading Zeiss spectrophotometer. Crystalline horseradish peroxidase was obtained from Nutritional Biochemicals, New Jersey, and standard solutions were prepared in 0·1 *M* sodium acetate buffer at *p*H 5.

Peroxidase was released from intact eosinophils and isolated granules when these were placed in a sodium acetate buffer of ionic strength 0·02 *M* and *p*H less than 5·5. The enzyme was released more rapidly as the *p*H was reduced to 4. The amount of peroxidase released from one million rat eosinophils was found to be equivalent to 0·5 μgm. of crystalline horseradish peroxidase. Microscopic examination of stained films revealed that the eosinophil granules had disappeared in those preparations in which peroxidase was released. Peroxidase was not released and the granules remained intact when preparations of intact cells or isolated granules were added to distilled water at *p*H 6. Similarly peroxidase was not released from intact cells or granules exposed to 0·15 *N* saline when the *p*H was reduced to 4. The enzyme remained in solution at *p*H 5 and ionic strength 0·02 following centrifugation of the solution at 28,000*g* for 30 min. in a Spinco ultracentrifuge. Activity remained inside a dialysis bag following dialysis of the solution against distilled water for 24 hr. at room temperature. Peroxidase activity was destroyed by boiling or lowering the *p*H below 2. The enzyme was precipitated out of solution when the *p*H of the solution was increased to 8, but the precipitate redissolved in 0·02 *M* sodium acetate buffer at *p*H 5. The precipitate containing the enzyme was re-dissolved to a concentration equivalent to 10 μgm./ml. horseradish peroxidase and the solution was faintly yellow in colour. Following the addition of a few crystals of sodium dithionite the solution became faintly green and spectroscopic examination revealed the presence of an absorption band in the Soret region at approximately 440 mμ. The significance of the spectroscopic findings is being investigated separately.

Recent studies by Hirsch and Cohn[6] have shown that granules isolated from rabbit neutrophils undergo lysis and liberate their contained enzymes when exposed to an environment of low ionic strength and low *p*H. In the present work eosinophil granules underwent lysis with release of peroxidase under similar environmental conditions. Hirsch and Cohn have suggested that following phagocytosis by neutrophils there may be a fall in the *p*H of the cytoplasm sufficient to cause lysis of the granules. It is tempting to suggest a similar mechanism for the lysis of eosinophil granules following phagocytosis by eosinophils.

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Inhibition of β-Glucuronidase by Endogenous Saccharate during Hydrolysis of Urinary Conjugates

THE use of β-glucuronidase as an agent for the liberation of a steroid from its urinary glucuronide conjugate has now become standard practice. The overall rate of the enzyme action is unpredictable, however, due to the presence in the urine of natural inhibitors and other substrates of varying affinity and ease of hydrolysis.

D-Glucosaccharic acid has been found to be a normal constituent of mammalian urine[1]; the excretion in adult human subjects is about 10 mgm. a day, and is doubled during pregnancy, returning to normal within a few days after parturition[2]. At the acid *p*H at which molluscan or mammalian β-glucuronidase preparations are normally used, there is partial conversion of saccharic acid to its 1:4-lactone, the potent and specific β-glucuronidase inhibitor[3]. This explains the progressive decrease in enzyme activity which occurs during incubation with urine[4].

Fig. 1 shows the inhibition by pregnancy urine of β-glucuronidase from rat liver, acting on phenolphthalein β-glucuronide. The urine was boiled for 15 min. after neutralizing, to destroy endogenous enzyme activity and to open existing lactone rings. It was then incubated for varying periods at 38°, after buffering with acetate to *p*H 4·5 or with phosphate to *p*H 6·1, and immediately tested as an inhibitor of β-glucuronidase in the usual 1-hr. assay. Whereas after incubation at *p*H 4·5 the urine became more inhibitory, there was no change at *p*H 6·1, where no

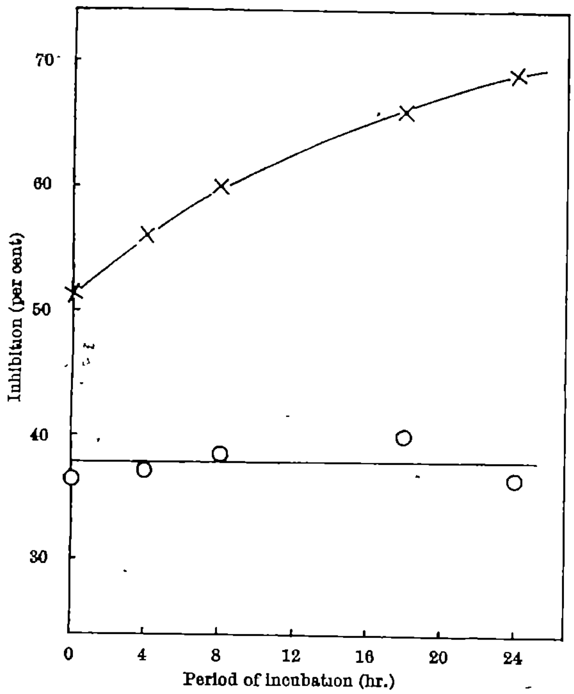

Fig. 1. Effect of incubating human pregnancy urine at pH 4·5 (×) or 6·1 (○) for varying periods, before testing at a concentration of 10 per cent as an inhibitor of rat-liver β-glucuronidase. The enzyme assay was carried out at the pH of the incubated urines with 0·63 mM phenolphthalein β-D-glucuronide as substrate

conversion occurs of saccharate to the inhibitory lactone[2].

It is therefore recommended that, if prolonged incubation with the molluscan or mammalian enzyme is necessary, acid urines should be neutralized before enzymatic digestion, to convert any existing saccharo-1 : 4-lactone into non-inhibitory saccharate, and the digestion should be carried out at as high a pH as is consistent with reasonable enzyme activity. The activity of mammalian or molluscan glucuronidase is unduly low (< 50 per cent optimal) at pH 6·1, however: bacterial β-glucuronidase preparations have optima at pH 5·5–7·0[4], but are inferior in potency to those from molluscan or mammalian sources, for example, limpet and female-rat preputial gland.

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Oxytocic Lipids in Human Amniotic Fluid

It has been known for some years that human amniotic fluid contains lipids which have an oxytocic action. Hanon *et al.*[1] reviewed their work on these substances in 1953. They showed that the active principle, which they called eutocine, would cause isolated preparations of guinea pig uterus to contract, and was distinguishable from histamine, acetylcholine, choline, and pituitary oxytocin. Eutocine could be dissolved in lipid solvents and was particularly soluble in ether. Eliminating cholesterol and phospholipids from the extracts caused no loss of activity, which seemed to be concentrated in the fatty acids and glycerides.

In 1958 Ferraris[2] confirmed the observations that an ether extract of amniotic fluid had oxytocic activity, and showed that the active substance would stimulate isolated strips of non-pregnant human uterus.

In the present work I have used mainly specimens of amniotic fluid obtained from pregnant women at or near term, when the membranes were ruptured artificially to induce labour. In a few cases samples of fluid were obtained earlier, when a pregnancy was interrupted by therapeutic or spontaneous abortion.

Extracts of amniotic fluid were tested on isolated uterine cornua from immature guinea pigs (*K* strain, 3–4 weeks old), maintained in Krebs–Henseleit solution aerated with 95 per cent oxygen/5 per cent carbon dioxide at 37° C. Emulsions or solutions of the extracts in Krebs's solution were added to the bath fluid and contractions of the uterus recorded with a writing lever applied to a kymograph. Oxytocic activity was estimated by comparing the responses with contractions of the uterus produced by known doses of ricinoleic acid, which is active in concentrations of 1–10 μgm./ml. The potency of the extracts was expressed in units, one unit being the equivalent of the oxytocic activity of 1 μgm. of ricinoleic acid.

The great majority of the active substances obtainable from amniotic fluid with lipid solvents was removed by two extractions with equal volumes of ether. In general 95 per cent or more of the ether-extractable substances passed into the ether phase at the first partition. Subsequent extractions of the aqueous phase with petroleum ether (b.p. 60°–80° C.), dichloromethane or chloroform yielded considerable quantities of lipid but little or no oxytocic substances. Further amounts of lipids possessing slight activity were obtainable by acidifying the aqueous residue and repeatedly extracting with ether.

Subsequent work was confined to preparations made by evaporating ether extracts of neutral amniotic fluid to dryness under reduced pressure, taking up the residue in ether and filtering to obtain a clear solution free from pharmacologically inactive lipoproteins.

In early pregnancy (10–20 weeks) the amniotic fluid had a mean ether-extractable lipid content of 0·13 mgm./ml. (range 0·04–0·43, 11 cases). The lipid extracts had an average oxytocic activity of 14 units/mgm. (range 1–40). No difference was detected between specimens obtained by operation and the samples from spontaneous abortions. In late pregnancy, at or near term, the ether-extractable lipid content was higher (0·64 mgm./ml , range 0·04–2·76, 112 cases) but the average oxytocic activity of the extracts (3·6 units/mgm., range 1·2–10·0) was less.

The responses of the guinea pig uterus to the active substances were not antagonized by atropine sulphate (1 μgm./ml.) or by mepyramine acid maleate (1 μgm./ml.), indicating that the preparations were not contaminated by acetylcholine or histamine. Ether extracts of amniotic fluid had an oxytocic action on isolated strips of human uterus, obtained at operation in the first half of pregnancy.

The active substances appeared to resemble the oxytocic lipids which Pickles *et al.*[3–5] have found in endometrium and menstrual flow. Similar methods were used partly to purify the oxytocic lipids from amniotic fluid.

On partition of the ether extracts between 80 per cent methanol and heptane, the methanol fraction was several times as active as the heptane fraction, though most of the lipid passed into the latter. By concentrating the active constituents in this way, preparations with activities 15–150 units/mgm. were obtained. The methanol fractions were subjected to chromatography on silica gel, eluting with a graded mixture of petroleum ether containing an increasing proportion of diethyl ether, and finally methanol. This procedure demonstrated the presence of four active substances. One of these was eluted by 25–75 per cent diethyl ether in petroleum ether and could be further purified by passage from ether into 2·6 per cent aqueous sodium bicarbonate. It appeared to consist of free acid.

While it would be speculative to propose that these oxytocic lipids play a part in parturition, it is clearly of interest that they are present in human amniotic fluid, and are capable of stimulating human uterine muscle.

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Tyrosine Metabolism in Melanoma

The urine in patients with generalized melanoma contains substances which cause its dark colour when exposed to air—urinary melanogens[1,2], of which there are two types[3]: (1) nitrogen melanogens, insoluble in ether or ethylacetate, giving a positive Thormählen test[4–6]—indole melanogens, as they are called, and (2) non-nitrogen melanogens, soluble in ether or ethylacetate, giving a negative Thormählen test—phenol melanogens.

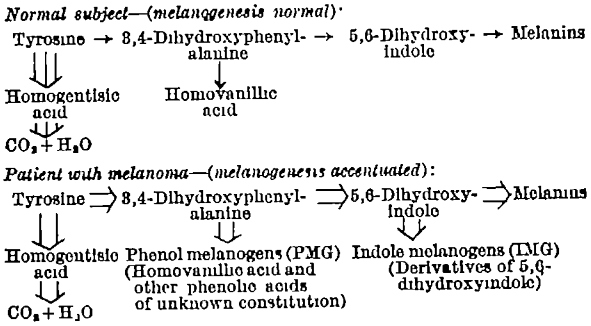
In our preliminary reports[7] we characterized phenol melanogens as a mixture of phenolic acids of an unknown constitution. We identified one of them as the homovanillic acid (HVA), the 3-methoxy-4-hydroxyphenylacetic acid[8].

A quantitative method for estimation of HVA was worked out[8] based on separation of HVA from ethylacetate extracts of urine, using paper chromatography[9]. The coloured spots obtained by detection of HVA with diazotized *p*-nitroaniline were cut out from the chromatograms, eluted and measured photocolorimetrically.

Using this method HVA excretion in the urine of patients with melanoma (metastases) was found to be 3–4 times greater than in normal people. After oral application of tyrosine, an increased urinary excretion of HVA occurred in these patients, whereas in normal health it was not changed[8].

Similar results were also obtained with indole melanogens. Concentration in the urine we estimated using a photocolorimetric modification of the Thormählen reaction according to Pechan[10]. It was found that after oral application of tyrosine an increased urinary excretion of indole melanogens occurred in melanoma patients[2,8].

Because HVA has recently been found to be a natural metabolite of endogenous 3,4-dihydroxyphenylalanine in normal people[11] and indole melanogens are derivatives of 5,6-dihydroxyindole[5,6], our findings appear to be in agreement with the well-known Raper–Mason scheme for melanogenesis:


Normal subject—(melanogenesis normal):
Tyrosine
3,4-Dihydroxyphenylalanine
5,6-Dihydroxyindole
Melanins
Homogentisic acid
Homovanillic acid
$CO_2 + H_2O$
Patient with melanoma—(melanogenesis accentuated):
Tyrosine
3,4-Dihydroxyphenylalanine
5,6-Dihydroxyindole
Melanins
Homogentisic acid
Phenol melanogens (PMG) (Homovanillic acid and other phenolic acids of unknown constitution)
Indole melanogens (IMG) (Derivatives of 5,6-dihydroxyindole)
$CO_2 + H_2O$

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Interrelation between Tryptophan and Nicotinic Acid in Human Viral Hepatitis

Continuing the chromatographic work carried out on tryptophan–nicotinic acid metabolism on urine of mice affected with experimental *MHV*-3 virus[1], we have investigated the effect of viral hepatitis on the relationship between tryptophan and nicotinic acid in man.

Samples of urine were collected over a 24-hr period from normal subjects, from twenty-four subjects affected with viral hepatitis at various stages of the disease and from nine subjects who had recovered either recently or some time before. For chromatographic analysis of the urinary metabolites involved in the tryptophan–nicotinic acid system Dalgliesh's technique[2] was used. Identification of the compounds was carried out by examination of the fluorescence by ultra-violet light (3660 Å.) and by means of the characteristic colour reactions.

Of the 24 cases still suffering from the disease, in 22 we found kynurenine, in 17, 3-hydroxykynurenine, in 10, 3-hydroxyanthranilic acid, and in 3 cases anthranilic acid. Frequently, acetyl-kynurenine (15 cases), kynurenic and xanthurenic acids appeared. It must be realized that various chromatographic tests were carried out on different days for each patient, and these tests did not always give results which were perfectly identical. Usually the chromatographic pattern presented the greatest metabolic alterations at the time when the disease reached its maximum.

From the studies carried out on 9 patients who had recovered for a few months, the chromatographic picture was almost completely normalized. (Only in 3 cases in the urine did we find any 3-hydroxykynurenine and that in small quantity.)

In man, therefore, even though a blockage of the reaction catalysed by kynureninase prevails, it is however associated sometimes with other metabolic disturbances, thus in some cases causing an accumulation of 3-hydroxyanthranilic acid in the urine.

The dissimilarity of results obtained from investigations conducted on mice and those on human beings suffering from viral hepatitis (the presence in the urine of mice inoculated with virus *MHV*-3 of kynurenine and 3-hydroxykynurenine, the presence at times in the urine of human subjects affected with viral hepatitis of 3-hydroxyanthranilic acid also—never found in the urine of mice) may be explained as follows: in our experimental conditions, the infection in mice took a particularly dramatic and acute course, so extensive and severe that a wide blockage was caused in the enzymatic activities ahead of the reaction catalysed by 3-hydroxyanthranilic oxidase.

The human beings whom we investigated were suffering from a milder form of viral hepatitis; therefore it can be assumed that the activity of kynureninase was not always completely abolished, thus resulting in the possibility of revealing other metabolic disturbances beyond this enzymatic activity.

These studies on hepatitis patients have revealed an important point. In the course of this disease, which causes direct alterations of the liver parenchyma, we did not find such great, characteristic and durable changes as those which occur in cancer of the urinary bladder[3].

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Thermodynamics of Biological Growth

According to some recent opinions, living organisms are nearer to open systems in the thermodynamic sense than to classical closed systems, although an organism plus its environment constitute a system which must be regarded as closed. While closed systems eventually reach equilibria characterized by minimum Gibbs free energy and maximum entropy, Prigogine[1] and de Groot[2] have predicted that open systems will approach steady states of minimum entropy production. On this basis, Prigogine and Wiame[3] have inferred that living organisms grow towards a state of minimum metabolism per unit mass, but this view and other discussions[4] on this theme are subject to two criticisms. On one hand, the theorem of minimum entropy production holds only for open systems very near to the steady state[5] and, on the other, it has never been tested experimentally for living organisms so that conclusions derived from it are weakened to some extent

A study of this problem has been initiated in this College to provide the experimental proof for the application of the entropy theorem to biological growth. Bacteria have been chosen as the representative organisms since these cells approximate closer to the theoretical models used in the derivation of the entropy theorem than are species containing more specialized organs within their structure. Bacterial cultures should tend towards a state of minimum entropy production per unit cell mass (σ) during their exponential phase of growth, provided the cells are well adapted to their growth medium and are growing at their maximum rate in a 'steady state'. As a first step in testing this hypothesis experimentally, the heat evolved by cultures of *Aerobacter aerogenes* growing in a synthetic glucose medium[6] has been measured in a simple microcalorimeter.

A microcalorimeter unit consisted essentially of a sterile culture tube, containing a known quantity (40 ml.) of medium, which fitted into a silvered Dewar flask with a silicone rubber bung assembly. The unit was immersed in a water bath maintained at 40·0 ± 0·01° C., with only the tops of an air-exit and thermometer pocket tubes above the water-level. Air saturated with water vapour was bubbled into the culture medium and served not only as an oxygen source, but also stirred the medium efficiently. Rises of temperature in the calorimeter were estimated from the resistance changes of a thermistor measured with a Pye Wheatstone bridge. The heat capacity of the calorimeter assembly and culture medium was determined by passing a known quantity of electrical energy through a Brightray heating coil immersed in the medium for about 2 hr. After switching off the current, a cooling curve was plotted for about 2–3 hr. from which the Newton cooling constant was calculated.

After the calorimeter had reached thermal equilibrium, an experimental run was started by inoculating the calorimeter with an arbitrarily fixed volume of suspension (*c*. 0·1 ml.). Exactly the same quantity of

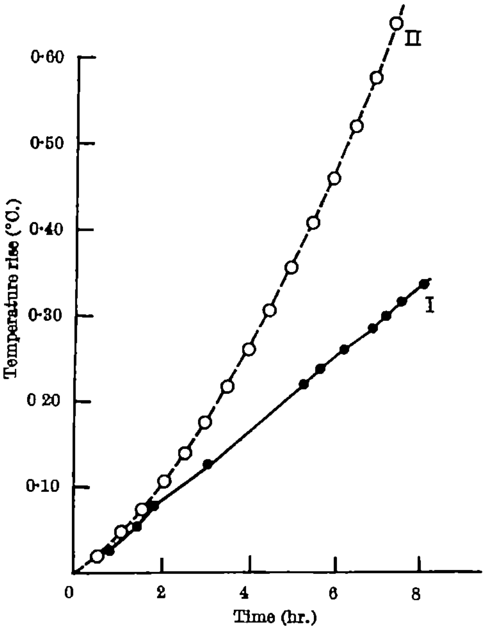

Fig. 1. Observed (I) and corrected (II) rises of temperature in the calorimeter during the aerobic growth of *Aerobacter aerogenes* in a synthetic glucose medium

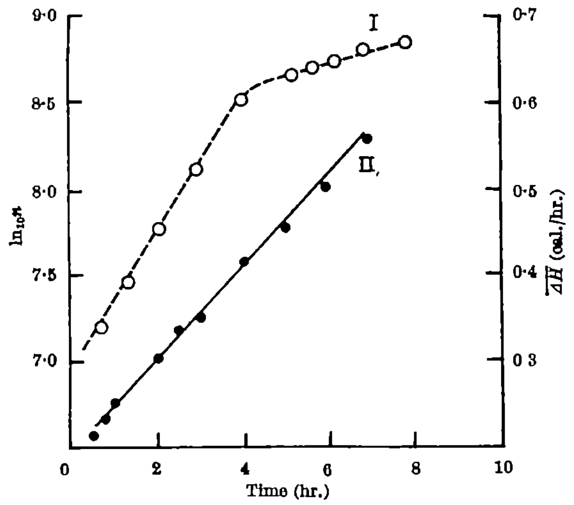

Fig. 2. Growth curve (I) and the rate of heat production, $\overline{\Delta H}$ (II). The cell population is n cells/ml.

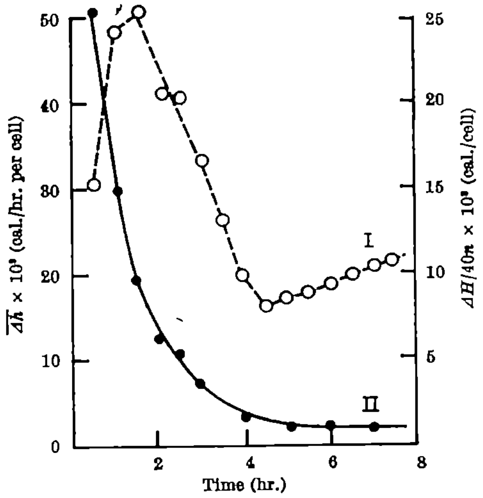

Fig. 3. The heat production per cell, $\Delta H/40n$ (I), and the rate of heat production per cell, $\overline{\Delta h}$ (II)

suspension was also inoculated into each of two ordinary culture tubes both containing the same volume of culture medium as the calorimeter: the growth of the culture in these two tubes was followed nephelometrically and their average cell count was taken to be that in the calorimeter chamber.

The total heat (ΔH) produced by the system and the rate of heat production ($\overline{\Delta H}$), after correction[7], showed no signs of tailing off after the culture had entered the stationary phase (Figs. 1 and 2). However, the variation of $\overline{\Delta H}$ per unit cell ($\overline{\Delta h}$) with time showed a different pattern (Fig. 3) : the curve quickly reached a maximum soon after the onset of the exponential phase, but then smoothly declined during growth and finally approached a minimum value asymptotically well into the stationary phase. This confirms Prigogine and Wiame's view[3] if Prigogine's suggestion[8], that the rate of Gibbs free energy change ($\overline{\Delta G}$) is zero in the steady state so that σ becomes simply related to $\overline{\Delta h}$, is conceded. On the other hand, the alternative hypothesis suggested earlier that σ approaches a minimum during the exponential phase cannot be rejected until some data for the $\overline{\Delta G}$, time relationship have been accumulated.

Further discussion of this problem and other relevant data will be published elsewhere together with the details of an improved adiabatic micro-calorimeter that by-passes the need for the large cooling corrections invoked by the conventional type described here.

I thank the Department of Scientific and Industrial Research for financial support for this project.

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Influence of Temperature on the Syntheses of Contractile Proteins in *Triturus* Embryos

The influence of temperature on the syntheses of contractile proteins, actin and myosin, in early embryos of *Triturus pyrrhogaster* Boie, was examined.

Approximately, 1,000 embryos were divided into five equal groups which were raised separately at five different temperatures (10°, 15°, 18°, 20° and 25° C.). The determinations of the first detectable trace of actin and myosin formation in embryos were carried out by a serological technique that has been outlined before[1,2], in the period of 12 hr. following fertilization. Anti-sera against G-actin and myosin were prepared by injecting intravenously into rabbits a solution of G-actin and myosin, isolated from the skeletal muscle tissue of *Triturus pyrrhogaster* by Szent-Györgyi's method[3], and were raised to their serological specificity by resorption tests with saline extracts of liver, spleen and skin tissue of *Triturus*. Titres of both sera were adjusted to 1 : 512 before the precipitin reaction with a saline extract of embryos. Constant volumes of sera and progressively decreasing amounts of antigen were used in order to avoid an excess of antigen.

It was found that actin first became detectable 84, 120, 132, 144 and 156 hr. after fertilization in

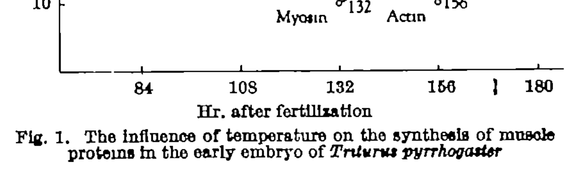

Fig. 1. The influence of temperature on the synthesis of muscle proteins in the early embryo of *Triturus pyrrhogaster*

embryos raised at 25°, 20°, 18°, 15° and 10° C. respectively. As to myosin formation, the first detectable trace was found 108 hr. after fertilization in embryos raised at 25° C., 168 hr. when raised at 20° C., 180 hr. at 18° C., then again 168 hr. at 15° C. and 132 hr. at 10° C., as shown in Fig. 1. Thus, actin formation in early embryos was markedly suppressed with dropping temperature under the above experimental conditions. On the contrary, myosin formation was most strikingly suppressed at 18° C. and from thereon was clearly promoted independently of falling or rising temperature.

This finding is unexpected, but the result is noteworthy, because the temperature of the natural breeding season of *Triturus* is about 18° C. We may imagine that many metabolic pass-ways are in competition for the materials that are required to keep a balance necessary for the growth of the embryo. For example, there may be a metabolite present which at the optimum temperature for growth appropriates the materials necessary for myosin formation.

The order of actin and myosin formation in embryos raised at 10° C. is opposite to that found in natural development. This result furnishes further evidence of the independence of actin and myosin formation one from the other[3,4].

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PHYSIOLOGY

Reconstitution of Cell Membrane Structure *in vitro* and Its Transformation into an Excitable System

THE formation of single, stable bimolecular lipid and proteolipid[1] membranes up to 10 mm.² in area has been accomplished routinely in 0·1 *M* saline solution by methods analogous to the formation of Hooke-Newton 'secondary black' in air soap films[2-4]. By forming such a membrane between two compartments filled with saline its transverse electrical properties can be measured, and controlled chemical investigations can be undertaken.

This experimental structure, invisible in transmitted light, appears as a faint grey sheen in reflected light at a large angle of incidence. It is grossly manipulable, resilient, self-sealing to puncture, liquid in the plane of the bilayer, stains with osmium tetroxide and is 60–90 Å. thick under the electron microscope. Electrical capacity is about 1μ F./cm.². Resistance is usually 10^7 but often greater than 10^8 ohm cm.² and is constant up to dieletric breakdown at 0·15–0·20 V. (2·5 × 10^5 V./cm.), whereupon the membrane shatters. It also breaks below *p*H 5·0 and above *p*H 9·0. In ionic gradients the unmodified membrane shows weak electrical polarization and poor ionic selectivity.

However, this inert structural barrier has been successfully modified by a variety of water-soluble macromolecules which spontaneously adsorb to it when added to the environmental solution. Some proteins adsorb but do not lower its resistance. Others adsorb and steadily lower the resistance to 10^3 ohm cm.² or less, probably by penetration and/or formation of channels.

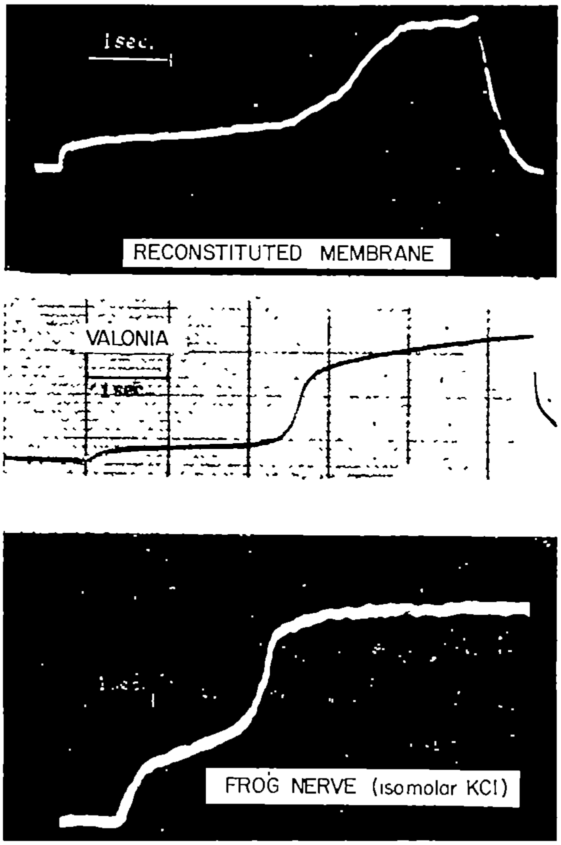

Fig. 1. Potential recordings of responses to applied rectangular currents. The membrane electromotance is very likely zero or close to zero in all three cases. Upper two are trans-membrane recordings, bottom record is proportional to trans-membrane potential of frog single node in 0·1 *N* potassium chloride (Middle figure reprinted by permission from Blinks, L. R., *J Gen. Physiol.*, **19**, 633; 1936)

One, as yet unidentified, heat-stable molecule obtained from bacterially de-sugared egg whites which can be precipitated by ammonium sulphate (50–100 per cent sat.), denatured by chloroform and has an estimated mol. wt. of 10^4–10^6 adsorbs, lowers the resistance and, in addition, regularly induces the following gating reaction to d.c. electrical stimulation. At a sharp and generally unidirectional threshold voltage, which can be varied by [Ca^{++}] between 15 and 50 mV., the resistance shifts 5-fold to a new steady value after a latency which changes inversely with applied potential (Fig. 1). Recovery is prompt, the phenomenon repeatable and indistinguishable in detail from the behaviour of the excitable alga, *Valonia*, in the 'variable resistance' state[5] and is similar to the resistance increase of the skin 'action potential'[6] and to the frog nerve 'action potential' in isotonic potassium chloride[7]. The above resistance-voltage kinetics, including the threshold, can be predicted quantitatively from the general theory of reaction-rates under the assumptions that the adsorbed molecules form a finite number of two state resistive channels (that is, gates) and that the number in each state is determined by the Δ F. between the two states and the applied potential.

Because these theoretical, mechanical, electron microscopic and electrical properties are like those attributed to the cell membrane and because this experimental membrane is made from solubilized lipids extracted from membrane structures of white matter, we suggest that it is reconstituted cell membrane structure. Evidently it can exist in stable form in the absence of all other cell components and, in addition, can show the cellular function of electrical excitability, and perhaps other cell functions as well, when the composition of the lipid phase and the nature of the adsorbed molecules are properly selected.

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Some Extremely High Acuities of Taste for Phenylthiocarbamide

IN human population groups a primary dichotomy (so-called 'non-taster' and 'taster' classes with some overlap) in the distribution of thresholds of taste for phenylthiocarbamide (PTC) was established by the early work of Hartmann[1] and others. Falconer[2] noted the irregular nature of the distributions of 'non-taster' material; and Lugg and Whyte[3], from the results (corrected for the effects of age and sex) of their investigations of southern Chinese, European, southern Indian and Malay ethnic groups, considered these distributions to be potentially (if not always actually) bimodal, a view which was supported by subsequent work[4] on Negrito and Senoi subjects. They[3] also questioned the presumption that 'taster' material is always unimodally distributed; but it was realized[4] that the distribution irregularities might be due to sampling errors rather than to intrinsic causes. The question remained open, however, and it was hoped that clarification of the overall picture of threshold distributions might come with the accumulation of additional data from work on other ethnic groups.

Results of work recently done in Hokkaido on Japanese and Ainu ethnic groups and on persons of mixed origin support the view that 'taster' material is, potentially, not unimodally distributed. There is appreciable evidence that, apart from the earlier suggested category of 'high acuity tasters', a category of 'extremely high acuity tasters' exists in these, if not other, groups.

The PTC solutions used in these investigations comprise a series integrally numbered from zero, the concentration of solution numbered n being $2{\cdot}60 \times 2^{-n}$ gm. PTC/l. A subject demonstrates his ability to discriminate between solution n and solvent (distilled water) in a sorting trial, the chance of success by guesswork being 0·125, and his most likely threshold value[3] is $n + 0{\cdot}5$. The taste-quality he reports is not necessarily the characteristic quality, bitter, experienced with more concentrated solutions.

It was among the Kintak Bong Negritos of Malaya that the tasters of previously highest acuity were encountered[4], one subject succeeding with solution 18. His reaction in the trial was such as to suggest that he may have succeeded with at least solution 19 if it had been possible then to test him further. In the Hokkaido work it has been found necessary to extend the range to solution 24 in order to carry out the survey effectively. Of fifty 'pure' or almost 'pure' Ainu and of fifty Japanese subjects, seven in each sample succeeded with solution 18 or higher number; and of an extremely heterogeneous sample of fifty persons of mixed extraction containing many children in the 10–14-year age-range, a factor predisposing to more successes with solutions of higher number, twelve successes were recorded. In all, three subjects succeeded with solution 19, eight with 20, four with 21, three with 22 (one of them describing the taste as bitter and the other two as astringent), and one, a 14-year-old Ainu boy who described the taste as sweet, with 23.

Solutions 18 and 23 contain 9·9 and 0·31 μgm. PTC/l. and are $6{\cdot}6 \times 10^{-8}$ and $2{\cdot}1 \times 10^{-9}$ molar, respectively. The subject takes 2·5 ml. of solution (or solvent) into the mouth in each step of a sorting trial, representing $2{\cdot}5 \times 10^{-8}$ gm. or $1{\cdot}6 \times 10^{-10}$ mole or some 10^{14} molecules of PTC with solution 18, and 8×10^{-10} gm. or 5×10^{-12} mole or 3×10^{12} molecules with solution 23. If Moncrieff's[6] estimate is accepted, namely, that 0·05 ml. of solution, properly applied, is sufficient for taste-testing, the number of molecules of PTC involved would be 2×10^{12} with solution 18 (not 8×10^{9} stated earlier[5] as the result of a miscalculation) and 6×10^{10} with solution 23.

The Negrito subject's success with solution 18 ranked him as the most acute taster of any substance among human subjects examined up to that time[5]. Yet, of the 150 persons examined recently in Hokkaido some 17 per cent rank either in his company or (the 14-year-old Ainu boy *in excelsis*) considerably above him. Tasting acuities of this higher order lie far beyond any previously recorded and were utterly unexpected when the work was begun.

Proper acknowledgement of help received will be made elsewhere, when the full account of this work and discussion of its implications in anthropological studies are published. Mention can here be made only of the many forms of assistance rendered by Prof. S. Watanabe, but for whose prior interest the Hokkaido programme could not have been undertaken.

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Hæmophilia and the Protease Inhibitor in Peanuts

Boudreaux and Frampton's report[1] on a hæmostatic factor in peanuts (*Arachis hypogaea*), orally active in hæmophilic patients, aroused great interest. Since these and other investigators have been unable to demonstrate restoration of blood coagulation in the treated patients, other mechanisms of action were suggested. Boudreaux and Frampton[1] considered vasoconstriction as a possible mechanism and they later found vasoconstrictive agents in ethanol extracts[2]. Verstraete *et al.*[3] extracted with ethanol from the skins of peanuts hæmostatically active anthocyanidines and flavones.

Peanuts contain a protease inhibitor[4], which might delay fibrinolysis. Retardation of fibrinolysis could then counteract the delayed formation of fibrin in hæmophilia, producing a hæmostatic effect[5]. This concept is supported by the findings that consumption of peanuts produced a decrease in spontaneous fibrinolytic activity in normal persons[5] and in patients with classical hæmophilia (Factor VIII deficiency)[6]. Increase in blood of an inhibitor against fibrinolysis has also been reported[7].

Thus patients deficient in Factor IX (*PTC*, Christmas factor) should also respond to treatment with peanuts. In our preliminary work[8] a group of 4 patients with Factor IX deficiency responded to flour prepared from raw peanuts similarly to a group of 6 patients with Factor VIII deficiency. Similar observations were made by Verstraete *et al.*[3]. Furthermore, Boudreaux, who first noticed the effect on himself, has since been found to be deficient in Factor IX and not in Factor VIII as originally reported (personal communication).

If the hæmostatic effect of peanuts is mediated through the fibrinolytic system little effect could be expected in patients with serious relapse in their hæmorrhagic condition, and only milder episodes could be improved. This is in agreement with observations made by several investigators, and could explain the variable and erratic clinical results. We have seen confirmation of this concept in our own work[8], and Moers and den Ottolander[9] reported especially on the ineffectiveness of peanuts in grave hæmorrhagic cases.

Large quantities of roasted peanuts are required. Boudreaux[1] recommended 180 gm. of the flour a day and the same amount was used by Verstraete[3]. The powder prepared by us from unheated peanuts with a cold extraction process had a high anti-protease activity and was used in a correspondingly smaller dose (8 gm. a day). Other authors using raw, unroasted peanuts[6,9] administered 20–50 gm. a day. For use in future studies we have made a concentrated preparation of the protease inhibitor as follows:

Cold prepared flour (200 gm.) from hexane extracted raw peanuts (supplied by Mecodumex Ltd., Copenhagen) was stirred for 1 hr. with 1 l. water and 2 gm. calcium hydroxide. After centrifugation calcium was removed with carbon dioxide and the final supernatant (800 ml.) was precipitated with 3 vol. acetone. The precipitate was isolated, resuspended in 200 ml. water, and made slightly acid (litmus paper) with citric acid (12 ml. of a 10 per cent solution was required). The suspension was precipitated with acetone followed by acetone and ether until dry. 5·7 gm. of a whitish powder was obtained, which contained nearly all the inhibitor activity of the original flour (assayed by the fibrin plate method against a plasminogen activator prepared from pig heart tissue).

This inhibitor preparation was tried (300 mgm. a day) in one of our patients (Factor VIII deficiency) with results similar to those obtained with the crude flour. The purified preparation was tested by Dr. Boudreaux for vasoconstrictor activity with negative results (personal communication). In samples of ethanol extract supplied by Dr. Boudreaux we found little or no protease inhibitor.

With the purified product available for trial on a large number of patients it should be possible to demonstrate first if a hæmostatic factor is present in the ethanol fraction or remains in the flour, and secondly whether our concept of a mediation of the effect through the fibrinolytic system, causing a change in the dynamic hæmostatic balance, is valid and can explain the limitations of this treatment.

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Acetylcholine Sensitivity of Rat Diaphragm maintained in Organ Culture

MUSCLE fibres in the diaphragm of adult rats are sensitive to externally applied acetylcholine only in the region of the nerve muscle junction and its immediate surroundings. If the motor-nerve is cut, the area of muscle membrane sensitive to acetylcholine increases until it involves the whole fibre surface[1].

Until now the development of generalized chemosensitivity has been examined only after the denervated muscle has remained *in situ* for the required time (about 1 week). Therefore, it was desirable to find out if a similar process of sensitization would occur in muscles kept *in vitro*, and for this purpose the organ culture technique described by Trowell[2] was used.

Strips of muscle, about 5 mm. wide, were cut from the diaphragm of rats. The muscle fibres and attached ribs were then cultured in the chemically defined medium *T*8 (ref. 2) at 36–37° C., with a gas phase of 5 per cent carbon dioxide in oxygen. After 5–6 days the muscles were taken out of the culture chamber and their response to acetylcholine was examined. Acetylcholine was applied to single muscle fibres by micro-ionophoresis through a fine pipette (less than 1μ at its tip), and it was found that the entire surface of many muscle fibres had become sensitive to acetylcholine. Potentials produced by acetylcholine applied to a muscle fibre at a region normally insensitive to the drug are shown in Fig. 1.

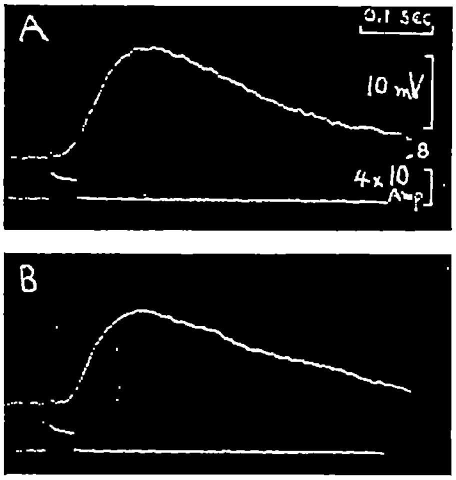

Fig. 1. Potential changes produced by micro-ionophoretic application of acetylcholine to a fibre, in a muscle cultured for 5 days at 37° C. Upper traces record intracellular potential while lower traces monitor the current which flows through the acetylcholine pipette and releases the drug. Acetylcholine was applied at a point about 1 mm. from the end of the muscle fibre in *A* and 1·5 mm. in *B*. Temperature, 14° C.

It was further observed that cholinesterase, which is normally concentrated at the site of the neuromuscular junction[3], is still active and localized in the same region of the muscle fibres after 5–6 days *in vitro*.

The main conclusions derived from these experiments are: (*a*) that adult skeletal muscle fibres can be kept alive in organ culture; (*b*) that the development of generalized chemo-sensitivity in muscle does not require the participation of other organs in the body. The muscle fibres themselves (and the synthetic medium) provide all that is required to produce the changes in the muscle fibre membrane which make it react to drugs.

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HÆMATOLOGY

Significance of High γ-Globulin Content in Burmese Serum

THE γ-globulin content in serum of Indians[1,2], Africans[3] and Burmese[4] was found to be significantly higher than that reported for Europeans[5,6] and Americans[7].

An explanation for this finding remains largely a matter of speculation. It has been thought that people with high γ-globulin are those with greater immunity consequent on repeated exposure to infections[8]. However, total quantity of γ-globulin cannot be used as a criterion of immunity. The total quantity of circulating antibody to any particular antigen was so small that its presence cannot be detected electrophoretically, and even after repeated inoculations, which result in hyperimmunity, there was no correlative changes in the electrophoretic pattern[8].

An elevated serum γ-globulin is one of the most common abnormalities in liver diseases[9]. It has also been reported that there are non-contagious diseases peculiar to the tropics and sub-tropics the aetiology of which is unknown or uncertain and which are responsible for much ill-health and high mortality in the communities concerned. It was suggested that in these cases liver damage is involved resulting from a toxic factor derived from food or intestine helminths[10].

These observations led to the present work to elucidate if high γ-globulin value in Burmese serum involves liver diseases. A mixed sex group of 103 normal Burmese, ages ranging from 17 to 47 years, who gave no history of liver impairment were examined. Total serum protein was estimated by the biuret method, as described by Reinhold[11]. Freeze dried serum (batch number 9) of Glaxo Laboratories, Ltd., was used as the standard. Serum protein fractionation was done electrophoretically with barbitone buffer *p*H 8·6, 3 *MM* Whatman paper and set for 18 hr. Excess bromophenol blue dye was washed with 'Dioxan' and ether. After scanning, areas under graph were measured for relative serum protein contents. Daily amino-acid nitrogen excretion in urine was determined by the formol titration method[12]. Ammonia nitrogen was determined to get the true amino-acid nitrogen.

It was found that Burmese serum protein measured 6·7 ± 0·028 gm. per cent (mean ± *S.E.*) and urinary amino-acid nitrogen daily excretion was found to be 0·5 ± 0·033 gm. (mean ± *S.E.*). Electrophoretic analysis showed serum protein values as given in Table 1.

Table 1. RELATIVE PERCENTAGE OF PROTEIN VALUES (MEAN ± *S E*)

Albumin	43 59 ± 1·61
α-1-Globulin	6·46 ± 0·94
α-2-Globulin	12·73 ± 0 59
β-Globulin	12·28 ± 0 87
γ-Globulin	24·94 ± 0·86

Results show low albumin value. Values of α-1, α-2 and γ-globulin are high. It was found that one of the fairly constant effects of parenchymatous hepatic disease is reduction in the serum albumin[13]. High amino-acid nitrogen excretion was also found in

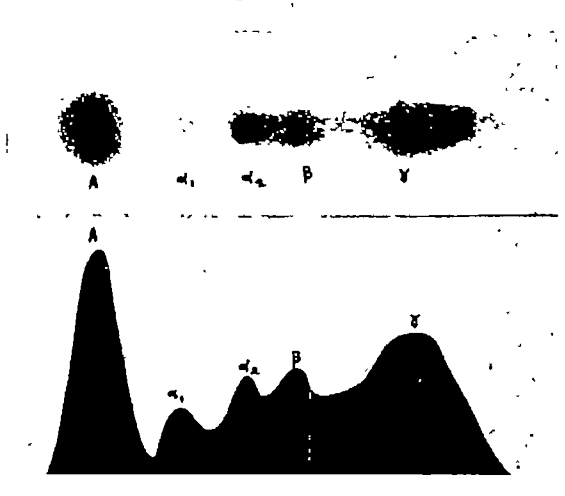

Fig. 1. Electrophoretic pattern in Burmese serum. *A*, Albumin; α_1, α-1-globulin; α_2, α-2-globulin; *β*, β-globulin; *γ*, γ-globulin

this series. Further work is in progress and details will be published elsewhere.

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PHARMACOLOGY

Effect of Monoamine Oxidase Inhibitors on the Behaviour of Rats in Hall's Open Field

Although numerous methods are available for assessing the potency of monoamine oxidase inhibitors *in vivo*, these almost invariably rely either on the direct measurement of tissue levels of catecholamine, serotonin or some metabolite thereof[1], or on the ability of the inhibitor to potentiate or nullify the effects of some other drug such as 5-hydroxytryptophan, tryptamine or reserpine[2]. Direct behavioural effects of monoamine oxidase inhibitors at low doses are usually slight or detectable only by use of conditioned response techniques[3].

In a series of aralkylhydrazines we have noticed a correlation between the monoamide oxidase inhibitory powers of these compounds and their effectiveness in producing changes in the behaviour of normal untrained rats in Hall's open field[4]. Individual rats were placed for a period of 3 min. in an open drum 6 ft. in diam. with 2 ft. high walls. The floor of the drum was divided into foot squares. The number of times the rat preened, reared and defæcated were counted as well as the number of fæcal boluses passed and the number of squares traversed.

The results are shown in Table 1.

Inhibition by monoamine oxidase *in vitro* was measured by incubating various concentrations of the inhibitor with guinea pig liver mitochondria for 15 min. at pH 7·4 and 25°. Tyramine was then added and the residual monoamine oxidase was assayed by the dinitrophenylhydrazine method[5]. The concentration giving 50 per cent inhibition (I_{50}) was determined by interpolation. In the experiments in column (*a*) semi-carbazide, which is required in the assay, was present together with the enzyme and inhibitor; in those in column (*b*) semicarbazide was added with the tyramine. Although there are some quantitative differences in the results obtained by these two different procedures, the general order of activity was not greatly affected.

The doses of drug producing behavioural changes are of the same order as those required to counteract the sedative effects of reserpine. The effects observed with the aralkylhydrazines persisted at least 24 hr., consistent with the very slow recovery of the inhibited enzyme[6]. The effects with harmine were much shorter (< 3 hr.), harmine inhibition of monoamine oxidase being readily reversible[7].

The relationship of the behavioural changes to the anti-depressant action of monoamine oxidase inhibitors is not clear, particularly as different aspects of behaviour were affected at the minimal effective dose with different compounds. It is, however, interesting to compare these results with those obtained with tranquillizers such as chlorpromazine where all aspects of behaviour studied in the open field were depressed. This effect has also been reported by Ryall[8].

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Table 1

Compound*	Approx. minimal effective dose (mgm./kgm.)† producing significant change from control behaviour (Groups of 8 rats)	Type of behavioural change at minimal effective dose	I_{50} (µmoles/l.) for monoamine oxidase inhibition *in vitro* (*a*)	(*b*)
p-Chlorobenzylhydrazine	2	Decrease in emotional defæcation	0·07	0·25
Benzylhydrazine	2	Decrease in emotional defæcation	0·2	0·4
3,4-Dichlorobenzylhydrazine	2	Decrease in no. of squares traversed and no. of times rearing	0·1	1·0
o-Chlorobenzylhydrazine	5	Decrease in emotional defæcation	0·25	0·2
3-Picolylhydrazine	10	Decrease in no. of squares traversed	1·3	1·3
4-Picolylhydrazine	>10	—	2·5	2·0
3,4-Dimethoxybenzylhydrazine	>10	—	2·5	1·2
α-Naphthylmethylhydrazine	>10	—	4·0	4·0
O-Benzylhydroxylamine	>20	—	>1,000	>1,000
Harmine	4	Decrease in no. of squares traversed	Not tested	0·5

* Hydrazines used as hydrochloride salts
† Drug given subcutaneously 3 hr. before test (except for harmine when it was 1½ hr.)

Variations in Inorganic Pyrophosphatase and Glutaminase in the Liver and Brain of Mice poisoned by Carbon Tetrachloride

J. Hagen[1] has shown that carbon tetrachloride poisoning in factory workers induces extensive biochemical and metabolic changes in the central nervous system, liver and kidneys. On investigating the action of carbon tetrachloride in mice, Frunder *et al.*[1-5] found significant changes in the metabolism of carbohydrates, proteins and lipids in the liver parenchyma.

We have examined the effect of carbon tetrachloride (oil solution) injected into mice[3] on liver glutaminase and pyrophosphatase, 2 hr. as well as 48 hr. after the injection, when the regenerating process is already in progress[4,5].

In Table 1 it is shown that 24 hr. after the injection, glutaminase is partially inactivated in the brain as well as in the liver. After 48 hr. there is a tendency towards recovery. The variations in pyrophosphatase are recorded in Table 2; it may be seen that, in the liver, the activity of this enzyme decreases 2 hr. following the injection, and continues to fall even after 48 hr. In the brain this enzyme decreases after 2 hr. but tends towards recovery after 48 hr. Glucose administration partially protects this enzyme.

Table 1. LIVER GLUTAMINASE WAS DETERMINED IN BUFFER SOLUTION AT pH 7·4 (REFS. 9 AND 10), BRAIN GLUTAMINASE WAS DETERMINED IN PHOSPHATE BUFFER SOLUTIONS AT pH 8·5 (REFS. 10 AND 11)

	M.mol. deaminated glutamin/gm. tissue	
	Liver	Brain
Normal controls	0·402	1·660
2 hr. after injection of carbon tetrachloride	0·280	0·860
48 hr. after injection of carbon tetrachloride	0·307	1·056

Table 2. VARIATIONS IN INORGANIC PYROPHOSPHATASE DUE TO ACTION OF CARBON TETRACHLORIDE

Enzymatic activity determined for pyrophosphatase in the liver (according to Rafter, ref. 7), and brain (according to Seal and Binkley, ref. 8), in total homogenates. Determinations were carried out by measuring the phosphorus using the Firske–Suborov method, using methol-methobisulphate as a reducer

	Liver		Brain	
	− Glucose	+ Glucose	− Glucose	+ Glucose
Normal controls	8·12	8·20	12·80	12·75
2 hr. after injection of carbon tetrachloride	5·40	6·81	8·90	10·20
48 hr. after injection of carbon tetrachloride	3·35	4·06	9 60	11·78

Our investigations confirm Frunder's concept[4,5] according to which carbon tetrachloride selectively attacks the cellular mitochondrial apparatus, both enzymes being sited in the mitochondria. It is believed that carbon tetrachloride alters especially —SH enzymes, both enzymes having also —SH groups. Reactivation by glucose apparently accounts for these effects as was shown by Rapoport and Schneider[6] for erythrocite pyrophosphatase.

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Structure-action Relationships among Drugs acting on Connective Tissues (Anti-rheumatic Agents)

CONVENTIONAL assays for anti-inflammatory (anti-rheumatic) drugs frequently measure the inhibition by a drug of granuloma formation, induced by subcutaneously implanting foreign bodies (cotton pellets, plastic sponges, agar gels, etc.) in small laboratory animals. Other *in vivo* assays for anti-inflammatory activity, for example, inhibition of exudate formation by formalin-blisters, indicate the relationship between the activity of a compound in suppressing tissue inflammation and as an inhibitor of *de novo* connective tissue formation. This latter property is readily assayed *in vitro* by measuring the action of a drug on induced biogenesis of cartilage in tissue cultures[1] or on the biosynthesis of mucopolysaccharides in connective tissue slices[2]. In both these *in vitro* systems, anti-inflammatory corticosteroids and salicylate strongly inhibit the incorporation of inorganic sulphate-^{35}S ($^{35}S_i$) into mucopolysaccharides, thus reproducing the known effect of these drugs on $^{35}S_i$ incorporation into connective tissue polysaccharides *in vivo*. Using the techniques described[2], it has now been found that these particular drugs and other anti-inflammatory drugs such as phenylbutazone, chloroquine, their respective active congeners (oxyphenbutazone, sulphinpyrazone, hydroxychloroquine, etc.) and cinchophene, each inhibit the incorporation of glucose-U-^{14}C, acetate-1-^{14}C and $^{35}S_i$ into cartilage mucopolysaccharides and of $^{35}S_i$ into corneal mucopolysaccharides *in vitro* (Table 1). These drugs were active inhibitors at concentrations in the incubation medium which corresponded to the plasma levels required for therapeutic action (for example, salicylate, phenylbutazone). Sodium aurothiomalate had no significant effect, but thiomalic acid (10^{-3} *M*) and many other thiols (thiosalicylic acid, cysteamine) were potent inhibitors of polysaccharide sulphation.

Table 1. EFFECT OF SOME DRUGS ON THE BIOSYNTHESIS OF MUCOPOLYSACCHARIDES *in vitro*

Drug	Concentration $\times 10^{-3}$ *M*	Cartilage from glucose-^{14}C (per cent)	PS-^{14}C from acetate-^{14}C (per cent)	PS-^{35}S Cartilage (per cent)	PS-^{35}S Cornea (per cent)
None		100	100	100	100
Salicylate	4	45	65	40	20
Salicaldehyde	1	21	42	28	5
p-Hydroxybenzaldehyde	1	84	107	95	95
Phenylbutazone	0·5	38	25	28	20
Di-*p*-carboxyphenylbutazone	1·2	100	100	100	100
Chloroquine diphosphate	1	32	59	26	30
Cinchophene	1	33	57	33	28
Hydrocortisone	0 25	35	39	54	62

Mucopolysaccharides (PS) were isolated from slices of young cattle costal (or tracheal) cartilage and corneas, incubated with either glucose-4-^{14}C, acetate-1-^{14}C or $Na_2{}^{35}SO_4$: ^{14}C and ^{35}S content of PS in drug-incubated tissue slices is expressed as percentage of PS-^{14}C or PS-^{35}S in tissues incubated without drugs.

Many chemical analogues of these drugs were tested, usually at 10^{-3} *M*, using the inhibition of $^{35}S_i$ incorporation into cartilage and corneal polysaccharides, as an index of activity. The potency could be correlated with at least two physical properties: (1) ability to form complexes with metal ions; (2) lipophilic character, favouring partition into lipid rather than aqueous phases.

Among salicylate analogues, potency declined in the series; *O*-cresotate, salicylate, 2,3-dihydroxy-benzoate, 2,4-, 2,5- and other dihydroxybenzoates = 4-aminosalicylate (PAS) = 5-sulphosalicylate (all inactive) and in the parallel series: ortho-vanillin, salicaldehyde, 2,4- and 2,5-dihydroxybenzaldehydes, 2,4,6-trihydroxybenzaldehyde, 2,4- and 2,5-dihydroxybenzaldehydes, 2,4,6-trihydroxybenzaldehyde, pyridoxal, pyridoxal-5-phosphate. Potency increased in the series: salicylamide, salicylate, salicaldoxime, salicylanilide, 3,5-dihydroxy-naphth-2-oate, salicaldehyde, *o*-monohydroxynaphthoates, *o*-monohydroxynaphthaldehydes — the latter were strong inhibitors at 2×10^{-5} *M* and approximately 100 times as potent as salicylate. Saligenin, pyridoxine, *o*-methoxybenzoate, *o*-hydroxyacetophenone, methyl salicylate, *m*- and *p*-hydroxybenzaldehydes, 2,4-dimethoxybenzaldehyde, *m*- and *p*-hydroxybenzoates and other hydroxynaphthoates were almost devoid of *in vitro* activity and formed only weak, or no, complexes with ferric ions in ethanol.

Phenylbutazone (butazolidine) exists at physiological *p*H predominantly as the enol form (about 90 per cent, calculated from the optical density at 264 mμ) and will form complexes with metal ions in ethanol. The methyl enol-ether will not and is inactive as a drug *in vitro* and *in vivo*[3]. Among phenylbutazone (PB) analogues, potency decreased with increasing water solubility in the series: PB, 4-*n*-propyl analogue, oxy-PB, mono-*p*-carboxy(phenyl)-PB, di-*p*-carboxy(phenyl)-PB. This last compound is inactive *in vitro*, only feebly active *in vivo*[4]. Some pyrazol-5-ones exhibited activity *in vitro*, which diminished in the series: 1,3-diphenyl-pyrazol-5-one, picrolonic acid, 1-phenyl-3-methyl-pyrazol-5-one, 1-phenyl-2,3-dimethyl-pyrazol-5-one (antipyrine, and inactive). These pyrazolones formed highly coloured complexes with ferric chloride in ethanol. With the possible exception of antipyrine, they can exist as enols in aqueous solution.

Among analogues of cinchophene (2-phenyl-quinoline-4-carboxylic acid), activity decreased in the series: cinchophene, 2-hydroxyquinoline-4-carboxylic acid, isonicotinic acid (inactive). Hydroxychloroquine (plaquenil) was less active than chloroquine (resochin), in turn less potent than mepacrine (quinacrine, atebrin) and santoquin (3-methylchloroquine). These antimalarials only inhibited polysaccharide biosynthesis after a measurable time lag (1–2 hr.), reminiscent of their delayed therapeutic action *in vivo*[5]. Their drug action was not reversible. The action of all other drugs tested was reversed by washing the drug-incubated tissues and transferring them to a drug-free medium.

8-Hydroxyquinoline (oxine) was a potent inhibitor *in vitro* and has been shown to exhibit anti-inflammatory properties *in vivo*[6]. 2-hydroxyquinoline was inactive *in vitro*. Oxine, salicylic acid, salicaldehyde[7] and a number of anti-inflammatory corticosteroids[6] will chelate cuprous and ferrous ions in non-aqueous media. These collected observations and the inhibitory activity of lipophilic thiols able to form chelates (but not cysteine and glutathione) suggest that ability to interact with metal ions in (or across) a lipid phase may be the chief determinant in conferring anti-inflammatory properties on a compound, rather than ability to uncouple oxidative phosphorylation *per se*[8,9]. Extension of this hypothesis to the corticosteroids may explain the greater anti-inflammatory activity of methyl derivatives (dexamethasone, medrol, paramethasone)[10,11] and enol ethers[12] than the parent steroids, and the striking increase in anti-inflammatory activity of steroid 16α-, 17α-diols upon transformation to the corresponding acetonides[11,13].

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OPHTHALMOLOGY

Anti-viral Activities of Several Iodinated Pyrimidine Deoxyribonucleosides

KAUFMAN[1] has reported that 5-iodo-2′-deoxyuridine (IUDR or IDU), the deoxyribonucleoside of 5-iodouracil, is effective in controlling herpes simplex keratitis in rabbit and human eyes. IUDR[2], 5-iodo-2′-deoxycytidine (ICDR), that is, the deoxyribonucleoside of 5-iodocytosine[3], as well as their 3′,5′-diacetyl derivatives, were synthesized with the view of examination of them as potential chemotherapeutic agents in cancer. Both IUDR and ICDR have been shown to inhibit the biosynthesis of deoxyribonucleic acid (DNA) and, in addition, to be incorporated as IUDR 5′-phosphate into DNA, in place of thymidine 5′-phosphate[4-6]. This communication confirms the efficacy of IUDR in the treatment of an infection of rabbit eyes with a DNA-virus, namely, herpes simplex, and describes a similar anti-viral activity for ICDR and 3′,5′-diacetyl-5-iodo-2′-deoxyuridine (Ac$_2$IUDR); in addition, a preliminary test of 3′,5′-diacetyl-5-iodo-2′-deoxycytidine (Ac$_2$ICDR) has indicated that this derivative is also active.

Both eyes of 14 and 17 rabbits in the first and second experiment, respectively (Table 1), were infected with the *MP* strain of herpes simplex after scratching the cornea; in all cases, marked lesions developed within 3 days. In the first experiment, the left eyes were treated with the test compounds dissolved in Hanks's balanced salt solution supplemented with penicillin (500 u./ml.) and streptomycin (100 μgm./ml.), while the right eyes were treated with only the antibiotic agents in the saline solution. Two drops of the solution were placed into each conjunctival sac at hourly intervals for 10 hr. and at intervals of 2 hr. for 14 hr.; this regimen of dosage was repeated during a second 24-hr. period. The animals were divided into 2 groups (Table 1).

After treatment for 48 hr., all the right eyes, to which antibiotic agents only were administered, showed severe corneal lesions, while the left eyes showed marked regression of the lesions from the pre-

Table 1. Comparison of Various Iodinated Pyrimidine Nucleosides in the Treatment of Herpes Simplex Keratitis

Exp. No.	Compound administered	No. of eyes treated	Concentration of compound mgm./ml.	Relative molar concentrations	Clinical effectiveness
1	IUDR	7	1·24	1·0	+ + + +
	Ac_2IUDR	7	1·7	1·1	+ + + +
	None	14	—	—	0
2	IUDR	6	1·0	1·0	+ + + +
	IUDR	6	4·0	4·0	+ + + +
	ICDR	6	1·0	1·0	+ + + +
	ICDR	6	4·0	4·0	+ + + +
	IUR	5	4·0	4·0	0
	None	6	—	—	0

treatment condition. The right eyes had large areas of epithelial ulceration with circumcorneal congestion and in many cases an anterior uveal reaction. Ulceration was minimal or absent in the left eyes and none of these eyes showed anterior uveal reaction. In the concentrations used, IUDR and its diacetyl derivative were both effective in controlling infection.

In the second experiment, a comparison was made of IUDR and ICDR, each at two equivalent concentrations (Table 1). In addition, it was shown that 5-iodo-uridine (IUR), the ribonucleoside of 5-iodouracil, a compound which undergoes metabolic degradation in a manner similar to that of IUDR, but exerts no inhibitory effect on the biosynthesis of DNA, is without anti-viral activity. The compounds were dissolved in the manner described above and 2 drops of the appropriate solution were placed into each conjunctival sac at intervals of 1 min. for 5 min. and thereafter at intervals of 0·5 hr., but for only 8 hr.; this regimen was repeated during the subsequent 2 days. All the control eyes, as well as those treated with IUR, developed severe corneal lesions, whereas in those eyes which received IUDR or ICDR in each of the concentrations used, the lesions regressed strikingly, in a manner similar to that observed in the first experiment.

This more intensive but abbreviated regimen of treatment appears to be as effective as that used in the first experiment and no evidence of corneal irritation was observed. In the concentrations administered, IUDR and ICDR appeared to be equally efficacious.

In a preliminary experiment, in which the anti-viral activity of ICDR at 5·1 mgm./ml. was shown, its diacetyl derivative also was active. Further experiments with these four compounds will be required in order to determine whether there are significant qualitative or quantitative differences between these iodinated pyrimidine deoxyribonucleosides. It is clear, however, that each compound has marked activity in prevention of spread and in causing the regression of the severe corneal lesions caused by this pathogenic DNA-virus.

The mechanism of action of IUDR as an inhibitor of the biosynthesis of DNA has been discussed in several papers[4,5,7–9] and has been attributed to the intracellular formation of phosphorylated derivatives of IUDR. The concept of obtaining a metabolically more stable compound than is the case with 5-bromo- and 5-iodo-2′-deoxyuridine, through the formation of the corresponding derivatives of 2′-deoxycytidine, has been discussed in some detail elsewhere[9]. Suffice it to say here that ICDR, which is also much more thermostable and soluble than IUDR, acts as a reservoir-form of IUDR and of its 5′-phosphorylated derivatives, and that these compounds inhibit the utilization of thymidine and its 5′-phosphorylated derivatives, normal precursors of DNA; in addition, as has been shown elsewhere[5,6], 5-iodo-2′-deoxyuridine 5′-phosphate, formed metabolically from either IUDR or ICDR, can enter DNA in place of thymidine 5′-phosphate. It is proposed that, in this manner, the replication of the DNA-virus either is inhibited or a 'fraudulent' viral DNA is formed that fails to replicate, or both. The corneal epithelium appears to offer an unusual opportunity for selective chemotherapeutic inhibition of the replication of a pathogenic DNA-virus, in circumstances that avoid the toxicity of these agents for reproducing mammalian cells.

The 3′,5′-diacetyl derivatives of IUDR and ICDR were designed to achieve greater lipid-solubility than is exhibited by the parent compounds, with the view of two possible applications: one, oral administration in systemic chemotherapy[9], and two, the local treatment of viral lesions of the mucous membranes and skin. The results of the experiments described herein show that the diacetyl derivatives are converted, presumably by the corneal epithelial cells, to the active metabolites. Although the relative activities of these and the parent compounds have not yet been determined, the clinical importance of the application of these agents in the chemotherapy of herpes simplex lesions of the cornea justifies this report of the confirmation and extension of the findings of Kaufman[1].

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Effect of Fluorinated Pyrimidines on Herpes Simplex Virus

Several reports have appeared recently regarding the effects of 5-fluoropyrimidines on nucleic acid metabolism[1–10]. One of these compounds, 5-fluorouracil, has been shown to exert a profound effect on the quantity of thymine component of nucleic acids, perhaps by interfering with the methylation of thymine[1,2,8]. Other investigators have shown that this fluoropyrimidine can be incorporated into RNA of tobacco mosaic virus, *E. coli*, mouse tumours and rat liver[3-7,9,10]. Horowitz *et al.* have also examined the effect of several other fluoro-derivatives as well as 5-fluorouracil on the synthesis of bacterial proteins and nucleic acids[11]. These investigators found that the 5-fluoropyrimidines invariably inhibited the synthesis of DNA in several strains of *E. coli*.

These observations suggested the possibility that one or more of the 5-fluoropyrimidines might have a similar effect on nucleic acid metabolism in the herpes simplex virus.

The virus was grown on the chorioallantoic membrane of the chick embryo. In view of the repeated passages of virus required for this experiment, the virulence and viability of the virus were checked at alternate passages by inoculation into mouse brain and by adding anti-herpetic horse serum to one or two allantoic membranes. The effect of 5-fluorodeoxyuridine (5-FDU) and 5-fluorouracil (5-FU) (both kindly supplied by Dr. W. E. Scott of Hoffmann-La Roche, Inc.) on the growth of herpes simplex virus and on nucleic acid synthesis was investigated by adding 5 μgm. of 5-FDU or 5-FU in 0·1 ml. sterile water to each membrane at two different time-periods. These analogues were either added at the time the allantoic membrane was inoculated with a virus suspension or 24 hr. after the initial virus inoculation (at which time pock-forming units are already visible). In the control experiments, saline was substituted for the fluoropyrimidines. All the membranes were removed after 48 hr., the number of pock-forming units were counted and the virus collected from the membranes according to Serry's method[12]. Nucleic acids were extracted according to the method of Brown and Martin[13] and measured by spectral assay. A modification of Ceriotti's method[14] was used for measuring DNA and the orcinol method[15] was used for RNA.

In those experiments in which 5-FU was used there was no apparent effect on virus growth (as measured by the number of pock-forming units per membrane) nor on the total amount of nucleic acid extracted (expressed as μgm. per pock-forming units). In both the control and 5-FU experiments the number of pock-forming units (48 hr. after inoculation with virus) averaged 250–300 per membrane.

When 5-FDU was used, there was a very marked decrease in the number of pock-forming units per membrane. Depending on the dilution of virus used at the time of inoculation, virus growth was inhibited by 70–100 per cent after 48 hr. Fig. 1 shows a control membrane containing 280 pock-forming units while Fig. 2 shows a representative membrane to which 5 μgm. of 5-FDU was added 24 hr. after inoculation with the virus. There are only 8 pock-forming units present in this membrane, representing more than 97 per cent inhibition of growth. When 5-FDU was added at the time of viral inoculation, no growth was observed in these membranes.

The results of these experiments indicate that 5-FDU is a potent inhibitor of herpes simplex virus. Since this virus attacks the corneal epithelium initially it should be particularly sensitive to the action of 5-FDU and preliminary clinical investigations indicate that this drug may prove to be effective in the therapy of herpes simplex keratitis. (It is interesting to note that a closely related compound, 5-iodo-2 deoxyuridine, has recently been used with apparent clinical success in the therapy of herpes keratitis[16].) Preliminary evidence also indicates that 5-FDU used in 0·1–0·5 per cent concentration does not affect the epithelium of the rabbit cornea nor does it inhibit the rate of re-epithelialization of abraded corneas. However, from observations on these membranes in which there was less than 90 per cent inhibition of herpes virus, there is the possibility that injudicious use of this compound may be followed by a rebound phenomenon which would indicate that all patients should be watched carefully for 2–3 weeks after therapy has ceased for evidence of a recurrence.

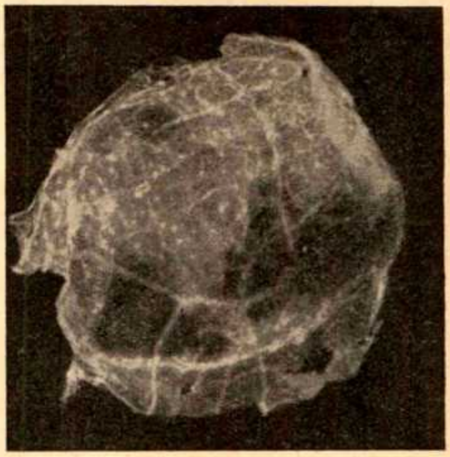

Fig. 1. Chorioallantoic membrane containing 280 pock-forming units (herpes simplex virus)

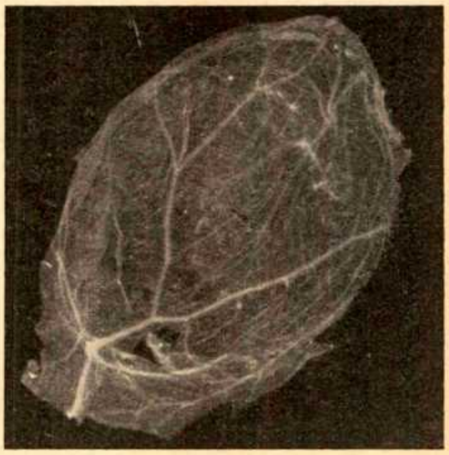

Fig. 2. Marked inhibition of herpes simplex virus growth (eight pock-forming units) on chorioallantoic membrane to which 5 μgm. of 5-FDU were added

The results with the other fluoropyrimidine used in this experiment are interesting since there was no apparent effect on viral replication nor on nucleic acid synthesis. Present evidence indicates that in certain bacteria 5-FU interferes with the synthesis of the thymine component of nucleic acids by inhibiting the enzyme responsible for the introduction of the methyl group[11]. These experiments indicate that 5-FDU inhibits the synthesis of DNA thymine in herpes simplex virus while 5-FU has no effect on DNA thymine synthesis in this virus. However, preliminary studies in our laboratory indicate that herpes simplex virus may contain some RNA and there is the possibility that 5-FU is being incorporated into the nucleic acids. This possibility is now being investigated.

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BIOLOGY

Production of Fæcal Pellets in the Wild Rabbit

Professional rabbit-catchers in Pembrokeshire, when asked what was the average number of fæcal pellets dropped by the wild rabbit (*Oryctolagus cuniculus* L.) in 24 hr., estimated the 'output' to be between 20 and 50 pellets. They based their estimates of numbers of rabbits present in a warren on these figures for pellets visible on the ground; which figures had, of course, to be multiplied by the number of days during which, they believed, a fæcal pellet survived above ground—about one week in wet, and several weeks in dry or frosty, weather—before it disintegrated or disappeared.

During the course of a four-year investigation[1] (grant-aided by the Nature Conservancy) of the wild rabbit in enclosures at Orielton, near Pembroke, this disappearance or 'decay rate' of rabbit pellets was found to vary as widely as the rabbit-catchers had estimated—between a few days to several weeks, depending on temperature, humidity and the activity of the dung-eating ground fauna. But the average production of fæcal pellets proved to be much higher than 20–50 in 24 hr.

An adult male wild rabbit at Orielton was taken from one of the large (one-acre) enclosed study warrens and placed in a contiguous enclosure sufficiently large—250 sq. yd.—to provide it with ample grazing. For cover, an artificial burrow was provided with a concrete-lined sleeping chamber to which this buck retired to rest during the day. His behaviour could be watched through a plate-glass window protected by an observation hut; and over several weeks he became accustomed to artificial light when this was switched on during periods of observation. Most of his time underground he slept, occasionally preening and re-ingesting fæcal pellets collected at irregular intervals from the anus.

Neither during re-ingestion nor during any other time spent underground was this male or any other rabbit, male or female (observed at intervals over three years in this artificial burrow and sleeping chamber), seen to drop any pellets or urinate below ground. The entry–exit passages and the sleeping chambers were thus kept dry and sanitary. All fæcal pellets that were not re-ingested were dropped outside on the grazing area.

Several weeks after this male had settled down in isolation, but within close proximity to other rabbits on the other side of the wire-netting fence, and when he appeared to be behaving naturally, all pellets were cleared from his enclosure on January 15, 1958. Each morning afterwards, until February 10, the pellets in the enclosure were collected, counted and removed. After an interval of seven weeks, on April 1, 3,254 pellets were collected—all surviving over this interval—and for 13 days following collections were made daily.

In result there was a very wide variation (see Table 1), from the lowest of 116 pellets after the first 24 hr. of the experiment to a top yield of 522 on April 14.

Table 1. Number of Fæcal Pellets produced by an Adult Male Rabbit

Date (1958)	No. of pellets removed	Remarks	Date (1958)	No. of pellets removed	Remarks
Jan. 15	All pellets removed		Feb. 4	346	
16	116	0·04 in. rain	5	327	0·04 in. rain
17	168	0·01 in. rain	6	213	0·01 in rain
18	222		7	308	slight snow
19	280		8	368	0·58 in. rain
20	290	0·02 in. rain	9	303	1·00 in. rain
21	192		10	331	0·90 in. rain
22	nil	snow covers ground	Apr. 1	3,254	(all in pen *D*)
23	250	on top of snow	2	444	
24	672	melted snow reveals	3	450	
		pellets covered 22nd	4	390	
25	250	0·22 in. rain	5	445	
26	247	0·31 in. rain	6	492	
27	220	0·025 in. rain	7	400	
28	260	0·47 in. rain	8	452	0·05 in. rain
29	206	0·07 in. rain	9	481	
30	372	0·01 in. rain	10	408	0·25 in. rain
31	283		11	365	
Feb. 1	319		12	512	
2	246		13	437	
3	387		14	522	

The daily production in January and early February was probably affected by cold weather (with snow on January 21–22). Over the first 26 days the average daily yield was 276 pellets. In thirteen days of April the average was 446 pellets a day. When an adult doe was introduced on April 14, production over four days averaged 448 pellets a day per rabbit (Table 2).

Table 2. Production of One Pair of Adults

April 15	822
16	927
17	1,067
18	770

Although figures for captive animals are open to objection, it is interesting to compare them with figures for free-ranging animals. In captivity at Orielton a 9-month-old tame rabbit in a hutch, supplied with abundant green food, produced 302 pellets in 24 hr. In captivity, the cottontail, *Sylvilagus floridanus mearnsii*, averaged 320 pellets a day at 3–4 weeks old[2]. Trippense[3] records that two adults of the same species averaged 265·6 pellets per 24 hr. Dalke and Sime[4] found about 500 pellets were produced by the cottontails, *S. f. mallurus* and *S. transitionalis*, per day over 44 days on a meagre diet of bark, twigs, and buds.

The daily variability and the wide difference between the winter and spring counts at Orielton are theoretically explicable as variations due to weather conditions affecting movement and appetite. In winter, rabbits (which are usually in fat condition in late autumn) appear to graze less and defæcate less. It is possible, but not proved, that in bad weather and snow, while remaining underground, they re-ingest more fæcal pellets than in summer. This would be a compensatory habit in idleness and shelter when grazing conditions are difficult.

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Ultrastructure of the Sterilizing 'Knobbed-Sperm' Defect in the Bull

IT seems to be generally accepted that the galea capitis of the mammalian sperm plays an important part in the fertilization process although the details of this process are still unknown.

In a previous investigation of the ultrastructure of the sperm head in the bull a characteristic structure, that is, the apical body, situated at the front edge of the galea capitis, has been described[1].

In order to obtain a better understanding of the functional mechanism of this apical body it seems relevant to compare the ultrastructure of sperm heads from normal fertile bulls with that of sperm heads from non-fertile bulls, which are known to show a defect in the front part of the galea capitis. This type of sterility does exist. The defect is known as the well-characterized, so-called 'knobbed-sperm' defect or 'acrosomal-defect' described by various workers[2–6]. The defect has been demonstrated mainly in the Friesian breed and is genetically determined. The semen of such bulls shows a normal sperm concentration with practically normal motility. The only abnormality found is a somewhat rounded and slightly eccentric thickening in the front part of the sperm head easily seen in indian ink smears.

In May 1961, thanks to the generous co-operation of three British colleagues, we obtained two ejaculates from such a 2-year-old sterile Friesian bull. While investigating the breeding problem in the herd, semen from this bull was found to show the typical 'knobbed-sperm' defect in the heads of the majority of sperms.

The samples were sent by air to Copenhagen in a thermos flask. On arrival a motility of about 60 per cent was found and counts on indian ink smears revealed the defect in 87·4 and 87·2 per cent of the sperm heads. Other primary sperm abnormalities amounted to 2·4 and 2·0 per cent.

About 48 hr. after ejaculation, specimens were prepared for thin sectioning and electron microscopy as previously described[7]. However, in addition to 'Araldite' embedding[8] an embedding in 'Vestopal-*W*' (ref. 9) was used.

From the examination of a rather great number of electron micrographs of sections of sperm heads from this bull we find it justifiable to conclude that only the galea capitis of the sperm, and in particular the apical body, shows any morphological abnormalities. A great many transverse, longitudinal and median sagittal sections were studied, but no other region of the sperm head showed any defects and especially the nuclear part of the head presented a normal ultrastructure.

A survey field of sperm from the sterile bull is shown in Fig. 1. A sperm head cut through the median sagittal plane is seen in the centre of the field. The apical body at the tip of this sperm head is cut in the same manner as that of the normal sperm shown in our previous publication[1].

The abnormal apical body is shown at higher magnification in Fig. 2 *A*. In addition, the appearance of a sperm head from a normal fertile bull of the Red Danish Milk-breed is given at the same magnification, in Fig. 2 *B*. The apical body is here shown in the closed form, that is, closely applied to the tip of the nucleus and still enclosed within the practically intact cell membrane.

Figs. 1 and 2 *A* show the abnormal apical body as a greatly swollen and rather deformed organelle. It is approximately 6–8 times bigger than in the normal sperm head. Most of the swollen part consists of a cyst-like structure, about 1μ in diameter, outlined by a well-defined wall or membrane. In the case shown the cyst contains two membrane-bounded circular or ellipsoidal bodies, in the interior of which vacuoles, vesicles, and fragments of membranes are found. In addition to the cyst-like swollen structure of the abnormal apical body, the basal part of this organelle seems to be lined with a rim of electron-dense

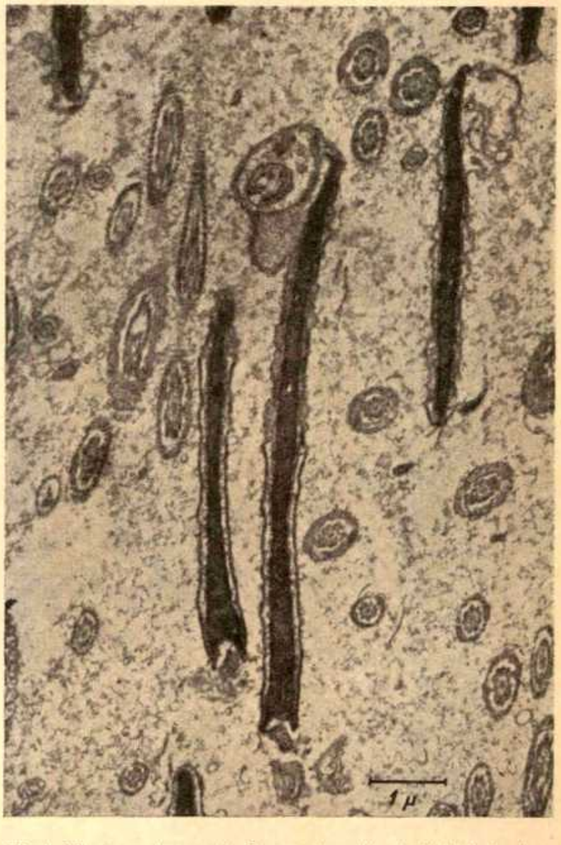

Fig. 1. Electron micrograph of sperm from the sterile bull showing the 'knobbed-sperm' defect. In the centre of the field a median sagittal sectioned sperm head with the abnormal swollen apical body. 'Vestopal-*W*' embedding; uranylacetate counterstained (× 10,000)

material of the same type as that which constitutes the galea capitis proper.

The observations presented in this preliminary report show that only the front part of the galea capitis, the apical body, is involved in the formation of the abnormality.

In our opinion this adds supplementary knowledge to observations made earlier on bull sperm showing the 'knobbed-sperm' defect. It is obvious that the swollen structure in the front part of the sperm head given by Hancock[6] represents the abnormal apical body described in this communication.

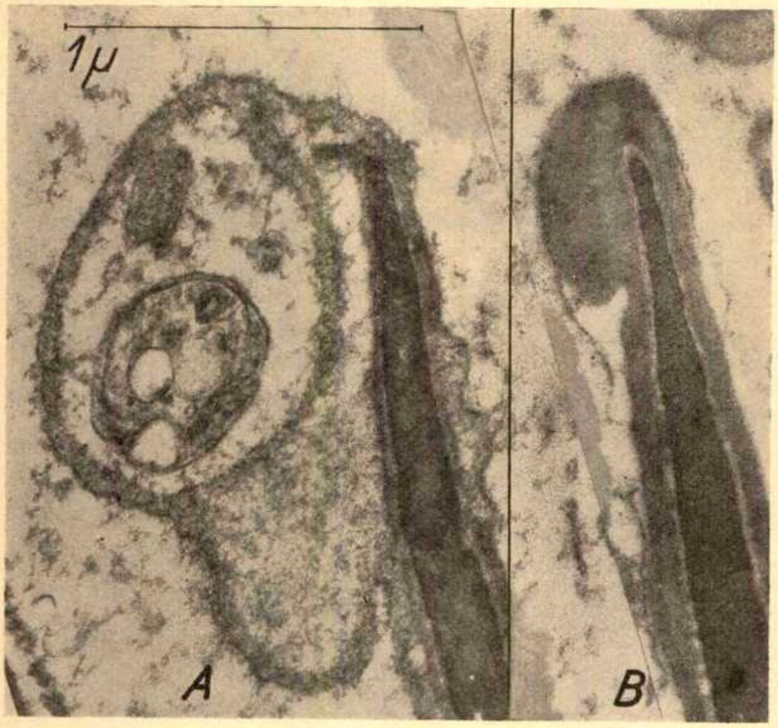

Fig. 2. *A*, Higher magnification of the same abnormal apical body, as shown in Fig. 1. Note the cyst-like swelling of the apical body and the two inclusions described in text. *B*, This electron micrograph shows for comparison the ultrastructure of the apical body of a sperm head from a normal fertile bull. Here the apical body is present in the typical closed form still enveloped by the intact cell membrane. 'Araldite' embedding. Uranyl-acetate counterstained (× 47,000)

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Influence of Uterine Position on the Response of the Mouse Embryo to Anoxia

THE exposure of the pregnant female mouse to the effects of a severe degree of anoxia results in the reduction of litter size and the birth of deformed young[1]. The object of the present investigation was to determine whether the chances of an embryo surviving the effects of anoxia were in any way affected by the position which it occupied in the uterine horn relative to its siblings.

Female mice of the Strong *A* line were used in these experiments and brother–sister mating was used throughout. The onset of pregnancy was determined by the presence of a vaginal plug, the day on which the plug was detected being taken as day 1 of the pregnancy. On day 10 of pregnancy each dam was placed in a low-pressure chamber and exposed to an atmospheric pressure reduced to 22 mm. mercury for a period of 5 hr., 15 min. being spent both in decompression and in recompression. On day 18 of pregnancy, the dam was killed and the uterine horns examined for the presence of live, dead and resorbed fœtuses.

The findings are shown in Table 1. It can be seen from this that the effects of anoxia fall most heavily on fœtuses which have implanted at the medial or vaginal end of the uterine horn, while those at the lateral or ovarian end of the horn tend to develop normally.

It has been suggested by McLaren and Michie[2] that a major factor limiting fœtal growth in the mouse may be the blood supply of the uterus, and they have pointed out that embryos at the ovarian end of the uterine horn may be particularly vulnerable in this regard. The sensitivity of the fœtus to the teratogenic activity of hypervitaminosis-A has been shown to be greater if the fœtus occupies the medial end of the uterine horn[3]. It would be possible to reconcile these two statements by postulating that the poorer blood supply at the ovarian end of the horn prevented the fœtus from being exposed to the effects of the vitamin to the same degree as at the vaginal end. One would have expected, however, that anoxia would affect most severely embryos at the ovarian end, if the blood

Table 1

Dam No.	Right uterine horn. From lateral (ovarian) to medial (vaginal) side	Left uterine horn. From medial (vaginal) to lateral (ovarian) side
1	0 0 0 0 *A*	*A* 0 0 0 0
2	0 0 0 0	0 0 0 *D*
3	0 0 0	*S* 0 0 0 0
4	0 0	*S* 0 0
5	0 0 0	*S* 0 0
6	0	*A* 0 0
7	0 *A*	*A*
8	0 0 0 0 *A* 0	*A* 0 0 0 0
9	0 *A* 0 0 0 0	0 0
10	0 0 0 0 *A*	*S* 0 0 0 0
11	horn empty	0 *A* *S* 0 0
12	0 0 *S*	horn empty

0, Normal young; *A*, absorbed young; *D*, deformed young; *S*, stunted young.

supply at that end is less adequate. It may be that the embryo at the ovarian end, normally in receipt of less oxygen, is therefore better able to withstand sudden lack of oxygen than his sibling at the vaginal end of the horn. On the other hand, since it is probable that implantation occurs at different times at the two ends, it may be that the dissimilar response to anoxia is determined by the fact that the embryos in one uterine horn are not all necessarily at exactly the same stage of development. Further experiments are in progress.

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Double Circulation in the Amphibian *Amphiuma tridactylum*

The nature of a double circulation (the separation of the pulmonary and systemic circuits) in amphibians is a highly debated subject. Some maintain[1,2] in accordance with the classical theory of Brücke[3] that blood is selectively distributed between the carotid, arches, the aortic and the pulmocutaneous arteries. Others reject this hypothesis[4,5] and advocate an entirely random distribution with no selective preference. Still others[6,7] offer support for a compromise and feel that a separate distribution may prevail under certain conditions. Most investigators arrive at their conclusions from various kinds of injection experiments most of which suffer from a lack of quantitation necessary for an exact evaluation of the mixing conditions. The delicate pressure relations within the heart cavities and larger vessels are also prone to be disturbed when applying such procedures. The material used in earlier experiments was very selective. Species of *Rana* were the case in point while experiments on Urodeles are very scarce.

One accepted method for a quantitative evaluation of the mixing problem would be careful analysis of the blood oxygen content in the incoming vessels to the heart compared with the proper conduits leaving the heart. No such analysis has ever been reported for any species of Amphibia in spite of the fact that such procedures have put an end to the discussion on mixing conditions in the reptilian heart[8,9].

In our laboratory the large urodele amphibian *Amphiuma tridactylum* (500–800 gm.) was selected for analysis of blood oxygen as outlined here. The large size of this animal renders it very suitable for withdrawal of blood samples with no resulting effect of loss of blood or disturbance of the normal dynamics of the heart. The animals were lightly anæsthetized in *MS* 222. The tip of one lung was cannulated 10 cm. cranial to the cloacal opening, ensuring access in order to inflate the lungs with selected gas mixtures. A small incision in the region of the heart allowed for exposure of one aortic arch and one pulmonary artery (pulmocutaneous arch). A small incision in the posterior half of the pericardium gave access to the sinus venosus and pulmonary vein. Simultaneous samples were collected from the pulmonary artery and aortic arch followed by immediate sampling from the sinus venosus and pulmonary vein. The sampling was done by inserting a 30-gauge cannula through the proper vessel. The cannula was connected by a small plastic adapter directly on to a micro-pipette used in analysis of the blood gas. The volume withdrawn was very slight, 25 mm.^{3}, and was anaerobically transferred to the blood gas analyser for analysis by the method of Scholander *et al.*[10].

In most experiments the lung was inflated with pure oxygen some time prior to an experiment. The skin was kept moist to maintain normal cutaneous respiration. The results from 8 animals are given in Table 1. These offer unquestionable evidence of a selective distribution of the blood going through the heart of *Amphiuma*. The selection is, however, easily disturbed and abolished. A factor of prime importance is the general level of pressure in the heart, bulbus arteriosus and various arterial arches. At low pressures a perfect mixing seemed to ensue. Of interest is the small arterio-venous differences in oxygen content even following lung respiration with pure oxygen. This serves to emphasize the importance of cutaneous respiration in aquatic Amphibia. If skin respiration was restricted by drying the skin the measured arterio-venous differences in oxygen content increased markedly. Blood returning from the skin is drained in the sinus venosus and contributes only to a general elevation of the blood oxygen. From Table 1 it is apparent that the selective distribution consists of a slight or no shunting at all of blood from the

Table 1. Oxygen Content of Blood from the Great Vessels of *Amphiuma tridactylum*

Animal No.	O$_2$ cont. (vol. per cent) Pulmo. cut. arch	O$_2$ cont. (vol. per cent) Aortic arch	O$_2$ cont. (vol. per cent) Sinus venosus	O$_2$ cont. (vol. per cent Pulmonary vein	Method	Remarks
1	4·8	7·7			Natelson (micro-van Slyke)	
2	5·3	11·5			,,	
3	6·5	10·1			,,	
4	4·8	6·7			,,	
4	5·3	8·2		8·4	,,	
5	6·3	8·6			Scholander	
5	6·8	8·1			,,	
6	6·1	6·3			,,	Blood loss, sluggish circulation
7	6·4	10·5			,,	
7	6·9	8·5	5·0	8·5	,,	
8	8·8	8·8			,,	Sluggish circulation Weak heart action
9	7·9	8·6			,,	
9	6·8	8·1	6·7	7·6	,,	
10	9·6	11·0			,,	
10	9·0	10·0	6·8	10·5	,,	
11	7·5	9·1			,,	
12	8·2	10·2	7·9	10·2	,,	
13	8·5	10·2	5·0	10·2	,,	Skin kept dry

venous side to the aortic arches, while the shunting of oxygen rich blood towards the pulmonary circuit could be larger. However, under conditions of unrestricted cutaneous respiration the difference in oxygen content between blood from the sinus venosus and the pulmocutaneous arches was small. It is concluded that a selective distribution of blood traversing the amphibian heart is possible and actually present in spite of the lack of a correlative anatomical separation.

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Heart Mechanism in a Conchostracan, *Limnadia lenticularis* (L.)

THE present evidence suggests that the crustaceans can be divided in two groups according to the mechanism of their heart beat: branchiopods which have a myogenic heart; the other crustaceans with their neurogenic hearts, thus resembling the heart of other arthropods[1-4]. The branchiopods so far investigated include cladocerans (*Daphnia*[3,5-9], *Simocephalus*[3]) and anostracans (*Artemia*[3-4], *Eubranchipus*[4]). So far, nothing was known about the physiology of the heart in Conchostraca and Notostraca[3].

One of the few European species among Conchostraca, *Limnadia lenticularis*, originally described by Linnæus in 1758 on the basis of Finnish material, is occasionally found in Finland, always as a temporary inhabitant of rock pools with acid, humified fresh water on the rocky islands in the south-west archipelago. Females living in some pools in the vicinity of the Lohm Marine Biological Station were collected for experiments. The occurrence of *Limnadia* in this locality in June, 1960, was pointed out by Mr. Aatos Petäjä.

The heart of *Limnadia* is tubular, extending through 4 entire segments, open at both ends and equipped with 4 pairs of slit-like lateral ostia. The blood is drawn through the posterior opening and the ostiæ and forced by simultaneous contraction of the entire heart to move forwards and out through the opening at the anterior end of the heart. The anterior part with the first pair of ostia is situated on the place of attachment of the animal to its shell. This part of the heart is larger and apparently more effective than its other parts.

Ether (1/2–1/4 saturated aqueous solution), acetylcholine (10^{-2}, 10^{-3}, 10^{-4}) and adrenaline (10^{-3}, 10^{-4}, 10^{-5}) were externally applied at temperatures of 11–13° C. and 18–20° C. and the heart-rate recorded *in situ*. The heart-rates recorded previously after the application of the drugs and those of animals kept continuously during the experiment in the solvent served as controls. The control heart-rates ranged from 26 to 53 min.^{-1} at 11°–12° C. and from 86 to 125 min.^{-1} at 19° C. for females with a shell length of 10–11 mm.

When immersed in the ether solution, the movements of the telson, the legs, antennæ and the gut ceased in about 20 min., while the heart continued to beat at unchanged frequency. Acetylcholine was without any effect, observable for 1 hr. Adrenaline in concentrations of 10^{-4} and 10^{-5} in 15–20 min. caused an increase of about 50 per cent in the heart-rate. Adrenaline 10^{-3} caused a transient increase of the heart-rate and afterwards a decrease which in 40 min. from the beginning of the experiment resulted in a complete arrest of the heart, this arrest being reversible.

These observations suggest that the heart-beat is myogenic in Conchostraca, as in the other branchiopods studied. As the cessation of active leg movement and the closure of shell caused by mechanical stimulation were found to be accompanied by a rapid decrease in the heart-rate, the heart probably is under nervous control in Conchostraca. Anostraca seem to lack cardioregulatory nerves[4]. Further knowledge on the physiology of Conchostraca[10] would be interesting from the comparative point of view.

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Rate of Replacement of the Molluscan Radula

DURING investigations of the mechanism of secretion and replacement of the molluscan radula an attempt was made to determine the rate of replacement. Carriker[1] collected the fæces of *Lymnaea stagnalis* and counted the number of broken radula teeth present—over 23 days he collected 613 teeth. As there are approximately 100 teeth in a transverse row it can be calculated from Carriker's figures that approximately 0·266 row of teeth is formed a day. This is obviously a minimum figure. It was decided to use an operative technique—*Lymnaea stagnalis* 2·5–3·5 cm. long were anæsthetized using 'Nembutal' and *M.S.* 222 (Sandoz)[2], an incision was made behind and below the left tentacle and the tip of the radula gland was cauterized over a small area. The survival-rate was very low; out of 93 animals only 11 survived for a sufficient length of time. The animals were killed over a period of 10 weeks and the radula isolated. Only the undamaged part of the radula gland continued secreting after the operation. The number of new rows were counted where possible, but in the case of two animals no new growth occurred while the old radula was shed at the normal rate. In these cases the number of remaining transverse teeth rows were subtracted from the average number of transverse rows (110) found in normal individuals.

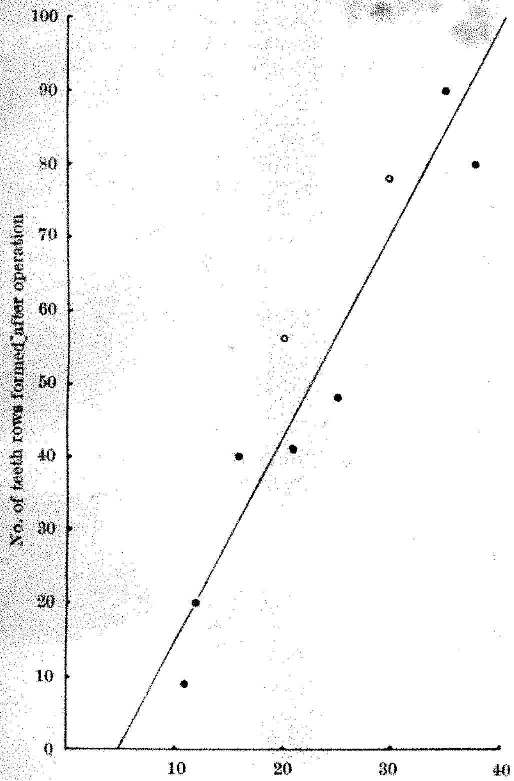

Fig. 1. Rate of radula replacement. ●, Number of new rows; ○, 110—number of remaining old rows

From Fig. 1 it can be seen that the animals took approximately 5 days to recover from the operation and that the rate of replacement of the radula was very approximately 2·8 rows a day, that is, an average radula (110 rows) would be completely replaced in about 40 days. In animals killed at 68 and 69 days none of the old radula remained. This very rapid replacement-rate accounts for the fact that, while there is a very great difference in size between the radula teeth of young and old individuals, in any one animal the teeth at the old and the new ends of the radula are approximately the same size.

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Fate of the 'Tail' during the Larval Development of *Raillietina cesticillus* in the Confused Flour Beetle, *Tribolium confusum*

Unparasitized confused flour beetles were fed cast proglottids of *R. cesticillus*, and were then maintained on whole wheat flour, without being re-infected.

During the early development of the cestode, the tail arises as a posterior lobe which gradually constricts at the base to form a slender stalk. Usually, some of the six larval hooks are included in the tail while others remain in the stalk or body. The tail is pinched off as a small, perfect sphere, the stalk remaining attached to the body. The tail is shed at the end of the procercoid-like phase of development, designated as stage 5 by Wisseman[1], and is therefore lost at the same stage as in the primitive pseudophyllidean life-cycle.

The cast tail does not disintegrate, but survives and proliferates while the body continues its normal development to the cysticercoid stage. The tail becomes indented and lobed, its shape becoming increasingly irregular as it enlarges. It usually begins to lose its spherical shape when development of the body has reached stage 10 of Wisseman, which precedes the withdrawal of the scolex into the posterior portion. One abnormal animal was observed in which the tail had not been cast, and was enlarged and misshapen while still attached to the body; the scolex was well developed.

The growth of the tail is unaccompanied by differentiation, and is unregulated and unlimited, often resulting in bizarre shapes. Eventually a very large, lobular mass of proliferating material is produced. The lobes are drawn out at the base into fine stalks and are constricted off as rounded bodies. The stalks remain projecting from the main cellular mass. The spherical bodies, which are usually without hooks, appear in turn to increase in size.

The proliferation of the cast tail is of no advantage to the species, and probably requires nutrients from the hæmolymph of the host which could be utilized by the developing cysticercoids. The process is not one of asexual reproduction; the cellular masses do not differentiate, and are not protected against digestion in the gut of the definitive host.

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Preservation of Tissue Culture Cells with Liquid Nitrogen

For prolonged storage of biological materials it is generally considered that temperatures of −100° C. or lower are necessary[1], and as thermodynamic changes have been demonstrated in mixtures of glycerol and Earle's solution in this temperature region[2] and in ice at −130° C.[3] it is preferable to use a lower storage temperature such as is provided by liquid nitrogen.

Storage in a small liquid nitrogen flask such as the Linde *LNR*-25-*B* (supplied by Union Carbide, Ltd., 8 Grafton Street, London, W.1) is now practical for small laboratories and it provides a temperature of −195° C. in the liquid and −185° C. in the gas.

In order to freeze tissue culture cells for low-temperature storage it is recommended that the average rate of cooling should be not more than 1° C. per minute down to −25° C.[1,4], and to do this several devices have been made[4–6]. With the liquid nitrogen flask it was felt that the normal continuous loss of cold nitrogen gas could be conveniently used as the coolant and the following simple procedure was devised.

It consists of a plug of expanded polystyrene ('Polyzote', supplied by Expanded Plastics, Ltd.,

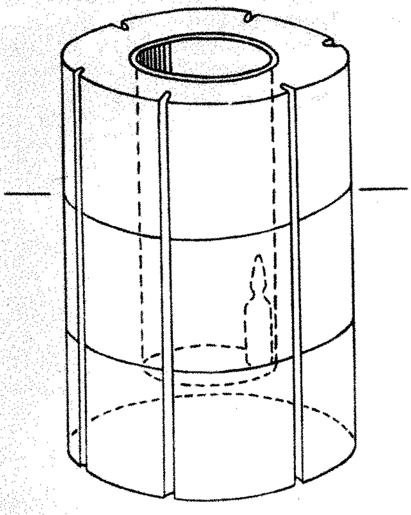

Fig. 1. Diagram of polystyrene plug freezer. Horizontal lines indicate level of rim of flask

Mitcham Road, Croydon, Surrey) of the same diameter, 3¼ in., and with 6 vertical grooves, each ⅛ in. wide and ¼ in. deep, as in the normal Linde-25-*B* plug (Fig. 1). It is made from 3 rings cut from a 3-in. thick slab of polystyrene, glued together with 'Bostik *C*' (made by B. B. Chemical Co., Ltd., Ulverscroft Road, Leicester), with a cardboard tube of 1½-in. internal diameter which lines a central hole that extends to 2 in. above the bottom of the plug. The hole inside the plug is therefore surrounded by about ¾ in. of insulation at the sides and 2 in. at the bottom. A group of 8 upright 1-ml. thin-walled glass ampoules can be lowered to the bottom of this well, and the temperature of the air around them read from a thermometer with its bulb among them.

Insertion of the loaded plug 6 in. into the neck of the flask gives the required rate of cooling when the ampoules contain dimethyl sulphoxide freezing solution. When less than 8 ampoules require freezing the number is made up with blanks containing medium only. After 3½–4 hr. a nearly constant temperature of about −100° C. is obtained, and the ampoules can be transferred to the body of the flask.

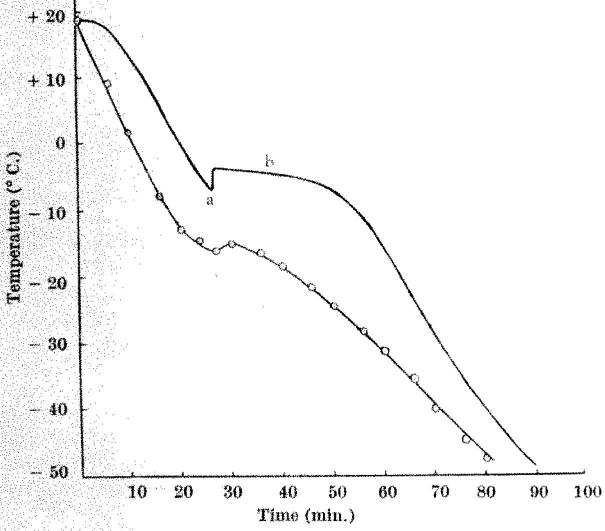

Fig. 2. Freezing curve. Continuous line is a tracing from a thermocouple inside an ampoule. Lower curve is plotted from thermometer readings at 2-min. intervals. The dip (*a*) in the upper record is produced by supercooling and the plateau (*b*) is produced during freezing of the dimethyl sulphoxide solution

The ambient temperatures, read either by thermocouple or thermometer, are generally about 10° C. lower than the temperatures recorded by a thermocouple in the contents of an ampoule. The temperature of the contents of an ampoule shows that an initial fall and supercooling precede a slow fall during freezing, which takes about 25 min.; thereafter rapid cooling is resumed (Fig. 2).

Although this is probably not the optimum curve for all tissue culture cells it has provided excellent results with a representative selection.

The superiority of dimethyl sulphoxide over glycerol[6–8] as an additive is confirmed under these conditions and we have used 10 per cent dimethyl sulphoxide with 15 per cent calf serum in Gey's salt solution in preference to other mixtures since R. M. Dougherty kindly made his experimental results available to us before publication[6].

For reconstitution, ampoules are thawed by being plunged into water at 37° C. and their contents diluted to 10 ml. or more with the appropriate growth medium modified only by reduction of the sodium bicarbonate to 0·035 per cent[9] and distributed at the rate of 150–200,000 cells per ml.

Consistently high recovery-rates have been obtained with this method, that is, 80–100 per cent of cells remained viable. Continuous cell lines frozen include several HeLa strains, and *MK*2. Primary cultures include human amnion, human embryo kidney and lung, and monkey kidney.

Primary cultures may be frozen immediately after trypsinization of the original tissue, and on reconstitution follow the pattern to be expected if not frozen. For example, amnion cells remain rounded for 1–2 days before growth begins and only reach confluence in 4–5 days; human embryo kidney cells show little change for 2 days and then grow rapidly to reach confluence in about 5 days.

However, if after trypsinization amnion and kidney cells are grown out for 4–5 days and then stripped off the glass with 'Versene' and frozen down they will, on reconstitution, start to grow where they left off and a confluent sheet can be obtained in 1–2 days, depending on the size of the inoculum.

This method of storage enables a wide variety of cells to be kept in the form of a tissue culture 'cell bank' which can be available for use within a day or two—an invaluable asset in the case of those cells which are difficult to obtain such as human embryonic cells and others which are only required at infrequent intervals.

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MICROBIOLOGY

Muller's Phenomenon

MULLER's phenomenon, which may be defined as the appearance of small circular areas or plaques of clearing around the growth of a *Staphylococcus pyogenes* on a serum-containing chromo-protein agar substrate, has been recently reviewed by Elek[1], who suggests that this proteolytic effect is due either to a non-pathogenic virus or to a hitherto unrecognized enzyme precursor present in sera and activated by a staphylococcal factor.

Five per cent human serum–milk–agar is suitable for the demonstration of this phenomenon (Fig. 1*a*), and on this medium 197 of 202 strains of coagulase-positive *Staphylococci* of human origin produced the plaques of clearing characteristic of this effect, while none of 41 coagulase-negative strains or of 130 strains of other common micro-organisms did so. The 197 strains of coagulase-positive, 'Muller-positive' *Staphylococci* were also fibrinolytic on a heated plasma agar medium, but the 5 strains of coagulase-positive *Staphylococci* which failed to produce Muller's phenomenon were non-fibrinolytic[2]. This finding supports the assumption of Christie, Graydon and Woods[3] that staphylococcal fibrinolysin is the agent responsible for Muller's phenomenon.

If the concentration of serum in a serum–milk–agar medium is increased to 50 per cent, as in that recommended by Fisk and Mordvin[4], plaques of clearing do not develop but a zone of proteolysis appears spreading outwards from the staphylococcal growth. With concentrations of serum less than 50 per cent plaques may afterwards develop in an irregular ring beyond the zone of lysis, or plaques, which have previously appeared around the staphylococcal growth, may be engulfed by the extension of this zone.

The staphylococcal factor which is essential for the development of this proteolytic effect has been prepared free from living staphylococcal cells. A Seitz-filtered or a centrifuged and heat-killed broth culture of a suitable strain of a *Staphylococcus*, that is, coagulase-positive, 'Muller-positive' and fibrinolytic, will produce plaques of clearing when incorporated in serum–milk–agar, incubated at 37° C. These plaques may take 2–20 hr. to appear, depending on the amount and activity of the staphylococcal factor and on the concentration of serum. The plaques may persist as separate and distinct areas of proteolysis so long as the plate is retained or they may coalesce, resulting in complete destruction of the milk in the medium, the outcome being dependent on the concentration of the staphylococcal factor and serum.

Proteolysis by *Staphylococci* can be achieved either by the direct action of staphylococcal protease or by the activation of serum plasminogen to plasmin by staphylokinase[5-7]. Protease activity is independent of the presence of serum and is unaffected by soya bean trypsin inhibitor. Proteolysis brought about by staphylokinase-activated plasminogen, however, is inhibited by soya bean trypsin inhibitor. Muller's phenomenon does not develop in a milk–agar medium without serum, the only evidence of proteolytic activity in such a substrate being a slight clearing of the milk around colonies of *Staphylococci* which produce protease.

Inhibition of Muller's phenomenon and of proteolysis can be demonstrated on serum–milk–agar in which soya bean trypsin inhibitor has been incorporated. Soya bean trypsin inhibitor will also prevent the development of plaques and proteolysis in serum–milk–agar containing the staphylococcal factor (Fig. 1*b*). It would appear, therefore, that staphylokinase is the staphylococcal factor necessary for the demonstration of Muller's phenomenon.

The conditions necessary for the manifestation of plaques have been investigated, and of importance are the concentrations of serum and staphylococcal factor, the nature and strength of the gel and the amount of sodium chloride in the medium. The nature of the test protein is also of great importance in that, on certain proteins such as milk and heated hæmoglobin, plaque production is more easily observed than on others. If living cultures of *Staphylococci* are used to demonstrate the effect, the nature and amount of nutriment incorporated in the serum–milk–agar medium are worthy of note in that they influence the number and size of the plaques.

Muller's phenomenon, it would appear, is a manifestation of proteolysis brought about by staphylokinase-activated plasminogen, plaque formation being determined by the physical conditions in the gel.

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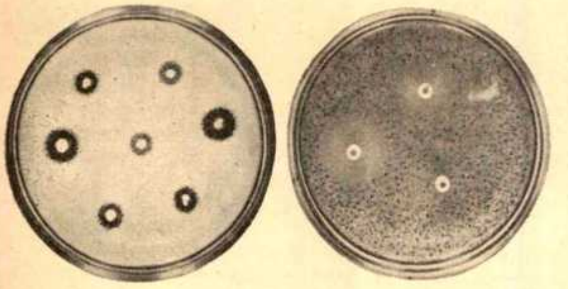

Fig. 1. *a*, Muller's phenomenon around seven strains of coagulase-positive *Staphylococci* on 5 per cent serum–milk–agar. *b*, Inhibition of Muller's phenomenon around two cups containing soya bean trypsin inhibitor on serum–milk–agar containing staphylococcal factor. No inhibition around control cup containing only diluent

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Synthesis of Optically Active γ- and δ-Lactones by Microbiological Reduction

MICROBIOLOGICAL reductions of α-keto-groups have been reported by Neuberg *et al.*[1]. Lemieux and Giquere[2] carried out reductions of β-keto-caproic and β-keto-caprylic acids. Microbiological reductions of γ- and δ-keto-acids to yield optically active lactones have so far not been described in the literature.

We used a simple method to test the ability of micro-organisms to reduce γ- and δ-keto-acids. About 300 mgm. cell material of the micro-organism to be tested was incubated in water agar at 30° C. for 48 hr., with and without added keto-acid (2 mgm.) and glucose (20 mgm.). The appearance of the characteristic odour of lactone is indicative for a positive effect.

A number of yeasts (*Candida* and *Saccharomyces* group), some moulds (*Penicillium notatum*, *Cladosporium butyri*, and *Cl. suaveolens*), and some bacteria (*Sarcina lutea*) produced an odour of lactone.

To be able to isolate the lactone formed and investigate its properties, these micro-organisms were cultivated on a somewhat larger scale on nutrient agar. About 12 gm. moist cell material and 10 gm. glucose were used for the reduction of 500 mgm. δ-keto-capric acid.

The hydroxy-acids were extracted from the filtrate and lactonized at 130–140° C. *in vacuo* for 1 hr. The crude lactone was purified. The rotation was measured in methanolic solution. The results obtained, starting with 500 mgm. δ-keto-capric acid, are given in Table 1.

Table 1

Micro-organism	δ-deca-lactone obtained (mgm.)	$[\alpha]_D$
Cladosporium butyri	71	−15°
Saccharomyces cerevisiae	355	+48·2°
Saccharomyces fragilis	229	+48·5°
Candida pseudotropicalis	252	+48·2°
Candida globiformis	233	+55·8°
Sarcina lutea	302	−29·2°
	1,400*	−36·8°

* From 2,400 mgm. keto-acid.

The odour of dextrorotatory and lævorotatory δ-deca-lactones was identical.

With *Saccharomyces cerevisiae* (baker's yeast) we carried out reductions of various keto-acids, each time using 10 per cent yeast slurry, 0·05–0·1 per cent keto-acid, 5–10 per cent glucose (incubation at 30° C. for 48 hr.). The results are given in Table 2.

Table 2

Keto-acid	(gm.)	Yeast (gm.)	Glucose (gm.)	Volume (ml.)	Lactone yield (per cent)	$[\alpha]_D$
γ–C9	10	1,800	450	18,000	77	+50·4°
γ–C10	10	680	680	10,200	85	+46·1°
γ–C11	10	1,800	450	18,000	72	+44·1°
γ–C12	10	1,330	1,330	20,000	60	+40·4°
δ–C8	10	1,000	300	6,000	13	+58·4°
δ–C9	8·8	1,800	1,800	18,000	55	+58·2°
δ–C10	200	12,000	12,000	102,000	47	+55·6°
δ–C11	3	600	600	6,000	63	+47·9°
δ–C12	7	600	300	6,000	46	+48·8°

Thus, it proved to be possible to prepare optically active γ- and δ-lactones by microbiological reduction. There is no exact proof of the optical purity of the lactones measured. Further details will be published elsewhere. Patents have been applied for[3]. We wish to thank Dr. J. Boldingh for his advice.

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VETERINARY SCIENCE

Infection of the Fowl by the Parenteral Inoculation of Oocysts of *Eimeria*

Under natural conditions infection with coccidia occurs as a result of the ingestion of viable sporulated oocysts, and this method of infection, that is, the inoculation of oocysts *per os*, is used experimentally to establish coccidia in laboratory animals.

Occasionally, however, other methods of infection, using different stages of the developmental cycle of the coccidia, are effective. For example, to obtain oocysts of *Eimeria necatrix* free from contamination with other species of *Eimeria* of the fowl, second-generation merozoites of the parasite can be inoculated *per os* or *per rectum* into susceptible chicks. Oocysts of *E. necatrix* are produced after a period of approximately two days, that is, the normal interval between schizogony and oocyst production. Similarly, the inoculation of first or second generation merozoites of *E. tenella* results in the production of oocysts after an appropriate interval. Such techniques, which involve the inoculation of a number of different developmental stages directly into the alimentary canal, are sufficiently reliable to be used routinely in this laboratory in maintaining pure infections of *E. tenella* and *E. necatrix* and in chemotherapeutic and immunological experiments.

Recently, Landers[1], in a series of observations on the factors necessary for the excystation of oocysts of *E. nieschulzi*, noted that oocysts excysted successfully following their inoculation into the thigh musculature of the rat, into the femoral and jugular veins and into the peritoneal cavity.

At Weybridge, similar experiments with oocysts of four species of *Eimeria* of the fowl have demonstrated that oocysts excyst successfully following parenteral inoculation into chicks (Table 1). Furthermore, schizogony has occurred in the sites normally invaded by the coccidia and oocysts have been produced after the normal interval of time. In some birds the prepatent period has been slightly prolonged.

Table 1. Parenteral Inoculation of Oocysts of *Eimeria* of the Fowl

Species	Route of inoculation	Number of oocysts	First appearance of oocysts days after inoculation
E. acervulina	Intravenous	650,000	6
	Intramuscular	650,000	Negative
	Intraperitoneal	650,000	6
E. maxima	i/v	100,000	7
	i/m	100,000	Negative
	i/p	100,000	Negative
E. necatrix	i/v	100,000	8
	i/m	100,000	Negative
	i/p	100,000	7
	i/p	150,000	8
E. tenella	i/v	50,000	7
	i/v	200,000	Negative
	i/m	200,000	7
	i/p	200,000	7
	Subcutaneous	200,000	6

In a majority of experiments the infections have been less severe than those which would be expected if similar numbers of oocysts had been inoculated *per os* and sometimes infection has not been established. The reason for the failures is not known. On several occasions, however, severe lesions of coccidiosis have been noted in the intestine, or in the cæca, and deaths have occurred.

Preliminary experiments with second generation merozoites of *E. tenella* have indicated that gametogony and oocyst production follow the intraperitoneal injection of merozoites but not their injection by the intramuscular or subcutaneous routes.

It appears, therefore, that one or more stages in the life-cycle of *Eimeria* possess considerable powers of migration. Limited migration of the first-generation merozoites of *E. tenella* and *E. necatrix* within parasitized epithelial cells was described by Tyzzer[2,3] and more recently local migration in sub-epithelial tissues of the sporozoites of *E. tenella* and *E. necatrix* has been described by Van Doorninck and Becker[4] and Pattillo[5].

Our results suggest that migration of the developmental stages of species of *Eimeria* may be a more important characteristic of the life-history than has previously been believed.

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[1] Landers, E. J., *J. Parasit.*, **46**, 195 (1960).
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GENETICS

Competition Experiments with Isoalleles

Isoalleles, that is, alleles with minor differences in activity which are indistinguishable except by means of special tests, are of considerable importance in evolution. For example, the evolution of dominance for the wild-type characters must have been a selection process among isoalleles with different activity, favouring the most active one. Hochman[1,2] has been able to perform competition experiments with wild-type isoalleles at the *ci* locus in chromosome IV of *Drosophila melanogaster*, using their different degree of dominance for discrimination. His results support the view that alleles with complete dominance have a higher adaptive value and supersede the hypomorphic ones.

The molecular basis of variations in degree of dominance is not known, but such phenomena are best explained by the assumption of varying capacity of enzyme formation. A case where an isoallele is biochemically defined has been examined by one of us (B. R.)[3,4], namely, a slightly hypomorphic wild-type allele at the *w* locus of *Drosophila melanogaster*. In females, heterozygous for *w*, this allele produces a little less red eye pigments than w^+. The difference is too small to be observed visually, but was revealed by spectrophotometric analysis. However, this allele is also characterized by a very low amount of isoxanthopterin in the testis of the males, and it was indeed first discovered because of this characteristic. It was then called *is* (isoxanthopterinless); but as it was later located to the *w* locus, its proper designation is w^{is}. It is of special interest to follow the competition between w^{is} and w^+ in population experiments, because this is a case where something is known about not only the biochemical activity of the isoalleles, but also differences in their chromosomal structure, extensive genetical tests[4] having shown w^{is} to be a small intra-genic duplication within the *w* locus.

Five such population experiments have been undertaken. Phenotypically all the competing animals were wild-type, but the gene frequencies of w^{is} and w^+ could be determined by taking out samples of males and subjecting them to chromatographic analysis. The isoxanthopterin (IX) compound belongs to the fluorescent pteridines, and its presence can be observed in ultra-violet light after chromatographic separation of the pteridines. This test can be performed on single males, the genotype of which can thus be determined as being w^{is} if the amount of IX is low, and w^+ if it is high.

The populations were kept in units containing six bottles with standard food, one of which was discarded and substituted by a fresh one each week. The bottles were connected by short rubber tubes to a narrow oblong chamber made of transparent plastic and with wire-netting at the ends. For determinations of gene frequencies a small sample of food was taken out of the one-week-old bottle. The sample contained numerous young larvæ, and was transferred to a fresh bottle, where the flies could develop under more favourable conditions than the population unit could provide. This was to assure an optimal development of the males to be analysed. Small size and undernourishment might otherwise influence the amount of pteridines. The newly hatched males were collected and aged for about a week before being analysed. The chromatographic separation was performed on the abdomen of single males. These were crushed on Whatman 1 paper, 10 on each sheet, and developed in darkness for 18 hr., using a solvent of butanol/5 *N* acetic acid in proportions 2 : 1. When observed in ultra-violet light the deep blue spots of IX could easily be recognized and the number of males with low and with high content could be determined. There was some variation in the normal amount of IX, and in the two first experiments this caused difficulties for the discrimination of the genotypes. When this could not be made quite reliable, the experiments were abandoned.

The w^+ allele was derived from two different sources, the wild-type stocks 602 and Canton-*S*. No attempts were made to render the stocks isogeneous before the start of the experiments. It was believed that the genotypic background would soon be equalized in relation to the competing alleles, except for the immediate vicinity of the *w* locus, and this could not be controlled even in extensive isogenization attempts

Table 1

Exp. No.	w^+ allele from	Date of onset	Environment	Duration in months	Gene frequency determinations No.	Gene frequency determinations No. of males in each
1	602	Sept. 1959	25°, constant	2·5	4	80
2	602	Sept. 1959	greenhouse	2·5	4	80
3	602	May 1960	25°, constant	17	4	40
4	Canton-*S*	Aug. 1960	25°, constant	13	10	20–60
5	Canton-*S*	Aug. 1960	greenhouse	9	8	20–40
			25°, constant	4	2	40–50

The data for the experiments are shown in Table 1. Each experiment was started with 20 heterozygous *is*/+ females and equal numbers of *is* and + males. The initial gene frequencies were thus always 0·5. When Exps. Nos. 1 and 2 were discarded because of discrimination difficulties, the number of flies in each unit was recorded and was found to be between 2,000 and 2,500. It is supposed that all the populations were numerous enough to make the influence of genetic drift insignificant.

The results of the gene frequency determinations are shown in Figs. 1 and 2. The length of one generation is taken as 14 days in Exps. Nos. 1, 3 and 4, which were kept in a constant temperature of 25° C. In the other experiments the length of one generation depends on the season. These populations were placed in a greenhouse, where the temperature never fell below 12° C., but in daytime rose up to 30° C. and was higher during spring and summer than during the dark months. On the basis of performed tests, the generation-length was estimated to 21 days during March–October and to 25 days from October to March.

Fig. 1 shows the situation during the first few generations in Exps. Nos. 1 and 2, 4 and 5. It is probable that the fluctuations during this initial

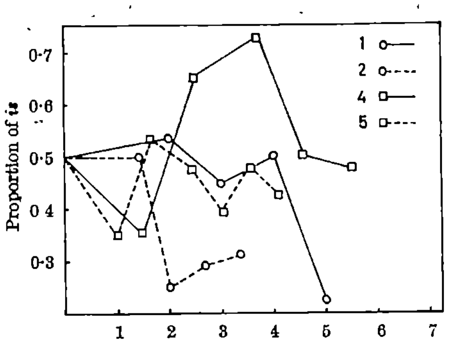

Fig. 1. Gene frequency changes during the initial stage of Exps. Nos. 1 and 2, 4 and 5

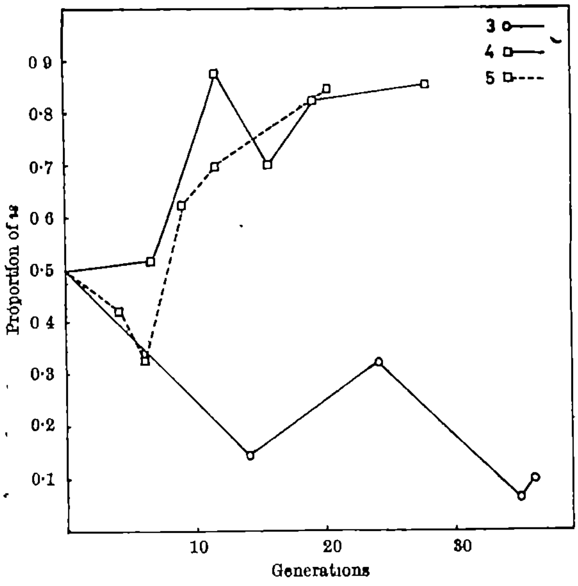

Fig. 2. Long-term gene frequency changes in Exps. Nos. 3, 4 and 5

period are due to an unequal distribution of the background genotype in relation to the alleles studied, and has little bearing on the adaptive values of these. The only definite trend to be seen is a decrease in the frequency of *is* in Exps. Nos. 1 and 2. This decrease is found again in Exp. No. 3, which was not studied during the earlier generations, but was kept going for more than 30 generations. It is shown in Fig. 2, together with the long-term progress of Exps. Nos. 4 and 5. A profound difference is found between the situation in Exp. No. 3, where *is* had to compete with the + allele of stock 602, and that in Exps. Nos. 4 and 5, where the + allele originated from Canton-*S*. In the first case the frequency of *is* has decreased to less than 10 per cent and presumably *is* will eventually disappear from this population, whereas in the latter *is* is superseding the + allele and is well on the way of being fixed. This holds for both experiments with Canton-*S* and the difference in environment does not seem to have had any other effect than to delay the progress in the greenhouse experiment.

The conclusions to be drawn from this investigation are that this special isoallele of w^+ is well enough adapted to compete successfully with normal w^+ alleles, but the outcome depends on the type of w^+ stock used. This suggests also that the w^+ alleles, which cannot at present be distinguished from each other, have different adaptive values. Finally, in one of two cases the w^{is} duplication has shown a selective advantage over the normal chromosome. This case represents an authentic instance where an increase of genetic material has taken place by means of the incorporation of a small duplication in the genome, which is postulated to have occurred repeatedly during evolution as a means to increased genetic organization.

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STATISTICS

A Fundamental Complementary Principle for Inductive Logic

The class $\{L_i\}$, of all finitely axiomatizable systems, is well known to be formalizable in a particularly simple way, for example, as Post[1] normal systems on the axioms and rules (A, R) of each L. Now already within this class there is a potential complementarity of the following kind; for given L if one fixes R then A may vary over the equivalence class of all true sentences in L, and likewise fixing A one can show, via Turing machines, that there is an equivalence class for R.

However, and this is the vital point, such complementarity is not realizable without a postulate as to how $\{L_i\}$ is to be related by some observer, O, to the external world U. The appropriate one is that A is to be taken as an interrogation (or selective punctuation[2]) of U the response S of which is a string leading from A to a true sentence $\bar{S}$ in some L. Now O's problem, having decided on an optimality criterio $L(S, \bar{S})$ for the relation between S and $\bar{S}$, is to fin the probability distribution of R.

One can set up a formalism on this basis analogou to many particle quantum mechanics in whic $L(S, \bar{S})$ plays the part of a Lagrangian function. Th analogy is to the many-particle theory becaus $L(S, \bar{S})$ must always contain the results of indefinitel many other punctuations. This in itself is of considerable interest because existing second quantu theory is in serious difficulties, due principally to th fact it is not known what makes the restriction to 4 co-ordinate dimensions. The above formalism doe not have this restriction so one can consider wha logical conditions on $L(S, \bar{S})$ would give it. Elsewhere[3] I show that there is a rather surprising answe to this question.

The principal interest of this formalism, however is that it is appropriate to investigating the relatio between an evolutionary system such as O and it environment U.

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[2] Goodall, M. C., *Nature*, 190, 480 (1961).

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Tuesday, June 12

Royal Society of Medicine, Experimental Medicine and Therapeutics Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Prof. C. H. Best: "Insulin and some of Its Scientific Offspring" (Dixon Memorial Lecture).

Wednesday, June 13

Institute of Fuel (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 9.30 a.m. and 2.30 p.m.—Second Symposium on "Flames and Industry".

Royal Institution (at 21 Albemarle Street, London, W.1). at 5.15 p.m.—Prof. P. P. Ewald, F.R.S. (Polytechnic Institute of Brooklyn): "William Henry Bragg and the New Crystallography" (First Bragg Lecture).*

Thursday, June 14

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. J. D. Bernal, F.R.S.: "The Structure of Liquids" (The Bakerian Lecture).

Friday, June 15

Institution of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Capt. T. St. B. Freer and Capt. R. McDougall: "Safe Altitude Information for Pilots".

University of London (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Harcourt Brown (Brown University, U.S.A.): "Scientists and Men of Letters—Maupertuis and Voltaire"*. (Further lecture on June 18).

Monday, June 18

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

British Society for the Philosophy of Science—(in the Joint Staff Common Room, University College, Gower Street, London' W.C.1), at 5.30 p.m.—Prof. H. Fain. "Some Problems of Causation".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer (with special interests in plant breeding) in Botany—The Registrar, The University, Hull (June 15).

Demonstrator in the Botany Department—The Registrar, University College of Wales, Aberystwyth (June 15).

Demonstrator (with, or about to obtain, a good honours degree) in Physics—The Registrar, The University, Keele, Staffs (June 15).

Lecturer/Assistant Lecturer (preferably with experience in the field of magnetic resonance or microwave techniques) in the Department of Physics—The Registrar, The University, Keele, Staffs (June 15).

Research Assistant (preferably with an honours degree in geography, geology or botany with chemistry advanced level), for work under Dr. M. M. Cole on the vegetation and soils of the southern Pennines—The Registrar, The University, Keele, Staffs (June 15).

Sub-Librarian (graduate with appropriate library qualifications and experience) in the Library of the University of York—The Registrar, University of York, King's Manor, York (June 15).

Lecturer in Mathematics and Director of the Computing Laboratory—The Secretary, The University, Exeter (June 16).

Manchester Royal Botanical Society Lecturer in Horticulture—The Registrar, The University, Manchester (June 16).

Junior Lecturer in Philosophy—The Registrar, Trinity College, Dublin, Republic of Ireland (June 19).

Assistant Chief Officer (with a degree in botany, agricultural botany or agriculture or an equivalent qualification, and preferably experience of seed improvement and staff administration) at the Official Seed Testing Station—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (June 20).

Assistant Librarians (2) (with a good honours degree, and preferably with experience and professional diplomas)—The Librarian, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (June 20).

Chair of Psychology; and a Chair of Sociology—The Secretary, The University, Exeter (June 22).

Lecturer and Assistant Lecturer in Physics (Theoretical and Experimental); and a Lecturer and Assistant Lecturer in Mathematics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton (June 23).

Lecturer or Assistant Lecturer (with good qualifications and teaching experience in physics and chemistry, and preferably a higher degree in education) in Education—The Registrar, The University, Keele, Staffs (June 23).

Senior Lecturers or Lecturers in Pure Mathematics and in Applied Mathematics at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 25).

Assistant Librarian (graduate or suitably qualified non-graduate) in the Science Library—The Registrar, The University, Nottingham (June 30).

Demonstrator (with a degree in biochemistry, agricultural chemistry, chemistry or physics, and an interest in applying this knowledge to problems in food handling and processing) in Food Science—The Registrar, The University, Nottingham (June 30).

Lecturer in Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield (June 30).

Lecturer in Geography at the University of Adelaide—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, June 30).

Research Demonstrator (with good honours qualifications in organic chemistry) in the Department of Chemistry—The Registrar, University College, Singleton Park, Swansea (June 30).

Assistant Experimental Officer (with a pass degree or H.N.C. in physics or engineering) in the Physics Department, to work on irrigation and associated problems of micro-meteorology and soil water—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (July 2).

Lecturer in Biochemistry at the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W.C.1 (July 2).

Chair of Statistics in the University of Otago—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or the Registrar, University of Otago, Dunedin, New Zealand (New Zealand and London, July 31).

Senior Research Assistant in the Meteorological Physics Section, to work in the field of infra-red spectroscopy, using interferometric techniques—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge (July 31).

Head (with qualifications, teaching and research experience necessary for recognition as a teacher of the University of London) of the Department of Chemistry—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Lecturer in Experimental Aspects of Animal Growth in the Department of Growth and Development—The Secretary, Institute of Child Health (University of London), Great Ormond Street, London, W.C.1.

Mathematics Master—The Headmaster, Queen Elizabeth's Grammar School, Blackburn.

Reader (with a high academic qualification in either physics, physiology, biology or psychology, and experience in research) in Ophthalmic Optics—The Secretary, Northampton College of Advanced Technology, St. John Street, Clerkenwell, London, E.C.1.

Research Posts in High Energy Experimental Nuclear Physics, involving the use of high energy machines at C.E.R.N., Geneva and the Rutherford Laboratory, Harwell—The Secretary to Council, Westfield College (University of London), Kidderpore Avenue, Hampstead, London, N.W.3.

Spectrochemist (under 45, with several years experience in a spectrochemical laboratory, preferably in one where research is also undertaken) with the East African Agricultural and Forestry Research Organization, to take charge of the Spectrochemical Section of the General Analytical Division, including the analysis of soil and plant materials, and initiating analytical research projects—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. R.C.264/348/06/T.

Tutor (graduate with good teaching experience) in Mathematics and/or Physics—The Bursar, College of Air Training, Hamble, Southampton.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Productivity, Vol. 1, January/February, 1962. (An International Journal and Abstracting Service for Industrial and Business Managers to help increase productivity in Factory and Office.) Pp. 56. Annual Subscription (6 issues) 70*s.*; U.S.A. and Canada 10 dollars. (London and New York: Pergamon Press, 1962.) [143

Bulletin of the British Museum (Natural History). Zoology. Vol. 8, No. 5: Marine Nematodes from Banyuls-sur-Mer: With a Review of the Genus *Eurystomina*. By William G. Inglis. Pp. 209–288. 24*s.* Vol. 8, No. 6: On a Collection of Freshwater Gastropod Molluscs from the Ethiopian Highlands. By C. A. Wright and D. S. Brown. Pp. 285–312 + plates 8–9. 12*s.* 6*d.* (London: British Museum (Natural History), 1962.) [143

Building Research Station Digest, (Second Series). No. 20: Layout and Equipment of Drawing Offices. Pp. 4. (London: H.M. Stationery Office, 1962.) 4*d.* [143

British Productivity Council. Producing for European Markets: Edited Text of Papers given at the Joint B.P.C.—Export Council for Europe Conference at the 1961 Factory Equipment Exhibition. Pp. 40. (London: British Productivity Council, 1962.) 2*s.* 6*d.* [143

The Scottish Field Studies Association. The First Ten Years. By B. W. Ribbons. Annual Report, 1961. The Bryophytes and Lichens of the Loch Tay Area By Ursula K. Duncan Pp. 31 + 4 plates. (Glasgow: The Scottish Field Studies Association, 1962.) 3*s.* 6*d.* [143

Forestry Commission. Forty-Second Annual Report of the Forestry Commissioners for the year ended 30th September 1961. Pp. 70 + 8 plates. (London: H.M. Stationery Office, 1962.) 5*s.* 6*d.* net. [143

B.B.C. Engineering Monograph No. 40: The Broacasting of Music in Television. Part 1: Introduction. By R. F. A. Pittinger. Part 2: Sound and Vision. By Lionel Salter. Part 3: Operational Technique. By E. G. M. Alkin. Part 4: Acoustic Treatment of Television Studios. By C. L. S. Gilford. Pp. 24. (London. British Broadcasting Corporation, 1962.) 5*s.* [143

The Year Book of the Royal Society of London, 1962. (No. 66.) Pp. 320. (London: The Royal Society, 1962.) 21*s.* [143

Education in the Soviet Union: Report of a College of Preceptors Delegation, May, 1961. Pp. 112 (16 illustrations). (London: The College of Preceptors, 1962.) 7*s*. 6*d*. [143

The Association of Scientific Workers. Science in Government and Industry: a Policy Statement by the Association of Scientific Workers. Pp. 8. (London: The Association of Scientific Workers, 1962.) 1*s*. [143

The Ramblers' Association. Annual Report, 1961. Pp. 16. National Parks and What They Are. Pp. 8. (London: The Ramblers' Association, 1962.) [143

Proceedings of the University of Durham Philosophical Society. Series A, (Science). Vol. 13, No. 18: The Berlese Theory of Insect Metamorphosis—The Views of Hinton and Poulton. By G. Heslop-Harrison. Pp. 174–192. Vol. 13, No. 19: Studies on the Substructure of Real Crystals by Optical Methods and by Magnetic Resonance. Part 1: Optical Methods. By E. E. Schneider. Pp. 193–201. Vol. 13, No. 20: Studies on the Substructure of Real Crystals by Optical Methods and by Magnetic Resonance. Part 2: Magnetic Resonance Methods. By E. E. Schneider. Pp. 202–216. Vol. 13, No. 21: Musical Sand: The Singing Sands of the Seashore, Part 1. By A. E. Brown, W. A. Campbell, D. A. Robson and E. R. Thomas. Pp. 217–230. Series B, (Arts). Vol. 1, No. 7: Some Problems in Comparative Linguistics. By Jeffrey Ellis. Pp. 54–62. (Durham: University of Durham Philosophical Society, 1961 and 1962.) [143

Imperialism: an Inaugural Lecture delivered in the University of Birmingham on 1st December, 1961. By Dr. H. S. Ferns. Pp. 20. (Birmingham: The University, 1962.) 2*s*. 6*d*. [143

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SECURITY IN BRITISH GOVERNMENT SERVICES

ALMOST exactly six years after the statement on the findings, but not the report itself, of the Conference of Privy Councillors on Security was published in March 1956 the Government has published the recommendations of the Radcliffe Committee, appointed in May 1961, to review security procedures and practices in the public service*. Although once again the Government decided that it would not be in the public interest to publish the report in full, the Committee's recommendations are supported with the text of the report to the maximum extent compatible with the public interest and the requirements of security. The Leader of the Opposition indicated his concurrence with the decision of the Government as to the parts withheld from publication, and Lord Radcliffe has agreed that the text, despite these omissions, preserves as nearly as possible in the Committee's own words the substance of the Committee's argument relating to security procedures and practices in the public service generally.

The White Paper in which the recommendations and arguments of the Committee are published indicates that the Government has already accepted the recommendations. Although the Radcliffe Committee formed the opinion that, while in certain respects improvements in the security services are still possible, the quality of the security organization and procedures and the general standard of security discipline are good. This confirms the earlier opinion of the Conference of Privy Councillors that there is nothing organically wrong or unsound about the British security arrangements. All in all, the White Paper is a far more useful document than the earlier statement, and merits attention by the scientist, for its findings in some matters are closely in line with the conclusions of responsible scientists on secrecy in relation to security and scientific advance, both in Britain and in the United States. Scientists will have only themselves to blame if they fail to ensure that due and continued attention is given to the Committee's recommendations for preventing abuse or excessive use of security measures as regards secrecy.

The report, in fact, corrects an impression that was derived from the statement of the Conference of Privy Councillors in 1956 who suggested that one of the chief problems of security was to identify the members of the British Communist Party, to be informed of its activities and to identify that wider body of those who are both sympathetic to Communism or susceptible to Communist pressure and present a danger to security. Thereafter, steps should be taken to see that secret information is not handled by anyone who, for ideological or other motives, might betray it. The Radcliffe Committee, however, while satisfied that much the most serious source of danger to-day lies in the intelligence services of the Soviet *bloc*, which are believed to be co-ordinated as between the various countries participating, does not regard the activities of the Communist Party of Great Britain as of the same order as a threat to security and consequently did not accept this assessment of the Privy Councillors. It believes that the central threat lies farther back, and that nothing is more important to effective security than the basic measures of physical security, if well planned and consistently executed. Safeguarding premises, documents and ciphers and eliminating carelessness are of the first importance.

No system of personnel security, however much elaborated, can be effective enough to modify the primary value of such physical measures, and the Radcliffe Committee further points out that counter-espionage against enemy agents is an indispensable part of the defensive system. None of the various defensive measures can safely be neglected in reliance on another, and the balance and sanity of this report offer some assurance against the eruption of any ill-judged measures of 'McCarthyism'. The Committee frankly recognizes that the social and political system of Britain is inimical to effective security, particularly the general public tradition that any individual is entitled to a fair hearing in respect of any accusation made against him and to fair treatment irrespective of political or public convenience. It further observes that security weaknesses of this kind are part of the price that must be paid for a social and political system that men want to defend.

It is indeed because of this that the Radcliffe Committee rightly stresses the need to persuade Government employees that security is the concern of everyone in the Government service. It is understandable that it should find patchiness in the security arrangements of the dozen departments it examined fairly closely rather than a uniform level of active concern for security, and that the subject was frequently treated as in practice of low importance. The practical danger seems to be this apathy rather than friction between the Security Service and the departments, of which it found no evidence. The Committee suggests that if any conflict of view should arise between the Director-General of the Security Service and the head of a department it should become the recognized procedure for the Director-General to seek an interview with the departmental head and state frankly his reasons for disquiet on security grounds. Much importance is attached to its being brought home to the Civil Service as a whole that security is a subject of active concern to those at its highest levels, and the Committee recommends that the structure of the interdepartmental committee in this field should be supported by measures designed to associate the supervising committee more directly with the work of the junior committees.

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* Security Procedures in the Public Service. Pp. vi+42. (Cmnd. 1681.) (London: H.M.S.O., 1962.) 3*s*. net.

On security organization in departments, the Committee makes a sharp distinction between the issue of security instructions and education in security. The former are, and should be, orders, and every member of the departmental staff who is handling classified information must be instructed in his or her personal responsibility for security and in the various security rules and procedure in force. The latter should be clear, firm and precise and the Committee adds that the preparation of instructions calls for the exercise of judgment and imagination and should be regarded as one of the most important tasks that fall to a departmental security officer. Generally, the Committee suggests that security in all its aspects should be included in the responsibilities of the principal establishment officer, and in support of this it cites the views expressed by the Plowden Committee. What is needed, it reiterates, is that the standards of the best departments should become general.

The Committee is on more controversial ground in its comments on the Civil Service Staff Associations and Trade Unions. It was disturbed at the number of Communist and Communist sympathizers who hold positions in these bodies either as permanent full-time paid officials, or as unpaid officers or members of executive committees. This massing of Communist effort in the Civil Service unions appears to be the outcome of apathy or neglect on the part of the general body of members rather than to any exceptional effort on the part of Communists themselves, but nevertheless the situation is regarded as most dangerous to security, and the Committee suggests that any department should have the right in respect of establishments or staff employed on secret work to deny access to or to refuse to negotiate with a named trade union official when it had reason to believe him to be a Communist under the definition used in the purge procedure. It is envisaged that the department would be required to challenge the union official formally and that he would have a right of appeal to the Three Advisers.

The Committee recognizes that this suggestion has drawbacks, if not risks of its own, and opposition to it will not be minimized in the absence of any evidence that Communist union officers have been detected in any act of espionage. Nevertheless, it is right to hold that it is essential that Civil servants should understand that trade union and staff association officials are not entitled, merely by virtue of their office, to be given secret information. The difficulty, however, is one that could well be resolved by a wise and far-reaching campaign of education, as the Committee itself seems to be aware when it observes that the dangers of the present situation are aggravated by the fact that very few people are conscious that the dangers exist. Of itself, the process of education in secrecy should foster loyalty if widely conducted, and specifically the Radcliffe Committee believes that Civil servants who participate in staff negotiations should be warned in general terms that the Communist representation among Civil Service union officials is disproportionately high and that they should be as careful to avoid disclosing secret information to union officials as to anyone else outside the Government service.

To the scientist perhaps the most welcome feature of the report is its discussion of security classification, even if no new constructive proposals emerge. Witnesses before the Committee were unanimous that the security system is at present trying to protect too much. Many documents are being given a security classification higher than is really necessary or in circumstances where no classification is really needed at all, and classifications originally necessary are retained after the need for them has disappeared. This view is so widespread that the Committee does not think it can be ignored, and it regards the situation as representing a serious defect in the security system. Security arrangements are probably more effective the more limited the field to which they are applied, and accordingly the Committee suggests that the time is ripe for a high-level re-appraisal as to what relative priorities of security ought to be given to which types of information. In the sphere of defence, for example, radical changes in strategic thought do not appear to have been accompanied by a parallel reassessment of security priorities, although a start has been made towards reassessing the relative priorities appropriate to the security of defence, economic and political information.

This reappraisal should be pressed forward, but the Committee recommends that it should be supported by amplification in each department of the standard definitions with examples drawn from its own field of activity. It recommends also that some limitation of authority to apply the higher security classifications should be seriously considered; alternatively, such classifications should be regarded as provisional until confirmed by an officer above a certain rank. Further, the Committee suggests extending to other fields the technique of the body which determines and regularly reviews the security gradings to be applied to classified information of a technical nature, while, with full awareness of the difficulties, it recommends that departments should make a determined effort to check the further accumulation of papers with obsolete classifications.

No one of these measures alone would probably have much effect in reducing the volume of classified information, but in the aggregate the effect of applying such measures firmly and intelligently might be really considerable and the security system would be strengthened at the same time. Constructive thought, however, is required continuously and some of this must come from the scientific and technical side if downgrading of information is to be achieved and the mass of restricted papers kept to a minimum.

Here, and again in the question of personnel security and what is known as the Positive Vetting Process, it is very largely a matter of common sense and of education in security of a type designed to encourage co-operation and understanding. Moreover, as the report also emphasizes, this is not a matter for the Civil Service alone: it involves industry also, at least to the extent of Government contractors.

The Radcliffe report is marked throughout by this refreshing air of common sense. It offers no support for the indiscriminate application or extension of security measures or procedures, and its respect for individual rights is as unmistakable as its recognition of the vital importance of an intelligent and adequate security system, even if no feasible system can offer complete assurance that every member of the public service who has access to secret information will prove trustworthy at all times and in all circumstances. Maintenance of a high standard of physical and documentary security will always remain of the first importance and the regular and proper observance of standards or procedures depends largely on the provision of adequate training and education.

Once again the Committee puts first a deliberate attempt to apply the principle that classified information should be kept to a minimum and disseminated no wider than is required for the efficient discharge of the business in hand. Those Civil servants or Service officers who are appointed to deal specifically with security questions should be given some formal training in security work; this training should be the responsibility of the Security Service and carried out by that Service. Even more vital is the much wider task of persuading the general body of public servants who are handling, or are liable to handle, classified information that security is a matter of practical importance and for this a sustained and skilfully directed educational effort to overcome the attitude of mind which questions the existence of any substantial threat to security is required. The Radcliffe Committee recommends that a programme on these lines should be drawn up. However, the effectiveness of any such programme will be greatly strengthened if scientists and technologists, who rightly emphasize the handicap to scientific and technical advance which any measure of secrecy in regard to scientific information represents, and also the futility of attempting to maintain secrecy in such matters for more than a limited period, associate themselves constructively with practical security arrangements. The difficult task of declassification could sometimes be assisted, for example, if scientists or technologists were prompt in directing attention to independent publication of such information as a result of work elsewhere. The report considers in some detail the problem of Press co-operation, but scarcely refers to the co-operation of the scientist and technologist. Such co-operation, however, is no less essential: in a free society, understanding, intelligence and goodwill are indispensable elements in any worth-while security system, and without them it is unlikely to function effectively. The ultimate safeguard of the individual as of the State rests on vision, sense and untiring vigilance.

MAMMARY ACTIVITY

Milk

The Mammary Gland and Its Secretion. Edited by S. K. Kon and A. T. Cowie. Vol. 1: Pp. viii + 515. 14 dollars; 100*s*. Vol. 2: Pp. ix + 423. 12 dollars. 86*s*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.)

BEGINNING in a small way nearly thirty years ago, research on the physiology and biochemistry of lactation (including research on the morphogenesis, histology and cytology of the mammary gland) has been providing, at a steadily accelerating rate, new knowledge on every aspect of mammary activity. The reasons for this rising output of significant new information are not far to seek. In the first place, in most western countries, as also in New Zealand, Australia, India and the U.S.S.R., milk production and processing together form a major industry, increasingly concerned with science and technology. As a consequence, funds for research, either from State or industrial sources, have become available both for the investigation of *ad hoc* problems and for more basic research in lactation. In the second place the important part played by milk and milk products in human nutrition, particularly in the nutrition of the young (a part emphasized by war-time experience), has been increasingly recognized, with renewed interest in the detailed reasons for their nutritional importance. In the third place, the astonishingly active processes taking place in the mammary gland, which were almost completely neglected for many years, have grown in interest and provided a fascinating field of research for an increasing number of research workers. In addition, a substantial inducement to many of these workers has been that many of the findings have had not only academic importance, but also applications in the dairy industry or in human or veterinary medicine.

The two volumes under review meet a need which has been felt for some years by students and teachers of mammary physiology and biochemistry for the bringing together in an authoritative text of the research findings that have emerged during the past decade or more on various aspects of mammary function. These recently published volumes provide such a text in the form of a connected series of short monographs, each written by a person or a small group that has made original contributions to knowledge of lactation. It is especially appropriate that the volumes have been organized and edited by members of the staff of the National Institute for Research in Dairying, Reading, since the stimulus and early impetus to much of the work recorded came from the pioneer investigations begun in that Institute in the early 1930's. This is particularly the case as regards the endocrinological control of lactation, the synthesis of milk constituents, the relation in the ruminant between diet, rumenal fermentation and milk quality and volume, and the composition and nutritional value of milk.

In all, there are twenty chapters—virtually short reviews, each with a comprehensive bibliography—in the two volumes. The chapters are conveniently associated into six groups as follow: (1) the mammary gland, morphogenesis, structure and growth; (2) functional physiology of the mammary gland; (3) lactation in man and farm animals; (4) biochemical activities of the mammary gland; (5) nutrition of the lactating animals; (6) nutritional value of milk (including a chapter on immunological aspects of colostrum). Though one or two of the individual chapters are a little discursive, all are a pleasure to read and of engrossing interest to any advanced student of mammalian (including human) physiology or biochemistry. Excellent author- and subject-indexes are provided.

The volumes, if a little expensive for British pockets, are very well produced and adequately illustrated; no library concerned with physiology or biochemistry, with human or veterinary medicine or with dairy science can spend 186*s*. to better advantage than by their purchase. H. D. KAY

QUEST FOR NATURAL LAWS IN PLANT ECOLOGY

Introduction to Plant Ecology

By Dr. Maurice Ashby. Pp. vii+249. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1961.) 25*s*. net.

The Plant Community

By Prof. Herbert C. Hanson and Ethan D. Churchill. Pp. xii+218. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1961.) 40*s*. net.

THE science of plant ecology is growing slowly out of its early descriptive phases and is becoming analytical and tentatively synthetic. It is no overstatement to say that the contribution of the present and next generation of ecologists may well be the establishment of principles analogous to those governing Newtonian physics or genetics.

Dr. Ashby's book, confessedly not new, is aimed to give pupils in school sixth forms and first-year university undergraduates a synopsis of these current aspects of plant ecology. He stresses the actual natural situations and the reactions of plants to them, making autecology the basis of his approach, using nine out of eleven chapters to develop a review of soils (thoroughly), root systems in relation to soil, effects of light, temperature and water on shoots, and biotic factors. He follows on with a study of "aggression", analysing factors concerned in spread and dispersal of species. One chapter is devoted to the nature of plant communities, succession and climaxes. A useful chapter and an appendix cover aims and methods of studying vegetation. A bibliography is provided.

The book is fairly well produced, having regard to its price, and contains lucid figures and some photographs. Even when price and costs are borne in mind, English text-books are not generally as attractive as American ones: an important point is arousing interest in schools. One has but to see how sixth-form pupils become absorbed in American books to appreciate the advances needed in book production elsewhere.

Dr. Ashby has trodden in the footsteps of the Pasteur of British ecology—the late Sir Arthur Tansley—and has woven into his work some of the modern developments of the topics Tansley the pioneer delineated. Thus we find chemistry, statistics and factorial interactions rightly stressed.

It is no fault of Dr. Ashby's or the American authors that plant ecology has become tainted with a most unfortunate form of anthropomorphism in the use of words like 'tolerance', 'sociability', 'fidelity', 'aggression', 'peaceful relations' and 'competition'. Perhaps the trend began with Darwin's use of the phrase 'struggle for existence'. Do we really have evidence of a plant psychology? Do we really have evidence that seedlings can act 'aggressively' (that is, launch an unprovoked attack)? Or are we as scientists so poor in vocabulary that we are unable to choose (or adapt classical) suitable words to express the interaction of physico-chemical systems concerned in given situations, for example, climate, soil and organism? Perhaps it is too late for ecologists to change their terminology; but the International Botanical Congress at Edinburgh in 1963 may find it possible to examine this unfortunate nomenclature.

Prof. Hanson and Mr. Churchill set out from a different starting point from Dr. Ashby's. They aim to supplement an elementary course by approaching the study of plant communities analytically and synthetically: a synecological study. Thus more than half their beautifully produced American book is concerned with the factorial interactions which influence groupings of species. It is not derogatory to suggest that in this liberally illustrated work, with many good photographs and tabulated information, some diagrams could have been more lucid than they are.

It is in the fourth part of the book that one glimpses the new synthetic approach to the classification of plant communities ('synsystematics'). Here are set out the inklings of the new principles, such as the predictive value of the presence of a given species, acting not merely as an indicator, say, of soil-type, but also as an index to the other species that will occur in the community. This book might well find a place on the shelves of school and university libraries to give reading matter to follow up an elementary course.

At a time of grave responsibility for agriculturists in the feeding of the world, the more an understaning of ecological principles and their application there is, the better for us all. Both books contribute to this end in different ways. L. M. J. KRAMER

MORPHOGENESIS

Advances in Morphogenesis

Vol. 1. Edited by M. Abercrombie and Jean Brachet. Pp. xiii+445. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 89*s*. 6*d*.

INVESTIGATIONS over the past thirty years or so have, for all practical purposes, converted the embryologist into a morphogeneticist. Embryology, *sensuo stricto*, has grown enormously; some allied kinds of investigations, such as in regeneration, now form a respectable part of it. The subject has also spread into interesting and valuable contact with other biological endeavours; one recalls the influence on the subject by studies in, for example, many aspects of cell biology, such as in genetics, in protein synthesis, on micro-organisms, in immunology, etc. The erstwhile embryologist now not only has his finger in a pretty big pie of his own, but also has a hungry eye focused on the pies of many others. Such gluttony is exciting, but it brings problems in its trail; more and more does it become difficult to see the main themes of the meal or even indeed to partake of it all.

In *Advances in Morphogenesis* the editors are making an encouraging and timely attempt to meet this situation. Their conception of morphogenesis is rightly a wide one—"any study of the new formation or of the remodelling of living material". In this and subsequent volumes they hope to "encourage the synthesis of some much-needed general ideas about mechanisms of development" and to "discourage the proliferation of clones of scientific thought". They hope to do this by publishing various kinds of review articles, giving authors, to judge from this first volume, space enough adequately to develop these general ideas.

There is always a danger in reviews of a lack of detail, and that those most interested know all about it anyway. The papers in this volume are generally successful in riding these dangers. It would be surprising if at least some of the essays did not provide food for thought for most morphogeneticists. The articles will also commend themselves to advanced students in the subject, for whom there has been insufficient reading matter of this kind.

The contributions to this volume range from a consideration of the structural and dynamic aspects of development of the teleost egg (Ch. Devillers), the embryology of ascidians (G. Reverberi), and the problem of the organizer from the chemical point of view (T. Tamada); through the morphogenesis of the eye (G. V. Lopashov and O. G. Stroeva) and limb (Edgar Zwilling), and regeneration of appendages (R. J. Goss) in vertebrates; to a rather less embryological approach in the differentiation of vertebrate pigment cells (C. E. Wilde), the action of morphostatic substances and the role of proteases in regenerating tissues and tumour cells (F. G. Lehmann), and determining factors in cell growth in relation to morphogenesis in plants (F. C. Steward and H. Y. Mohan Ram). It is not possible to comment individually on these contributions in a short review such as this; they are generally lively and stimulating, although not all the articles are entirely original. Of particular interest, perhaps, is the inclusion of material on plant morphogenesis; a greater *rapport* between botanists and zoologists will be of considerable benefit to both. The standard of production of the book is high and it contains but few errors. In addition to the usual reference lists associated with each article, the volume contains comprehensive author- and subject-indexes.

L. J. Hale

ASPECTS OF SOCIOLOGY

Hobhouse Memorial Lectures, 1951–1960

Pp. vi + 284. (London : The Athlone Press, University of London, 1962. Distributed by Constable and Co., Ltd.) 25*s*. net.

TEN annual lectures delivered under the auspices of the Hobhouse Memorial Trust are brought together in this volume. Although at first sight there seems to be little relation between subjects ranging from the philosophy of history, through psychology and archæology to the Welfare State, a certain coherence is given to the volume as one reads on and notes from the tributes paid by the various contributors to the memory of the late Prof. L. T. Hobhouse that each reflects some facet of Hobhouse's wide interpretation of sociology. Hobhouse was one of the great pioneers in a subject which has had a hard struggle for recognition in Britain, though elsewhere, notably in the United States, it is much studied and exerts great influence.

The lectures included in this volume were delivered between 1951 and 1960. Dr. Charles Singer opens with an exceptionally well-written and stimulating account of "Technology and History", and is followed by Sir Cyril Burt on "Contributions of Psychology to Social Problems" in what is by far the longest, but probably the most outstanding, of the ten lectures, illuminating the border country where the spheres of psychology and sociology overlap. Other lectures reprinted which are concerned with problems of social change and development are those of Prof. D. M. Mackinnon "On the Notion of a Philosophy of History", Sir Aubrey Lewis on "Agents of Cultural Advance" and Sir Mortimer Wheeler on "Aspects of the Ascent of a Civilisation".

A second group of lectures deal with social policy. Prof. W. A. Robson in his lecture on the "Welfare State" argues that the aim should be to bring about conditions which, while ensuring a relatively high general level of welfare, will be such as to evoke the exceptional efforts of men and women of outstanding ability. Gunnar Myrdal, who combined the roles of professor and international civil servant, examines "Realities and Illusions in Regard to Inter-governmental Organizations", while Prof. A. Macbeath poses the question "Can Social Policies be Rationally Tested?".

The remaining two lectures do not fall readily into any classification. One is a valuable, balanced and most interesting account of "The Curious Strength of Positivism in English Political Thought" by Mr. Noel Annan, provost of King's College, Cambridge, and the other, by Mr. T. H. Marshall, discusses "International Comprehension in and through Social Science". Mr. Marshall believes that the chief need in sociology is for the deeper study of the differences which distinguish one culture from another, and would like to see comparative studies, for example, of Europe and Asia in which European and Asian sociologists collaborate, each studying both cultures.

Taken together, though their quality is somewhat uneven, the ten lectures give an excellent idea of the contributions which sociology can make to our present problems and to our knowledge of society.

K. G. Fenelon

LARGE STRUCTURES OF HYDRAULIC ENGINEERING

Irrigation and Hydraulic Design

By Prof. Serge Leliavsky. Vol. 3: Hydraulic Structures for Irrigation and other Purposes. Pp. xvii + 765 (825 figures). (London: Chapman and Hall, Ltd., 1960.) 273*s*. net.

THIS, the third volume of the author's monumental work on irrigation and hydraulic design, is mainly concerned with the larger structures of hydraulic engineering. The first volume dealt with the hydraulic effects following the introduction of a structure into a river including seepage under structures, and the second with the planning and design of irrigation schemes and associated minor structures such as regulators, barrages and siphons. The present volume, comprising Chapters 9–13 of the whole work, discusses structures which are not necessarily connected with irrigation, but may be intended for navigation or hydraulic power production. It may therefore be of interest to a slightly different section of engineers than the second volume, and it is fortunate for such specialist readers that each chapter is largely self-contained.

The scope of the book is sufficiently well described by the chapter headings, namely: "Diversion Headworks in Alluvial Rivers" (9), "Locks" (10), "Arches" (11), "Dams" (12), "Electrification of Irrigation Works" (13), and it is written in the lucid style which we have come to expect of this author. The text is extremely well illustrated, as will be evident from the number of figures, and includes a number of dimensioned drawings of existing structures, the most detailed of these being a set of working drawings showing the arrangement of the steel bars in a reinforced concrete skeleton of a completed power house.

As one might expect from an author with wide experience in Egypt, many of the works chosen for detailed examination were built on the Nile and much space is devoted to the formidable difficulties associated with structures founded on alluvium. For example, much of the first chapter is concerned with the types of cut-off utilized in this situation, the construction of temporary sudds, de-watering and methods used in building the floor of such structures. In the second chapter also, particular attention is given to the design of locks with continuous concrete foundations, which are necessary where the soil is particularly treacherous. This leads to a certain lack of balance of the treatment, which is particularly evident in the chapter on dams. This chapter takes up more than a third of the book and deals with the structural design of gravity, arch, buttress and hollow dams. The gravity dam is treated in great detail and includes an analysis of the thermal movements and stresses in dams and the effects of uplift. In contrast, the treatment of the others is somewhat superficial, although a number of references is given to supplement the text. This is perhaps natural, for gravity dams have been in existence for centuries and a well-organized body of theory and experience is available for their design, while arch dams are a relatively recent innovation and their structural design is not yet standardized.

Throughout the book, as in previous volumes, the approach is historical and discursive and the author has been at pains to show the gradual evolution of design methods and modifications in the light of experience. Such a treatment is invaluable to the student and research worker, and it may also commend itself to those designers who are not only interested in the more accepted design methods but also in the fringe areas of their subject where progress is still being made but advances are not yet consolidated.

G. H. Lean

MINING RESEARCH

Proceedings of an International Symposium on Mining Research

Held at the University of Missouri, February 1961. Edited by George B. Clark. Vol. 1: Pp. xii+1–472. Vol. 2: Pp. vi+473–859. (London and New York: Pergamon Press, 1962.) 200*s*. net per set.

The proceedings of the symposium held at the University of Missouri in February 1961 have been reproduced in two volumes, containing a total of 50 papers. Volume 1 contains 31 papers, 19 of which are concerned with explosives and blasting. Three out of the 19 papers deal with the composition of ammonium nitrate—fuel-oil explosives, and four are concerned with their application. There are twelve papers on various aspects of blasting, one basic research into detonating pressure, and the remainder on applied research including the evaluation of explosive performance, techniques of blasting, efficiency of blasting, pneumatic cartridge loaders, pre-splitting, nuclear and chemical explosive cratering, blasting in cherty metallic iron formation, bench blasting with ammonium nitrate explosives and millisecond delay-firing.

The remaining contributions in Volume 1 consist of fundamental studies of liquid–solid systems in pipelines and steel wave behaviour in percussive drill-steels. Applied papers included the gyro-theodolite, mine environmental studies (a review), the life of drill steel equipment, the Castaing microanalyser, exploratory boring, applied statistics to determine mine working, methane drainage, ore analysis by X-ray and by γ-ray spectroscopy, and statistical analysis to plan sampling programmes.

Volume 2 contains 19 papers. There are several basic studies in rock mechanics, including direct measurement of strains by photoelasticity, behaviour and strength of rocks, correlation of rock properties, sonic techniques in exploring roof strata and roof bolting. Contributions to applied research in rock mechanics include block caving, subsidence, changes in stress accompanying wall closure in steeply dipping stopes, determinations of stress around an underground mine opening and the determination of dynamic elastic constants of rocks.

In addition to rock mechanics, Volume 2 contains papers on the use of jet-piercing for blast-hole production in taconite, disturbances resulting from shot-firing and also basic researches comprising studies of shock-waves in solids from explosives, effects of transient stress-waves in rocks, distribution of stress beneath a statically loaded drill-bit, stress-pulses in percussive drilling and the basic mechanics of coal ploughing.

The papers presented on explosives and blasting and on rock mechanics alone represent a substantial addition to the literature in these fields of study. The remaining papers, a little less than half the total, cover several aspects of mining research and include some interesting and very useful individual contributions. Half the papers presented come from the United States and the remainder from Great Britain, Sweden, France, Japan, West Germany, the U.S.S.R., Canada, Czechoslovakia, Austria and India.

The standard of the papers is high, and they represent a nice balance between fundamental studies, applied research and field trials. The work will be invaluable for reference purposes as well as providing much new information of immediate practical value. Its appeal will be not only to the mining engineer, but also to the civil engineer, engineering geologist, minerals engineer, etc. Most of the papers have a list of references or a bibliography attached, and there is a useful index at the end of Volume 2.

A word of praise is due to the editor for the presentation of the collected papers, which are conveniently arranged and suitably concise The large number of graphs, line diagrams and photographs provide neat, adequate illustrations, and the two volumes are well produced with large clear print and attractive binding.

The work constitutes an important and welcome addition to the literature on mining research, and is a good example of the high standard of research presentation which can result from an international symposium.

W. G. Thomas

Short-range Guided Weapons

By J. Clemow. (Temple Press Monographs on Rockets and Missiles.) Pp. vii + 79. (London : Temple Press, Ltd., 1961.) 15*s*. net.

THIS is the first monograph of a series, the later numbers of which will cover missile control, missile guidance, missile aerodynamics and propulsion—both solid-fuel rockets and ram-jets. This monograph is meant to serve as an introduction to the series. After considering the advantages of guided weapons and outlining the main parts of a guided missile it considers the same areas as will be covered by subsequent monographs. The last chapter deals with the hypothetical design problem of a particular ship to air missile and indicates some of the overall system problems involved in guided missile design. A selected bibliography is included.

This monograph provides a very adequate introduction to the subject, particularly bearing in mind the limitations which are inevitably imposed by security and the problem of covering a very wide range of techniques. It will be particularly interesting to those who have some scientific and technical background and as a useful introduction to the remainder of the series for any young professional missile engineer beginning his career.

In the hypothetical design problem some of the introductory general remarks are rather dogmatic and perhaps stem from the author's particular experience. Some of the remarks would certainly be qualified by other experts in the field. This chapter does give an indication of the complexity of the system problems involved, but with the limitations set by security and the allowable details it inevitably presents a somewhat oversimplified picture.

J. D. CLARE

Rhythmic Activity in Animal Physiology and Behaviour

By Prof. J. L. Cloudsley-Thompson. (Theoretical and Experimental Biology: an International Series of Monographs, Vol. 1.) Pp. vii + 236. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 54*s*. 6*d*.

THE rhythms with which this book is concerned are mainly the diurnal, lunar, tidal and seasonal ones, expressing themselves in an animal's behaviour or in those aspects of its physiology which border on behaviour. Prof. Cloudsley-Thompson's approach is broad and his erudition considerable. His treatment of his material is highly individual. His style is breezy rather than rigorous and some of his writing shows signs of haste (for example, "They claim these rhythms appear to be statistical rather than overt, for they become apparent only when the data of several days is averaged", p. 109). The result is a stimulating, unorthodox and somewhat slapdash review, most valuable perhaps to the outsider or newcomer to the field. Such a reader can scarcely fail to be interested by Prof. Cloudsley-Thompson's exposition and will be duly introduced not only to all the main problems on which attention is focused at present but also to the names and attitudes of the leading investigators. The ecological aspect is emphasized. On the Brown–Pittendrigh controversy, the author concludes: "Until further evidence is available it will be wise to keep a fairly open mind". The rhythms the periods of which do not happen to coincide with those of environmental cycles receive attention when relevant to the main theme. There is a useful bibliography.

G. P. WELLS

Bird Study

By Prof. Andrew J. Berger. Pp. xi + 389. (New York and London: John Wiley and Sons, Inc., 1961.) 72*s*.

BIRD STUDY is well named, for it is a book about the study of birds from all angles, including observation in the field and anatomical details. Though written primarily for the student it will be helpful even to the most advanced ornithologists, who will find much in it to interest them and give them material for thought.

The author, Prof. Andrew J. Berger, is an American, and the background of his work is the vast bird population of the North American continent. He writes, "the emphasis of this book is on the living bird and primarily of North American birds", and he never forgets the student to whom, as already said, this volume is chiefly addressed.

It is no doubt in consequence of this that he is not very much concerned with other readers, including natives of Britain, who may occasionally find the use of American popular names a trifle confusing; for example. the many references to "the robin", which remarks do not concern the pert little red-breasted bird so familiar in England, but a handsome member of the Turdidae of similar coloration, this latter being named after the European robin by the early settlers.

However, this is of small importance where there is so much of general excellence, including a large number of photographic illustrations and many diagrams and line drawings. These are illustrations in the full sense of the word and assist the text of the eleven chapters that cover so much ground, beginning with an introduction to the bird. moving on to "Field Identification", and continuing to the last chapters on "Conservation" and "Systematics".

FRANCES PITT

Approaches to Thermonuclear Power

By Dr. R. F. Saxe. (Nuclear Engineering Monographs, No. 10.) Pp. x + 65. (London: Temple Press, Ltd., 1960.) 12*s*. 6*d*. net.

THE book begins by discussing the conditions necessary to the operation of a fusion reactor, not considering problems outside the core such as losses of energy in the field coils or the regeneration of tritium in a deuterium–tritium reactor. A theoretical outline of plasma behaviour at high temperatures is then given, adequate for the purposes of the book. However, it would be better to say that instabilities grow at rates dependent on the Alfvèn speed rather than on the sound speed in the plasma. Next the linear pinch is treated, the early experimental results being quite well described. The fourth chapter reviews toroidal machines and curious explanations appear. To give two examples, a typical characteristic for a symmetrical double Langmuir probe is shown, and the text describes this in detail as the behaviour of a single probe. Again, it is stated that deuterium cannot be used for spectroscopic measurements of temperature because of its large Stark effect, though deuterium is stripped completely in hot plasmas and does not radiate at all. In the last chapter, mirror machines are better handled. There is a final résumé in which ion energies seem to be regarded as equivalent to ion temperatures, a most dangerous confusion. This monograph would considerably enlighten the inquiring engineer for whom it is written; but the physics is sometimes misleading and the description of achievements optimistic.

P. F. LITTLE

UNCONSCIOUS MENTAL IMAGERY IN ART AND SCIENCE*

By ANTON EHRENZWEIG

THE psychoanalytic concept of the unconscious is often refuted as unscientific because it is impossible to prove unconscious processes empirically. This is a narrow view. The unconscious is not a physical fact, but merely an explanatory concept which allows us to understand phenomena that could not be accounted for otherwise. Neither for that matter can an electron be observed directly. A few flickerings on a screen may be seen; all the rest is deductive reasoning producing explanatory concepts that are not open to immediate demonstration. In the case of the unconscious it follows from its definition that it cannot be observed directly.

I myself had to expand the use of the term 'unconscious' to a new field. I needed the concept of an unconscious mental imagery underlying creative thought, in order to break the deadlock which has held up the progress of psychoanalytical æsthetics for half a century. The beginnings of psychoanalytical æsthetics were highly promising. Freud had analysed the typical structure of dreams in which he recognized the working of the so-called primary process characteristic of the unconscious. In order to interpret the dream he had to assume that the primary process distorts meaning in a very specific way, for example, by condensations, displacements, representations by the opposite and the like. By undoing these distortions Freud was able to show a new meaning behind our dreaming activity which until then was usually dismissed as meaningless and the result of mental dysfunction. It showed the value of the new interpretation technique that Freud was able to transfer the whole inventory of dream symbolism to other fields, pre-eminently clinical, and also to a new understanding of æsthetics. It could be generally assumed that wherever typical primary-process forms of condensations, displacements, etc., occurred the primary process of the unconscious had been at work.

One of the first applications in the æsthetic field was surprisingly the joke, a border subject of æsthetics. Classical æsthetics had recognized that the specific structure of the joke had something to do with its laughter-raising quality. The joke liked to express too much by too little. Polonius in "Hamlet" speaks of brevity as "the soul of wit". The search for the joke's structure was akin to similar searches for æsthetic form. Men like Hogarth would link æsthetic feelings of sublimity with large awe-inspiring structures or gracefulness with slender small-scale shapes. But gradually the æstheticians lost heart in establishing empirically valid links between subjective feelings and objective structures outside. There was too much disagreement about what was beautiful. What seemed ugly at first sight gained beauty as it became more familiar. Æstheticians lost prestige by invoking allegedly universal laws of beauty in order to discredit the modern art of their time. Hanslick's polemics against Wagner are the best-known example. Such laws did not need refutation. It was only necessary for the modern art of the time to become universally accepted to prove the futility of the æsthetic laws. This frequent failure contributed to a gradual change of aim in nineteenth-century æsthetics. Instead of searching the objective structures in the physical outside world, æsthetics turned to analysing the subjective experience. Fechner and Lipps helped in this re-orientation of æsthetics towards psychology.

It seems paradoxical though gratifying that Freud, himself a psychologist and as an æsthetician influenced by Fechner and Lipps, should have solved the æsthetic border problem of the joke's structure in a truly classical manner. In his unjustly neglected book *The Joke and its Relation to the Unconscious* he applied the structural analysis of dreams to a similar analysis of the joke. He was led into æsthetics by the disconcerting reaction of some patients when he interpreted primary-process material. They laughed, as though he had told them a joke. Unabashed Freud went on to examine ordinary jokes and discovered that they expressed a half-hidden obscene or aggressive idea in the distorted and distorting way characteristic of the primary process. 'Seeing through' these distortions arouses laughter.

Freud started his review of jokes with a well-known joke by Heine cast in the form of a condensation. Heine condenses two words, 'familiar' and 'millionaire'. In the joke a poor man boasts about the intimate way in which he was entertained by a very rich relative. But instead of speaking about the rich man's gratifying familiarity, he distorts the intended word and speaks of the 'famillionaire' treatment he had met. His relative had not really been friendly, but instead displayed the superficial politeness that emphasized the social gulf between him and the poor man. Telescoped words like 'famillionaire' also occur in dreams. As words are rare in dreams, we find more often condensed images. Surrealist artists tried to imitate dream images deliberately by producing such condensations. Salvadore Dali would paint a chest-of-drawers sprouting human limbs. The joke and the dream share other primary-process structures. Displacement is one of them. The unconscious generally tends to express its symbolism in inconspicuous details which escape detection. The interpretation of dreams must often shift the displaced emphasis to those unobtrusive details. Such unexpected displacements of emphasis are also witty. Freud quotes one of the many jokes about the so said uncleanliness of Jews. One Jew meets another in the vicinity of a public bath and asks him: "Have you taken a bath?" and gets the surprised answer: "Why, is one missing?" The answer displaces the emphasis of the question to the inconspicuous word 'taken', giving it the stronger meaning of 'taken away', 'stolen'. Another important primary-process form in dreams and jokes are representations by the opposite which in a joke acquire a sarcastic meaning. In terms of normal rational thought primary-process distortions are nonsensical. We have to interpret or —in the case of the joke—have to guess their hidden meaning. I maintain that the unconscious mind understands the concealed meaning because on an unconscious level words and images lose their precise definition and become undifferentiated, until a limit is approached where all distinctions are submerged in a single oceanic image.

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* Substance of a lecture given before the British Society of Aesthetics on April 4.

But let me first continue my account of Freud's success as an æsthetician. I stated that Freud by identifying the joke's structure as a typical primary-process structure solved a border problem of æsthetics in a classical manner; subjective feeling was firmly related to objective structure. But Freud did more. If the joke was cast into the forms of the primary process this proved that it was shaped spontaneously by the unconscious mind. This spontaneity can be demonstrated by a purely behaviouristic observation. If we catch a witty person in the act of inventing a really new joke, we shall notice how he will suddenly stop in his tracks. An absent-minded expression will creep into his face and then, as from nowhere, the new joke occurs to him. He will communicate it as soon as possible as though he was not quite sure of the full implications of his witticism. There is a good deal more to be said about the spontaneity and truly unconscious quality of a new joke. What matters to the æsthetician is Freud's discovery that any spontaneously created structures can be expected to show the imprint of the primary-process forms which he first discovered in the dream.

One could have expected, therefore, that Freud would continue his incursion into æsthetics and solve the problem of art form at least so far as it is created spontaneously and unconsciously without conscious deliberation and planning. Art was dear to Freud's heart, who was a master of the German language. Freud was pleased when he found that he could transfer the whole inventory of dream symbolism for a better understanding of myths, fairy tales and literary works of art. But if he could thus reach the unconscious content of art, he failed to explain its unconscious structure. I stated that it was legitimate to expect that the spontaneous forms of art should carry the impression of the primary process, perhaps even more so than the relatively shallow joke which expresses only lightly concealed obscene and aggressive ideas and not—like the dream and art—deeply repressed unconscious phantasy. Otto Rank was one of the first psychoanalysts to explore the structure of art in this way and searched art for primary-process forms such as condensations. He pointed out that the German word for poet, *Dichter*, meant the condenser. Myth, folklore and also art abound in dream-like phantasmagorias, composite monsters like sphinxes, chimæras, winged angels condensed from animal and human forms. But these occasional intrusions of primary-process structures did not add up to an exhaustive catalogue of all spontaneous forms in art. Apart from the impossibility of emulating Freud's achievement in the complete survey of all possible joke forms—nobody has succeeded in cataloguing all possible art forms—it proves impossible to relate a catalogue of art forms, however incomplete, to primary-process structures. Art form generally in its often stringent and logical coherence seems a far cry from the nonsensical twists of the primary process in the dream and the joke. Half a century has gone by since Freud's book on the joke and its relation to the unconscious was published, and psychoanalysis has not made substantial progress towards solving the central problem of æsthetics—the character and origin of the forms of art. I think we have to accept the failure as definite.

Acceptance of failure does not exclude progress because it allows examination of the possible reasons for the failure. The most celebrated example for a fertile interpretation of failure is the invention of non-Euclidean geometry. It came after centuries of vain attempts at proving all Euclidean postulates. The acceptance of that failure led to a new conception of space which contradicts commonsense visualization, but nevertheless helps to explain phenomena that otherwise would remain unexplained.

The difficulty in accepting the failure was certainly connected with the difficulty, if not impossibility, in visualizing non-Euclidean space. It keeps shifting and destroys stable focusing points. A similar difficulty in visualization may be involved in tracing the unconscious roots of art. One can interpret our failure to discover those roots in two ways. One can either deny that art has an unconscious sub-structure (this is what the classical theory of psychoanalysis, as expounded by E. Kris, ultimately did) or else one can assume that the unconscious sub-structure exists, but for certain reasons resists conscious visualization. This will be my approach.

I could never accept the current theory which confines the structuring process of art exclusively to the conscious and preconscious mind. This view is inconsistent with Freud's treatment of the joke, which according to Freud is spontaneously created by the primary process of the unconscious. The spontaneity of artistic creation can be far more profound. Though a large part of the artistic composition is usually produced by deliberate planning excluding spontaneity, important components of the composition may emerge without any premeditation and even run against the artist's explicit intentions. Yet the artist, if he is inspired, will accept them as integral parts of the artistic structure. Such form elements must be conceded roots in the unconscious mind that are even deeper than the relatively superficial spontaneity of the joke. Certain spontaneous form elements of art even share with the joke an initial impression of unorganized chaos. The nonsensical condensations, displacements, representations by the opposite that go into the making of a joke impress us as nonsensical chaos and distortions of normal rational thought so long as we have not guessed the joke's half-concealed meaning. But once we have laughed at the joke—which is the signal of sudden enlightenment—the formulation of the joke loses its chaotic quality and will appear as a cogent and neatly turned formulation of a witty idea. Freud in his detailed survey of jocular structures behaves very much like a *connoisseur* who does not tire of pointing out the relative æsthetic merits of various jokes. The same transformation applies to the spontaneous form elements of art, such as the chaotic-looking scribblings and textures of artistic 'handwriting' or the endless undulations of primitive song with its inarticulate *portamenti* and *vibratos*. We cannot adduce a conscious law for the application of such inarticulate form elements and must leave their execution entirely to intuition and spontaneity, but they still obey some æsthetic discipline which is no less cogent for not being open to conscious visualization and formulation.

When I first wrote on psychoanalytic æsthetics about fifteen years ago, I commented on the crudity of our conscious powers of visualization. which cannot possibly do justice to the complexity of the artistic structure. The chief limitation of conscious visualization is borne out by the extensive experiments of *gestalt* psychology. In normal vision we are forced to select from the available sum of stimuli only a limited number which can be drawn together into an easily focusable *gestalt* pattern. The rest recedes into an indistinct background. The visual field is thus always bisected into a selected figure standing out from a ground the details of which are lost. Now bisecting the visual field into relevant figure and irrelevant background forms is precisely what the artist cannot afford to do. I have just written of the hidden discipline ruling the seemingly accidental and spontaneous scribblings of artistic handwriting. Only a bad artist will treat such textures as accidental and less-significant background forms and concentrate on the prominent large-scale composition (the selected 'figure' of *gestalt* psychology). Every single detail however paltry-looking has to be firmly related to the overall structure. This need constitutes the extreme complexity of any work of art. It defeats our normal powers of vision, which as we have seen cannot scan the entire visual field in a single act of attention. I concluded at the time that the artist must have powers of vision transcending our normal conscious faculties of visualization. There must be an unconscious undifferentiated type of visualization which is free from the compulsion of bisecting the visual field into figure and ground and can scan it in its entirety with impartial equality.

Much to my delight recent experiments in subliminal perception confirmed this hypothesis. It was found that our subliminal perception can, in a fraction of a second, register far more stimuli than can our normal conscious powers of visualization and is able to register details belonging to the background with equal acuity as the elements of the figure.

'Subliminal' is only another term for unconscious. We cannot demonstrate subliminal perception directly, but have to infer it (as a purely explanatory concept) from conscious phenomena which could not otherwise be satisfactorily explained. Nevertheless, we can make statements about unconscious techniques of perception owing to the gradual transition between conscious and unconscious (subliminal) processes reflected in a successive waning of our powers of visualization. If we cut down with the help of a tachistoscope the duration for which a picture is projected on a screen, the weakening of the physiological stimulus tends to produce a kind of vision that is strangely akin to our dream vision. The flicking picture becomes increasingly vague and incomplete and is easily distorted in the manner of the primary process. When the subliminal threshold is reached even the faintest and vaguest flicker disappears and the screen remains empty so far as conscious experience is concerned. Vision has turned subliminal and unconscious. Subliminal vision proves its kinship with dreaming by the ease with which traces of the subliminal picture reappear in later dreams. Recently a New York psychoanalyst, Charles Fisher, took the same short cut which Freud took to the unconscious when he discarded hypnosis as a method of treatment. Fisher, after exposing subliminal images, asked his observers to make drawings by uncontrolled 'free' association. Traces of the subliminal images duly emerged. For the sake of experimental rigour Fisher intermixed truly blank slides among his exposures and significant differences resulted.

The same series beginning with vagueness and distortion, followed by the gradual dissolution of precise spatial relations and ending in total blankness, is found also in dreams and in creative work. Recent psychoanalytic research into dreams penetrated below the nonsensical but still distinct primary-process structures. At first precise space relationships become uncertain. The dreamer may feel opposite an immense wall (an example of the so-called 'dream-screen') and at the same time feel also inside that wall. The psychoanalyst–anthropologist, Roheim, treats this conflict between an undifferentiated inner dream space (dream 'womb') and a more articulate outer dream space as part of a basic dream conflict. Roheim often uses strangely undifferentiated language. The dreamer, he says, may have the experience of entering his own dream womb. The vague space experience may disintegrate ultimately into a completely blank dream-filled intense emotion pointing to the existence of unconscious-subliminal phantasy.

This 'full' blankness, like the hidden discipline of 'chaos', would be the result of failure in our conscious powers of visualization. Our conscious vision has learned to differentiate sharply not only between figure and ground, but also between articulate patterns as such in order to construct a precise space. *Gestalt* psychology once assumed that this ability or rather compulsion to select and differentiate patterns was an inborn faculty. But the survey by von Senden of case-histories of people born blind who acquired vision by an operation in adult life show the incredible difficulties encountered by them as they were faced by the undifferentiated colour patches filling the visual field. Many of them, sometimes due to lacking guidance on the part of the physicians, faltered in their purpose and could not muster the effort for learning to project patterns into the chaos. Some of them felt profound relief when blindness overcame them once again.

The facts of subliminal vision suggest that we retain on an unconscious level the infantile type of vision that does not yet differentiate the visual field. But what is primitive weakness in the child becomes creative strength in the artist who, according to my hypothesis, resorts to an undifferentiated low-level vision so as to control the overall structure of the work of art. Let me put on record that it was the need for this æsthetic control which first suggested to me the hypothesis of an unconscious undifferentiated vision and so anticipated the confirmation by experiments in subliminal perception. No such confirmation is as yet forthcoming for other facets of the hypothetical undifferentiated vision in the unconscious. But æsthetic facts suggest to me an unconscious-subliminal vision that is altogether free from normal spatial differentiation. I spoke of certain dreams which suspend the normal differentiation between inside and outside space. But in art the suspension of differentiated space seems to go farther. For example, it would be difficult to explain the well-known effect of the golden section in any other way. Try to divide a line according to the rules of the golden section and you will see how difficult it is. The section rests on a simple enough mathematical formula: $a : b = b : (a + b)$, that is to say, the smaller section relates to the bigger section as the bigger section does to the undivided whole. But comparing the two proportions in order to make them identical is difficult if not impossible because of their confusing spatial distribution. a is juxtaposed to b, but b is contained within the whole $(a + b)$ into which it is 'telescoped'. The eye movements taking the two proportions are quite different in each case and do not allow proper comparison. Yet the golden section has undoubted æsthetic merits; to have this merit it must be perceived without much ado, if not consciously then unconsciously by a vision that is not bound by the normal distribution in space. Again, the hypothesis of an unconscious vision cannot be demonstrated directly, but serves only to explain the otherwise unexplainable.

It could be said that the golden section is mathematically simple, but obscure as a visual fact, a contrast that may have given it its mystic panache. Once we assume that it can be perceived by a spatially undifferentiated unconscious vision, its mystique disappears. Mathematical simplicity and perceptual obscurity are also found in the serialization of musical elements. Mathematically it could not be simpler, that is, a permutation of a finite number of elements such as the twelve tones of the chromatic scale. But the permutation of the tones destroys all vestiges of repetition and recognizable pattern and so produces chaos in our commonsense experience. It is easy, therefore, to dismiss serialization as an intellectual artifice unrelated to direct musical appreciation. Æstheticians were not slow in invoking the *gestalt* laws of perception in order to reject serialization as perceptually meaningless. But the æsthetician better beware of imposing rules on the artist and is better advised to accept the facts of art even if they do not fit in with current laws of psychology. As serialization wins the day, as it inevitably must, we are driven to the conclusion that its regularity must be perceived, if not consciously, then by an unconscious undifferentiated mode of hearing that is not bound by the normal sequence in time. It would be capable of recognizing a theme in any possible permutation of its constituent tones, whether they are played back to front, mirrorwise or in any other sequence.

If we assume this unconscious lack of differentiation, of time as well as of spatial distribution, then the initial impression of chaos turns into a hidden order. The double aspect of undifferentiation, conscious chaos against unconscious order belongs to the core of psychoanalytical æsthetics. Psychoanalysis has accepted all along that the primary process of the unconscious was undifferentiated and did not distinguish between different categories of things or even between opposites. Freud compared the undifferentiated meaning of unconscious symbols to archaic languages which, like Latin, do not differentiate between opposite meanings. The Latin word *altus* means both high and deep. The words hostility and hospitality go back to the same Latin root *host*. I said at the time that the reason why the unconscious mind can see through condensations like 'famillionaire' (which fuses friendliness and hostility) is found in its failure to differentiate between opposites and expressing them by the same symbol. Psychoanalytic writing, as a rule, does not realize that there may exist an unconscious type of vision for which these undifferentiated concepts become actual percepts so that different things can be actually seen as the same thing or high and low give the same space experience. A recently published psychoanalytical book dealing with man's view of the world casually mentions a patient's dream in which a man talked to a chair. The author marvels at a vision that does not distinguish between a man and a chair and treats it as a rare intrusion of psychopathological imagery into a dream. This interpretation is a far cry from my own, which assumes that all unconscious-subliminal imagery fails to make the ordinary distinctions of space and time.

Creative thinkers, however, have known that such a vision underlies creative work. The French philosopher, Henri Bergson, describes the structure of creative imagination (intuition) in just this way, namely, as a kind of vision where the normal differentiation of space and time ceases to operate and where incompatible things can extend into each other. Bergson advises the reader to evoke in himself a state of intuition by trying to visualize incompatible things (as a man and a chair would be) occupying the same spot in space. The main point which I wish to make is that unconscious undifferentiation may be a primitive infantile mode of vision, but in creative work becomes a sophisticated achievement. If the painter undoes the division between figure and ground and holds the entire visual field in a single glance he certainly regresses to an infantile mode of vision which has not yet learned to differentiate the visual field. That the child has undifferentiated concepts is a familiar fact. For him all males are still 'dada' while he has already learned to treat his mother as a distinct individual. But as I have maintained 'dada' would be more than a primitive concept. It is probably also a percept. The baby sees all males as having the same appearance as we adults fail to perceive fundamental individual differences if the biological incentive is lacking. The flies buzzing around the lamp-shade really look the same to us, as do all Chinese before we have established bonds of love and hate with single individuals.

The artist and scientist suspends the normal differentiation between concrete things in order to refine his power of abstraction. Prof. E. Gombrich, the well-known art historian, once poked his gentle but fierce fun at the pretensions of modern abstract art by pointing out—in my view correctly—that the modern artist's much-vaunted power of abstraction differs little from the child's still weak capacity for properly differentiating his environment. When Picasso gradually pares down the naturalistic shape of a bull into an abstract cipher he reverts to the vision of the child playing with his hobby horse who treats a mere stick like a living animal. Nor is it increased power of abstraction that prompts the drunkard to lift his hat to the nearest lamp-post. But Gombrich does not really debunk creative abstract art because, as I was so anxious to show, creative imagination turns primitive weakness into a sophisticated achievement, a dynamic transformation that belongs to the essence of the sublimation process as psychoanalysis conceives it. It is perhaps wrong or an oversimplification to say that the creative mind passively 'regresses' to the child's primitive vision. He actively undoes the basic differentiations of time and space itself into new types of visualization such as may have never been actually experienced in childhood.

The abstract concepts of science resemble abstraction in art; they are based on undoing commonsense differentiations between concrete things. A scientific abstraction extracts from different, often incompatible, things a common property and turns it into a new abstract concept. What is not sufficiently appreciated is that this 'seeing together' involves a capacity for undifferentiated vision. A new abstract concept at first sight seems empty of all mental imagery. But it is empty only in the way in which a blank dream or a subliminal image appear empty though they are filled with unconscious imagery. I am of course speaking only of truly creative abstraction in science. It differs from a sterile empty generalization as truly creative abstract art differs from a flat and empty ornament. Creative abstraction in art as well as in science is supported on a subliminal level by the multitude of incompatible visions that gave birth to it in the first place and which cancelled each other out on the way up to conscious visualization.

The need for seeing incompatible images 'together' is more clearly discerned in periods of transition when science is still groping for new concepts or models that would resolve existing contradictions. I owe a hypothetical example to a physicist friend of mine who once discussed the two complementary models of light which represent two conflicting images. Light is visualized in one context as a wave, in another as a stream of solid bodies. My friend suggested that a future scientist possessing powers of abstraction superior to our own may have no difficulty in visualizing light in terms of a more abstract concept that is neither a wave nor a solid body, but both at the same time. Such a potent abstract concept could only be formed by suspending temporarily the commonsense differentiation between incompatible things. So long as we have not achieved this high degree of undifferentiation it seems as incomprehensible to us as is the undifferentiated world of the child.

Psychoanalysis knows as yet little about the lower levels of perception that become activated during creative work. So far it has been more concerned with the content of the unconscious and its fantastic symbolism. The study of creative work requires more. The complexity of scientific and artistic work is such that it presses us to look for explanations in a subliminal sub-structure of conscious imagery. Its misleading impression of unstructured chaos has held up depth-psychological investigation even longer than has the irrational content of unconscious phantasy. We have only caught a passing glimpse of its hidden order and efficiency. Our descent into the depth of mental imagery has just begun.

PSYCHOTIC ILLNESS AND ARTISTIC PRODUCTION

SUBSTANCE OF A FRIDAY EVENING DISCOURSE BEFORE THE ROYAL INSTITUTION

By Prof. G. M. CARSTAIRS
University of Edinburgh

MENTAL illness is no respecter of persons. It afflicts rich and poor, intelligent and stupid, the gifted and the untalented alike. From the patient's point of view, its visitation is usually unwelcome, bringing suffering and handicap; in this it has some resemblance to the mixed blessing of unusual artistic sensibility. Like the artist, the psychotic sees the world differently from ordinary men; for him it often assumes a personal, or a threatening, implication—but above all it is full of the unexpected.

In our everyday life we tend to surround ourselves with familiar things, familiar sights, sounds, routines, gestures; and familiarity dulls perception. We notice this when we first arrive in a strange country —the sights and sounds of that first day are more vividly registered than they will be in a few days' time. The artist and the schizophrenic in their different ways seem to preserve that acuteness of perception which Sir Herbert Read has described as "the innocent eye"; but the schizophrenic's awareness is not just the freshness of a child's outlook on the world. It is a heightened awareness indeed, but one heightened by dread. He is aware of an element of threat in his surroundings. This is especially true during the active stage of the illness, which will sometimes recur with renewed intensity after a period of relative calm.

The depressed patient lives in a world drained of life, colour and hope. He is painfully aware that he himself has changed, and he is filled not with self-pity (although his state arouses compassion in others), but with self-loathing which may lead to self-destruction. Fortunately, severe depression is a disorder of limited duration: the sufferer can emerge from the depths and return to active life again. This was the experience of one of the earliest painters whose mental illness has been fully documented.

Hugo van der Goes (1440–82), an early Flemish master, twice experienced depressive illnesses of psychotic intensity. He achieved celebrity while still a young man and his work was in demand in places as far apart as Vienna and Edinburgh. His *Trinity Altar* from Holyroodhouse includes portraits of James III of Scotland and of Queen Margaret of Denmark among the attendant figures. Van der Goes was a man of restless energy, but he was not a happy man. He was subject to bouts of depression, and, like many depressives, he began to drink to excess in an attempt to relieve his feelings. At the age of thirty-five he temporarily renounced his vocation and retired to a monastery. It was not until five years later, however, that he became frankly deranged, crying out in despair that he was doomed. The monks, mindful no doubt of David's playing to Saul in similar circumstances, tried to exorcise his fit by playing music before him; but it was only gradually that he returned to something like his former state. In 1481 he was able to paint again; his *Death of the Virgin* (Bruges) was completed in that year, but it was probably his last work: in the following year his madness returned and he died soon afterwards. He himself protested that it was his own fame and success which caused his mind to become unhinged: but this sounds like the typical self-depreciation of the melancholic. Critics, wise after the event, have discerned qualities of tension and seriousness in his work; but it is surely remarkable for the technical mastery which survived rather than for any revelation of his serious illness.

It was a different matter in the case of Piranesi (1720–78). He too was a man noted for precocious gifts and exceptional energy. Born in Venice, the son of a stonemason, he was already drawing the buildings around where he lived at the age of eight. and his work was the talk of the town by the time he was fifteen. He was apprenticed to an uncle who was an architect, but his early years were punctuated by violent disputes both in Venice and in Rome, where he studied for a time under Tiepolo. He quarrelled with his uncle, with his father and with Vasi, who taught him engraving.

At the age of twenty-one he made a characteristically impetuous marriage, after a courtship lasting five days, and set up the engraver's workshop which was to establish his lasting fame. From this date until his death at the age of fifty-eight he produced nearly 1,200 etchings, many of great complexity. For several generations his visions of the antiquities of Rome were the models for teaching in every European school of architecture. In 1745, however, appeared an extraordinary series of etchings—the *Carceri d'invenzione*, or *Imaginary Prisons*. Tradition has it that they were the product of visions seen in the delirium of a fever.

Six years later, when at the height of his career, Piranesi returned once again to the *Carceri* and brought out a second edition in which the fantastic prisons are even more eloquently inhuman, vast and sinister than before. This juxtaposition of belligerent impetuousness, violent energy and preoccupation with human despair and hopelessness suggests very strongly that Piranesi was a manic-depressive who experienced episodes of melancholia.

The great Spanish painter Francisco Goya (1746–1828) was another man in whom spells of furious energy alternated with fits of black despair. Like Piranesi, he was an impetuous quarrelsome man, involved in duels and pursued by irate husbands. He is remembered for his ruthlessly candid appraisal of the pride and folly of the very society of which he was an idol. At times he was seemingly almost overwhelmed by the malignant folly of mankind, as when he created his series of etchings *The Disasters of War*, a sardonic commentary on the Napoleonic invasion of Spain; at times too in his *Caprichios* he hinted at disturbing personal visions, as in the title page "The Sleep of Reason begets Monsters". Goya lived to be over eighty, and even in his old age he was beset by fits of melancholy, during one of which he painted the series of Pinturas Nigras, of which one of the most terrible is *Chronos Devouring His Children*: with macabre humour, Goya chose to hang this picture in his dining-room.

Melancholia is a condition which we can readily understand: we have all sometimes felt depressed. But schizophrenia brings about bizarre and quite unfamiliar distortions of reality. In its early stages it can sometimes stimulate a more intense though usually rather sinister awareness of the mystery behind normal phenomena. One is compelled to wonder in what does this 'mystery' lie: perhaps it is an attribute of the beholder. Earlier in the present century painters of the surrealist school—men like Dali, Magritte and Yves Tanguy—were quite frankly envious of the schizophrenic's faculty of living in a dream-like state, even though the dream should often prove a nightmare. In the early 1920's the term 'paranoid' had a positive value in æsthetic parlance, and surrealists made it their aim to paint frankly psychotic imagery. This was their tribute to the 'magical' quality of schizophrenic phantasy, a quality which was attributed to its origin in the unconscious mind.

The onset of an acute schizophrenic reaction can be accompanied by distortions of perception. Patients will describe strange lights in the sky, a changed appearance of familiar objects. Volunteer subjects who have taken hallucinogenic drugs such as mescalin or lysergic acid diethylamide have described a rainbow-like appearance around the edge of things. Perhaps this is what Edvard Münch saw when he painted his *Madonna* and *The Shriek*. A similar vision is apparent in the early stages of the remarkable series of paintings of cats executed by Louis Wain in the course of his schizophrenic illness. Wain's cats undergo remarkable transformations under the influence of his mental disorder: at first realistic, charming and full of life, they become stylized, fragmented into angular symmetrical patterns, horrific and bizarre, and finally disintegrate into a shapeless kaleidoscopic mass.

Another item in the Guttmann–Maclay collection provides an involuntary record of a violent schizo-affective episode in the life of an anonymous Victorian flower-painter, between the years 1863 and 1865. His paintings are at first meticulously drawn, studied and cold. Then, in May 1864 they change; warm colours and ripe sensuous forms appear, while on the facing page of his book he apostrophizes the woman he loves. The succeeding paintings become more and more ecstatic and confused: a woman's form appears among the foliage, a snake and a solitary eye. The confusion reaches a climax—and then quite suddenly it is over: a new series of neat, dispassionate flower-paintings (dated 1865) supervenes.

Another patient from Maudsley Hospital was an art student who already showed considerable technical skill: but during her schizophrenic illness her paintings became slapdash, hasty, almost incoherent: she began to write in phrases and words to emphasize her fear of overwhelming disintegration. Later, as her personality rallied again, she expressed herself much more coherently in a finger-painting of two nightmare faces.

These series of paintings have shown the artists' struggle with the illness which threatened to overwhelm them. In a lesser degree, perhaps, all art is an attempt to exorcise an inner disease; certainly Freud was not alone in his experience that a period of restlessness and minor ill-health seemed to be the prelude to all his bursts of creative work.

Some of the greatest art has been the fruit of suffering: never more so perhaps than in the tortured magnificence of the works of Van Gogh's last four years.

There are a number of defences against the schizophrenic nightmare. One of these is to rally one's intellectual resources in an attempt to define and contain the threats which arise in phantasy: this is the obsessional defence. Few painters have been so constantly assailed by those monsters begotten in the sleep of reason as Hieronymous Bosch. In painting after painting executed with astonishing precision of detail he has conjured up a whole universe of demons. His bestiary, of course, is drawn largely from the traditions of the Middle Ages; but he depicted them with a new sense of urgency because he was persuaded that the fate of the world and the destiny of mankind hung in the balance. It has long been known that he believed the alchemists to be in league with the devil to overthrow the world, hence the profusion of alchemical symbolism in his works; but recent research has revealed that he was a member of a secret millenial cult, the Brethren of the Free Spirit, and their counter-symbols can also be found in his paintings.

A few years ago I had occasion to treat a young, self-taught Canadian painter, whose pictures showed certain affinities with those of Bosch, except that where Bosch was obsessed with the imminent destruction of humanity, this patient was for a time pre-occupied exclusively with his own tortured ruminations, his own phantasies and his sense of being trapped and helpless. He depicted these feelings eloquently in a series of paintings, particularly in one entitled *The Maze*, in which he drew his own prostrate form with his skull sawn open to reveal a maze, enclosed with no escape. In each compartment of the maze he showed one of his recurrent, usually painful, obsessive memories.

This patient won through, after some years, to a relative peace of mind. He is still painting, but his work has lost its former tragic intensity. Bosch, if one can judge by his works, never ceased to be tormented by his acute awareness of the peril which threatened mankind. His favourite image, to be found in many of his works, is the owl which represents human folly.

Where, I wonder, is the contemporary artist who can turn his "innocent eye" on the nightmare realities of this era with its threat of nuclear annihilation? We need a Goya or a Hieronymous Bosch to-day to quicken our sense of the urgency of the human predicament before it may prove too late.

DETERMINATION OF THE HELICAL CONFIGURATION OF RIBONUCLEIC ACID MOLECULES BY X-RAY DIFFRACTION STUDY OF CRYSTALLINE AMINO-ACID–TRANSFER RIBONUCLEIC ACID

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CRUCIAL steps in protein synthesis appear to involve interaction between transfer ribonucleic acid (RNA), to which amino-acids are attached, ribosomes, and the messenger or informational RNA which determines the amino-acid sequence of the protein[1-4]. More information about the 3-dimensional configuration of RNA molecules and the base sequences in them would greatly help these processes to be understood. In elucidating the structure of deoxyribonucleic acid (DNA), X-ray diffraction analysis was indispensable: it guided the building of the Watson–Crick model[5], and detailed diffraction

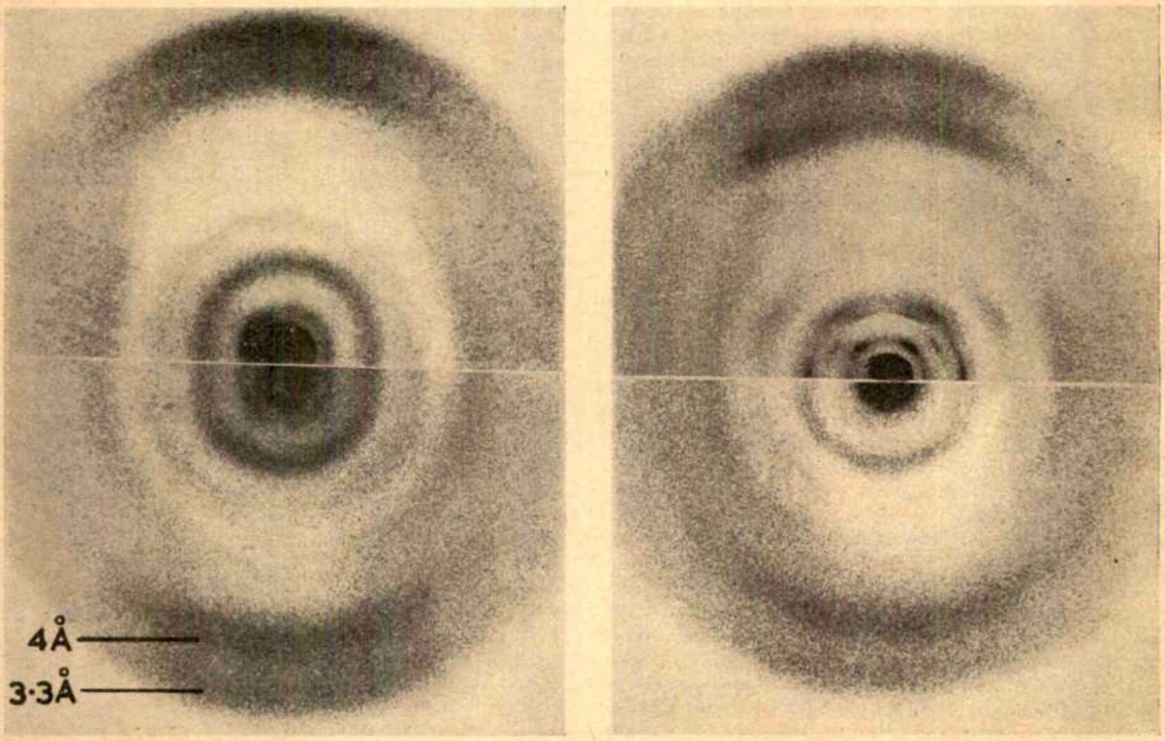

Fig. 1. Similarity of X-ray diffraction patterns of RNA. Top left, ribosome RNA from rabbit reticulocytes (supplied by Dr. R. A. Cox); bottom left, RNA from tobacco mosaic virus (supplied by Dr. H. Schuster); top right, crystalline yeast transfer RNA; bottom right, non-crystalline yeast transfer RNA (the innermost ring is missing from this pattern, which is taken at 57 per cent relative humidity, whereas the others are at 75 per cent)

data from crystalline fibres of DNA enabled the structure in its various configurations to be established[6-10]. The Watson–Crick hypothesis of DNA replication[11] was thus placed on a firm stereochemical base. In the case of RNA, however, X-ray diffraction has been of little use because the RNA was amorphous and the diffraction patterns were too diffuse to be interpreted[12].

Although the diffraction patterns of fibres of RNA had a broad similarity to those of DNA, it could not be established with certainty that the molecules were helical, whether there were one, two or three polynucleotide chains twisted together in helices, or whether there was more than one type of helix.

X-ray diffraction studies of synthetic ribopolynucleotides were less help than was hoped. The relation of the carefully established helical structure of polyadenylic acid[13] to that of RNA was not clear. Various complexes of polynucleotides gave DNA-like patterns[14]. The complex of polyinosinic and polycytidylic acids was of special interest because it gave a crystalline diffraction pattern resembling that of DNA and a non-crystalline pattern like that of RNA (ref. 15). This suggested that RNA might have a structure like DNA. The same conclusion was drawn from X-ray studies of soluble RNA (ref. 16) and from molecular model-building[17]. However, the most commonly found RNA pattern looked different from DNA patterns and no molecular model could be constructed which would correlate with it. On the other hand, nucleotide compositions of amino-acid transfer RNA from a wide range of sources[18-22] are very similar, and compatible with a DNA-like structure.

Physico-chemical investigations of RNA solutions[23-25] also provided much evidence that RNA molecules were probably helical. It was shown that RNA molecules contained hydrogen-bonded regions in which the bases were stacked on each other. Close correspondence of the behaviour of optical rotation for DNA, synthetic ribopolynucleotides and RNA strongly suggested that these regions were helical. Such investigations, however, gave little precise information about the helical regions; and, since the correlation of optical properties with configuration was largely empirical, the very existence of helical regions could not be established with certainty.

We have now obtained conclusive evidence that RNA molecules are helical and have determined the structure of the helix. This has been achieved by crystallizing yeast transfer RNA and by obtaining from it X-ray diffraction patterns of quality comparable to those of DNA. We give here a preliminary account of this work and of light microscope observations of liquid-crystalline forms of the RNA. We have concentrated on transfer RNA because the molecule was small and likely to have a regular structure, and because the prospects of isolating it intact seemed greater than with other types of RNA.

Preparation and Crystallization of Transfer RNA

Amino-acid transfer RNA was prepared from baker's yeast by the method of Brown, Kosinski and Carr[18,19]. Low-molecular weight RNA was extracted from the cells with 1 per cent 'Cetavlon' in 0·45 *M* sodium chloride; transfer RNA was purified by fractional precipitation with 'Cetavlon'–sodium chloride mixtures, and 'Duomeen 12'–$Mg(CH_3COO)_2$; residual protein was removed by treatment with phenol. Most of our observations have been made on preparations which were not fractionated with 'Duomeen'. The binding of amino-acids to the RNA was dependent on the presence of cytidine triphosphate, indicating that at least part of the terminal trinucleotide[26] was missing. The DNA content was found to be less than 0·1 per cent by the diphenylamine reaction[27]. 'Cetavlon' content was estimated as less than 0·1 per cent by Mr. P. J. Cooper, using a modification of a method for detecting quaternary ammonium groups[28].

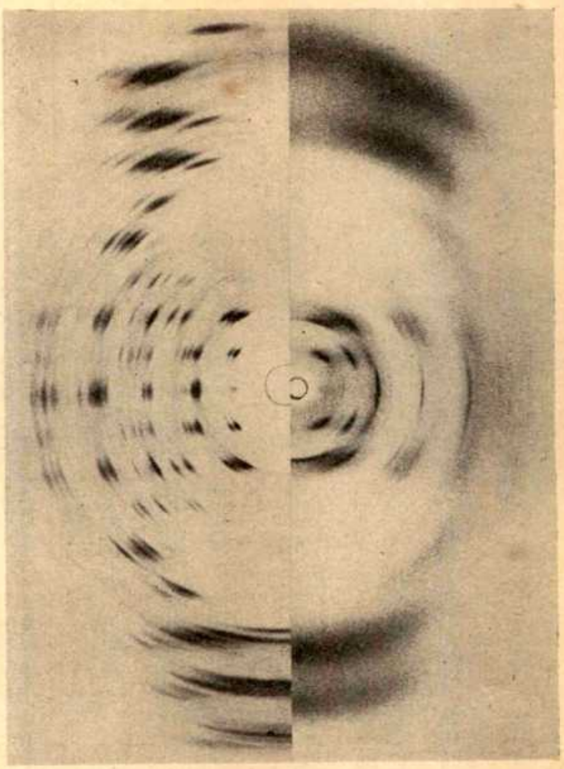

Fig. 2. Comparison of diffraction patterns of (left) DNA in the *A* configuration (supplied by Dr. L. D. Hamilton) and (right) crystalline yeast transfer RNA. Both are taken at 75 per cent relative humidity, with the fibres tilted 14° from the perpendicular to the X-ray beam. The patterns are joined along their meridians. Layer lines are horizontal, the equatorial layer line passing through the centre of the picture

Crystallization was induced by allowing a concentrated solution of RNA to dry in a controlled manner over a period of hours. Rapid drying, which does not inhibit crystallization of DNA, completely prevented crystallization of RNA. Only the sodium salt has so far been successfully crystallized. Excess sodium chloride prevented crystallization. Potassium and rubidium salts gave some crystallinity but the lithium salt did not. Magnesium inhibited crystallization. The best patterns were obtained by crystallization at about 0° C.; it was found possible at this temperature to obtain oriented fibres by drawing them from the material before it had completely solidified. With specimens prepared at higher temperatures, the overall distribution of intensity in the patterns was the same, but orientation was inferior and the positions of the sharp reflexions indicated that a mixture of two lattices was present.

X-Ray Diffraction Patterns of RNA

Fibres of all types of RNA which we have examined —RNA from tobacco mosaic virus, *E. coli* and rabbit reticulocyte ribosomes, and non-crystalline transfer

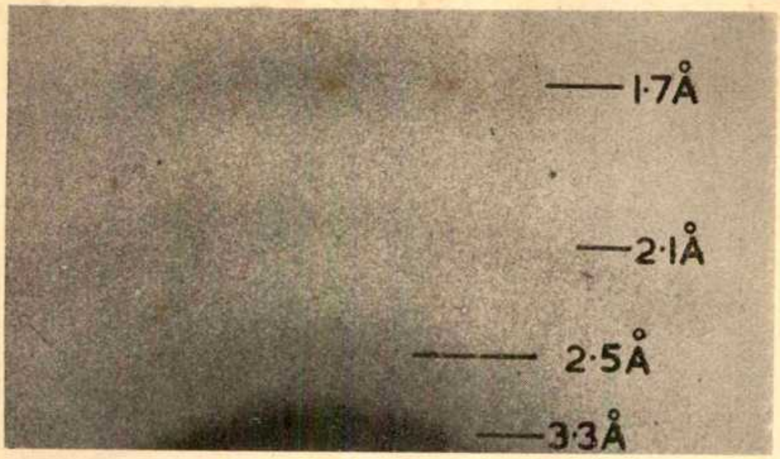

Fig. 3. Part of a heavily exposed diffraction pattern from crystalline transfer RNA, showing the eleventh layer line. The faint reflexion on this line appears to be meridional, and corresponds to 2·5 Å., the internucleotide spacing along the axis of the molecule. A similar reflexion is observed with *A* type DNA

RNA from yeast—give almost identical diffraction patterns (Fig. 1). When transfer RNA crystallizes, the pattern is better oriented, more clearly defined and, in the central part, the diffraction is split into sharp crystalline reflexions (Fig. 2). However, the broad distribution of intensity remains the same, showing that the molecular configuration has not been altered by the crystallization.

The crystalline pattern has a striking resemblance to the *A* type pattern of DNA (Fig. 2). This resemblance was not apparent when diffuse patterns alone were available. The positions of the sharp reflexions differ from those in DNA, because the crystalline packing is different; but it is clear that the broad distribution of intensity, which is determined by the molecular configuration, has maxima and minima closely corresponding to those of the DNA pattern on the various layer lines. This means that the two structures are essentially the same.

The diffuse patterns of RNA could not be interpreted without comparison with the crystalline patterns, because several basic features of the pattern could not be distinguished. The diffraction pattern of a helix may be recognized by absence of diffraction on the meridian on all layer lines, except for an upper layer line reflexion corresponding to the separation of nucleotides along the helix axis. In the diffuse patterns, lack of orientation prevented it being seen that meridional diffraction was absent on the first and second layer lines, obscured the fact that the strong outer reflexions at 3·3 Å. and 4 Å. were non-meridional, and made it impossible to distinguish equatorial reflexions from those on the first layer line. Another difficulty was that the 3·3 Å. and 4 Å. reflexions did not appear to lie on layer lines. With the crystalline patterns, these difficulties are very largely removed and the 3·3 Å.–4 Å. reflexions are seen to be resolved into several overlapping components which correspond roughly to those of the DNA *A* pattern (Fig. 2). When the fibre is inclined to the X-ray beam so that higher layer lines are recorded, the RNA and DNA patterns are very similar: no tenth layer line is seen but a reflexion on the eleventh at 2·5 Å. (Fig. 3) is present (the numbering of layer lines is taken from the *A* pattern). We believe that this reflexion corresponds, as in DNA, to the separation of nucleotides along the helix axis. Still higher layer lines are not seen separately, but the general intensity distributions for RNA and DNA are very similar (Fig. 4).

The relative intensities of diffraction on the sixth, seventh and eighth layer lines are for RNA somewhat different from those for DNA. In poorly defined patterns the seventh layer line appears to be weak; but in the best patterns there are indications that the intensity maximum on the seventh layer line has moved outwards so that it tends, owing to disorientation, to overlap with the maximum on the eighth layer line. This kind of overlap gives the impression in the poorer patterns that the reflexions do not lie on layer lines. Preliminary model building shows that the differences between the outer regions of the RNA and DNA patterns, as well as some differences in the central region, may be accounted for by a twisting of the *A*-type model of DNA about the helix axis and by a small movement of the phosphate groups relative to the base and sugar groups. Moreover, when DNA (sodium salt) is dried, the maximum on the seventh layer line moves in the way we describe (Fig. 5). At 10 per cent relative humidity the intensity distribution is very similar to that of RNA, the maxima not lying on layer lines. Dr. D. R. Davies directed our attention to the fact that unpublished patterns of dried DNA, obtained by Drs. Franklin and Gosling (see also ref. 29), had, on the higher layer lines, an intensity distribution like that of RNA.

Structure of the Transfer RNA Molecule

Intensity measurements of the diffraction patterns of transfer RNA indicate that, as with DNA, at least 50 per cent of the material is crystalline. The clarity and sharpness of the patterns suggest that most of the molecules have, apart from base sequences,

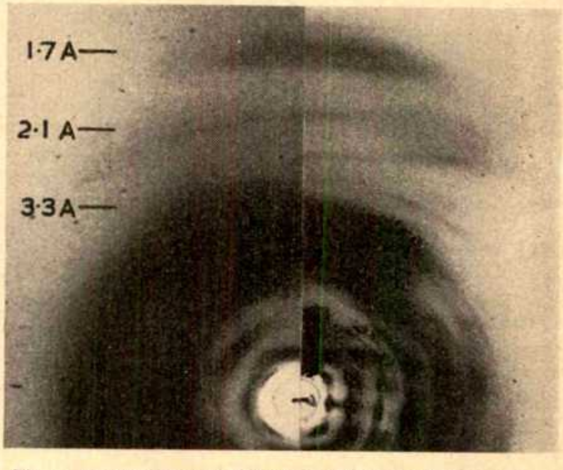

Fig. 4. Heavily exposed diffraction patterns of (left) crystalline transfer RNA, and (right) DNA in the *A* configuration, the fibres being tilted to record diffraction on higher layer lines. The diffraction in the upper region corresponds to smaller spacings and is similar in both patterns

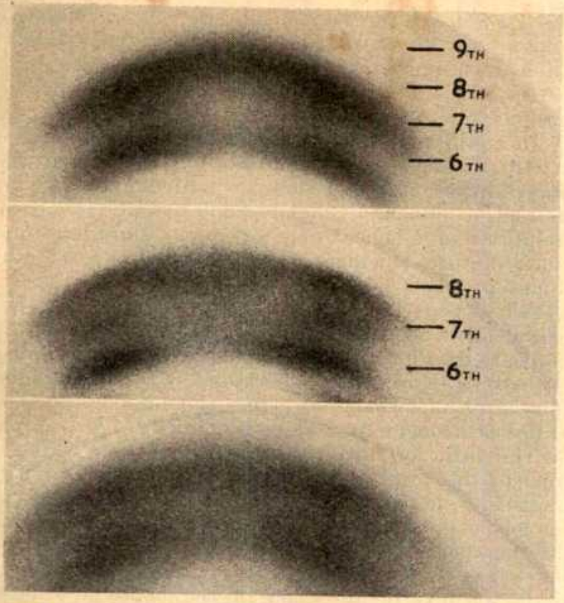

Fig. 5. Diffraction on sixth to ninth layer lines from (top) crystalline yeast transfer RNA at 75 per cent relative humidity, (middle) sodium DNA at 32 per cent relative humidity, and (bottom) sodium DNA at 10 per cent relative humidity

identical and highly regular structures. The fact that the transfer RNA molecule has a structure like DNA implies that the RNA molecule contains Watson–Crick pairs and complementary sequences. The sharpness of the patterns is not sufficient to exclude the possibility that there are minor deviations from complementarity. However, we think it likely that the sequences in the molecule are exactly complementary from one end of the helix to the other.

It is known that each molecule consists of one polynucleotide chain of about 80 nucleotides[19,22]. The most likely structure consistent with our results is therefore one in which the polynucleotide chain is folded back on itself[16] so that the sequence of atoms in one-half of the chain runs in the opposite direction to that in the other, the two halves of the chain thus being equivalent to the two 'anti-parallel' chains in DNA. The molecule is about 100 Å. long and contains roughly $3\frac{1}{2}$ helix turns. The pitch-length varies with humidity from about 28 Å. to 30 Å. The separation of nucleotides along the helix axis direction is 2·5 Å. The base pairs are tilted about 20° from the perpendicular to the helix axis. The distance between two ribose groups at the end of a molecule is about 12 Å. Model building shows that the portion of polynucleotide chain joining these groups, and forming the bend at one end of the molecule, need contain no more than three nucleotides. Also there is no difficulty in accommodating in the structure the hydroxyl group of the ribose. It is very likely that there is a hydrogen bond from the hydroxyl, either to the ring oxygen atom of the neighbouring ribose or to an oxygen atom of the adjacent phosphate group. Such an extra bond should help to stabilize the structure. This may explain our observation that the structure is largely unchanged over a wide range of relative humidity (32–92 per cent).

Base analyses of the RNA preparation support the proposed structure. Because of base-pairing there should be equal numbers of adenine and uracil groups and of guanine and cytosine (if we ignore the bases at the folded end of the helix and the remnant of trinucleotide tail). These equalities are observed[19]. Investigations of the properties of transfer RNA in solution suggest that 70–75 per cent of the material is helical[30]. We believe all the molecules are similar, and, because there is no evidence of uncoiling in the crystallites, our results suggest that the overall helical content in the solid state is higher. It may be that the molecules partly uncoil in solution but are entirely helical in the solid state because the high concentration of sodium ions present gives the helices extra stability.

It is not clear at present how our results relate to those of Brown and Zubay[16] on soluble RNA, with amino-acids attached, from *E. coli*. Their X-ray data indicated that a proportion of the molecules were in a configuration resembling the *B* form of DNA. It is, however, apparent in the light of our recent work that the patterns indicate also the presence of molecules with a structure like the one we describe here. Moreover, it has since been shown[19] that soluble RNA preparations of the kind studied by them contain only 65 per cent transfer RNA.

Crystalline Arrangement of Transfer RNA Molecules

The sharp reflexions in the crystalline pattern of RNA can be indexed on a hexagonal lattice and the indices of absent reflexions show that each molecule is surrounded by six neighbours each of which is displaced, in the helix axis direction, by one-third the pitch-length. When crystallinity is optimum, at 75 per cent relative humidity, the unit cell dimensions are $a = 40{\cdot}7$ Å., $c = 29{\cdot}0$ Å., the c axis being in the helix axis direction.

It is noticeable that, whereas the crystalline reflexions in the central region of the pattern are sharply defined, diffraction on higher layer lines is diffuse. DNA patterns of this kind are referred to as semi-crystalline. The explanation of the sharpness of the central reflexions is that the molecules interlock to form a highly regular arrangement of helices (Fig. 6). The diffuseness of the outer reflexions is apparently due to each helix being turned like a

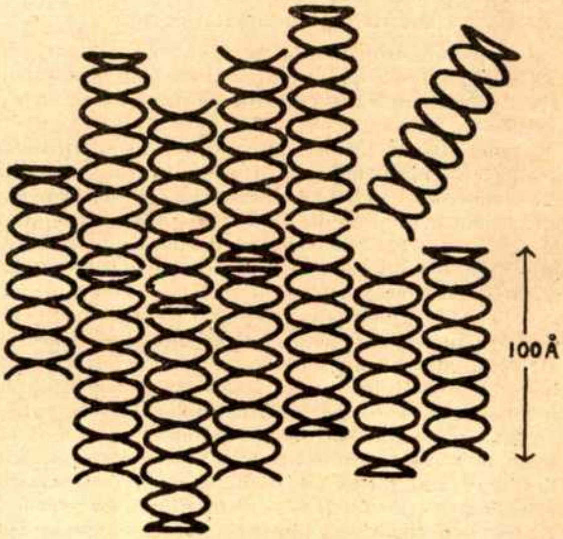

Fig. 6. Diagram of the arrangement of double-helical transfer RNA molecules in a crystal. The lines represent polynucleotide chains. A single molecule is shown at top right

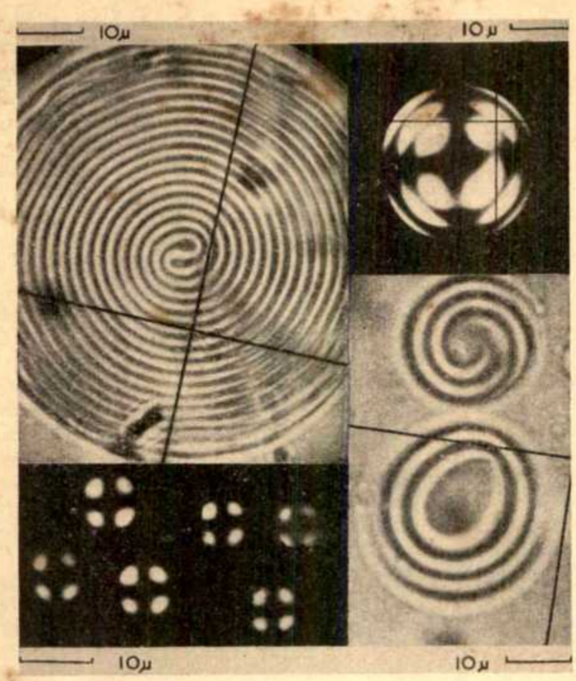

Fig. 7. Liquid crystal forms of transfer RNA. Top left, large spherulite showing double spiral contour due to variation of refractive index (unpolarized light). Bottom left, a group of simple spherulites (crossed nicols). Top right, spherulite showing circular contours of refractive index (crossed nicols). Bottom right, the same in unpolarized light, compared with a small spherulite of spiral type

screw about its axis by a random amount. In addition the short length of the molecules, 3½ helix turns, would cause the upper layer lines to spread vertically over a width one-third of their separation. It is possible that slight irregularities in the structure also contribute to the diffuseness.

If there were appreciable gaps between the ends of the molecules one would expect diffraction at angles corresponding to the length of the molecules; no such diffraction has been observed up to 200 Å.

Polarizing Microscope Studies of Transfer RNA and its Liquid Crystal Forms

Solutions of transfer RNA, on drying, show in the polarizing microscope a number of striking phenomena which corroborate the conclusions drawn from X-ray studies.

A dried layer formed at an interface with air is negatively birefringent. This indicates that the molecules are rod-shaped, negatively birefringent, and orient, as one would expect, parallel to the interface. Similarly, the sheared material in a drawn fibre of the kind used in the X-ray experiments has negative birefringence. The value of the birefringence is 0·07, which is consistent with the fibres consisting entirely of helical molecules like those of DNA in the *A* configuration, the high birefringence being due mainly to the bases lying roughly perpendicular to the length of the molecule.

The rod shape and negative birefringence of the molecules are confirmed by the properties of various liquid crystal forms which develop in concentrated solutions of transfer RNA on standing. As expected, tactoids are negatively birefringent and, in spherulites, the direction of the greater refractive index is radial. Furthermore, the small axial ratio of the tactoids (~ 1·5 : 1), and the very small difference of refractive index between ordered and disordered regions, both indicate that the axial ratio of the molecules is not large. In agreement with this, the structure we propose has an axial ratio of 5 : 1.

In addition to simple spherulites (Fig. 7), there is a remarkable type of spherulite which, viewed from most angles, shows a double-spiral due to varying refractive index. Similar bodies were found in solutions of α-helical poly-γ-benzyl-L-glutamate and their nature elucidated[31–33]. A similar form was found in gels of DNA[32].

A new type of spherulite is found in the RNA, the spiral form being replaced by concentric circles. It appears that both types of spherulite contain similar structures, except that the spiral type has a radial line of disinclination[34], whereas the circular type has a line of disinclination along a diameter.

The variation of refractive index in these bodies is due to the varying direction of the molecules. The molecules lie parallel in layers with the length of the molecule in the plane of the layers. The direction of the molecules in one layer is, however, inclined at a small angle to that in the neighbouring layer. As a result, the direction of the molecule rotates on passing through a succession of layers, so that the whole assembly is twisted. The inclination of the molecules in one layer to those in the next shows that the packing forces between the molecules must be asymmetric and therefore that the molecules are asymmetric. A helical molecular structure has the required asymmetry.

The twisted liquid-crystalline structure appears to be essentially the same as that found in cholesteric liquid crystals[35]. With RNA the regularity of the twisted structure and the perfection of the spherulites indicate a high degree of molecular homogeneity in the preparations.

The homogeneity of the preparations is also indicated by the finding that, when the RNA dries between microscope slide and coverslip, solid birefringent domains form in which the optic axis is often exactly parallel over an area of many square microns (Fig. 8). The parallelism suggests that these areas are single crystals; presumably they correspond to the microcrystals that give the crystalline X-ray patterns. In agreement with this, some unoriented specimens of crystalline RNA give diffraction rings in which the spots corresponding to individual crystals can be seen separately (Fig. 8).

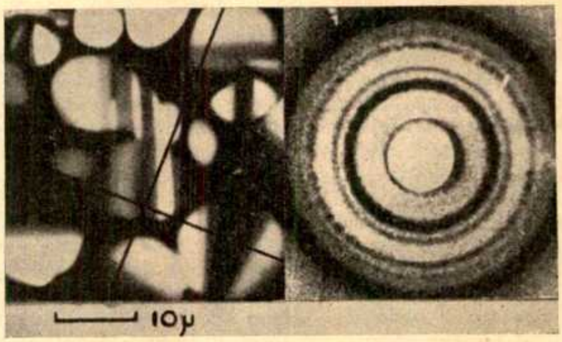

Fig. 8. Left, birefringent crystalline regions in a thin film of dried transfer RNA (crossed nicols). Right, central region of diffraction pattern from an unoriented specimen of crystalline transfer RNA. The third ring from the centre consists of spots corresponding to separate crystals in the specimen. The second ring from the centre is from another crystalline phase and shows no spots

This effect has not been observed with unoriented DNA; the diffraction rings are uniform and the crystallites very small.

Relation of the Structure of the Transfer RNA Molecule to its Function

Various speculations may be made about the transfer RNA molecule. (1) Molecular model-building shows that the fold at the end of the molecule must consist of at least three nucleotides with free hydrogen-bonding sites on the bases. These might be the nucleotides (probably three[36]) which specify each amino-acid in the code for amino-acid sequence, since their bases could form hydrogen bonds with coding RNA. (2) The crystalline packing we observe for RNA molecules in the solid state might be operative during protein synthesis, by packing transfer RNA molecules with helical regions of ribosomal or coding RNA. (3) The probable function of many of the nucleotides in the molecule is to react specifically with an amino-acid-activating enzyme so that the correct amino-acid is attached to the RNA molecule. The specific pairing in the molecule might serve to prevent the bases, which react with the enzyme, from hydrogen bonding to the coding RNA and thus interfering with the functioning of the adaptor. The role of pseudo-uridylic acid in the molecule is obscure; it could form a Watson–Crick pair with adenine in the double helix[17]. (4) The deduction of complementary sequences within each molecule suggests the molecule might be self-replicating. To replicate, the molecule would unfold and a second polynucleotide chain would form on the parent chain. If the two halves of each chain contained complementary sequences, both chains would be identical in their helical regions. After separating, each chain would fold into a double helix. However, the three or more unpaired bases in the folded ends of the parent and daughter molecules would not necessarily be identical.

Structure of the Helical Regions in Virus and Ribosome RNA

When we obtained crystalline diffraction patterns from transfer RNA, the nature of the diffuse patterns obtained from other types of RNA became clear. There is no doubt that they are essentially the same as the crystalline pattern, although there are minor variations: every feature of diffuse patterns can be accounted for in terms of the crystalline pattern, and the small though characteristic changes with humidity are the same. We are confident, therefore, that all types of RNA so far studied contain helical regions with a configuration essentially similar to that of *A* DNA.

The base compositions of virus and ribosome RNA are such that at least 10 per cent of the material cannot exist in a DNA-like structure with complementary sequences. In agreement with this, solution studies[25] indicate that the helical content of tobacco mosaic virus RNA is 88 per cent and that of ribosome RNA is 77 per cent. These studies also indicate that the molecules consist of single polynucleotide chains and that, where parts of the chain contain approximately complementary sequences, these parts may fold back on themselves to form double helices in which the two parts of the chain are anti-parallel[37]. The X-ray results show that the chains are anti-parallel. This provides further evidence that the helices in virus and ribosome RNA are formed by folding back of the chain.

It might be thought that the diffuse nature of the virus and ribosome RNA patterns was evidence that the helical regions were not regular. This is not necessarily so, for the patterns of virus and ribosome RNA are no more diffuse than that of non-crystalline transfer RNA. The differences between the non-crystalline and crystalline patterns of transfer RNA appear to be due to improved orientation and regular packing of molecules in the crystalline material, and not to increased regularity of the molecule. There is therefore little to suggest from the X-ray results that the helical regions in virus and ribosome RNA are less regular than those in transfer RNA.

It is therefore possible that the base sequences in the helical regions are exactly complementary. However, in the absence of crystallinity, the X-ray evidence is not adequate to decide between three possibilities for the structure of virus and ribosome RNA. (1) The helical regions might consist entirely of Watson–Crick pairs, separated by non-helical regions (which could prevent crystallization). (2) The base sequences in the helical regions might contain, in addition to Watson–Crick pairs, other base-pairs, some joined by only one hydrogen bond[37–39]. These pairs would cause minor irregularities in the helix, because their dimensions would differ from those of the Watson–Crick pairs. (3) Where there are deviations from complementary sequence in the folded regions of virus and ribosome RNA, the non-pairing part of the polynucleotide chain might form a small loop projecting from the double helix, all the bases in the helix forming Watson–Crick pairs[40]. Such loops would interfere with regular packing of one helical region with another. Some degree of regular packing does occur; this is shown by the presence of a fairly well-defined ring near the centre of the patterns of virus and ribosome RNA. While the X-ray evidence does not prove that there are no loops containing unpaired bases, it is probably not in favour of their existence. The diffraction patterns of random polynucleotides could cast light on these problems[14] but a difficulty is that the degree of randomness is seldom precisely defined.

Can X-ray Diffraction Analysis provide the Base Sequence of Transfer RNA?

The number of bases in a DNA molecule is too large for a determination of base sequence by X-ray diffraction to be feasible. However, in transfer RNA the number of bases is not too large. The size of the crystals observed by means of X-ray diffraction and polarization microscopy suggests that it should not be too difficult to grow crystals large enough for single-crystal X-ray analysis. If a pure preparation of transfer RNA specific for one amino-acid were used, one might expect that the crystals would be free of the disorder at present found and would be as perfect as those in microcrystalline DNA. If such crystals were obtained, and provided that all the molecules had identical structure and base sequence, a complete analysis of the primary structure of the molecule could be made, including the sequence of the bases and the form of the fold at the end of the helix.

We thank Prof. J. T. Randall, Drs. R. A. Cox and S. Arnott for advice and discussion; Mr. Z. Kosinski for help in preparing RNA; Mr. P. J. Cooper for technical assistance; Mr. Z. Gabor and Mrs. F.

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NEWS and VIEWS

Food Science and Technology at the Food and Agriculture Organization : Dr. Z. I. Kertesz

Dr. Z. I. Kertesz (United States) has been appointed chief of the Food Science and Technology Branch of the Nutrition Division of the Food and Agriculture Organization. He succeeds Dr. A. G. van Veen of The Netherlands, who has been appointed to the new chair of international nutrition, Cornell University, Ithaca, New York. Dr. Kertesz joined the Food and Agriculture Organization in September 1961, following more than thirty years with the Department of Food Science and Technology, Cornell University. During the past fifteen years he has also carried out surveys of the food industries in Europe and Australia, and in Ceylon, Korea, Turkey, Libya, Ethiopia, Taiwan, and Thailand. During 1952–53 he was nutrition officer for the Organization in Ceylon. During 1950–52, Dr. Kertesz was editor of the journals *Food Technology* and *Food Research*.

Applied Mathematics at Nottingham :

Prof. R. Hill, F.R.S.

Prof. Rodney Hill was appointed professor of applied mathematics at the University of Nottingham in 1953 after holding a research fellowship and readership at Bristol. His research has been mainly in the field of continuous mechanics of solids and has been concentrated in recent years on the fundamental questions of uniqueness and stability, especially for materials of non-linear type. His researches have been published in a large number of important papers in scientific periodicals and in his book on plasticity, which has been recognized since its publication as a standard work on this subject. He has also been editor of the *Journal of Mechanics and Physics of Solids* since 1952. He gained the degree of Sc.D. in Cambridge in 1959, and the distinction of his contribution to applied mathematics was recognized by his election to the fellowship of the Royal Society in 1961. Prof. Hill has resigned his chair at Nottingham to devote the year 1962–63 to writing and studying without academic responsibilities. Although his plans after that year are still uncertain, he will certainly be working and carrying out research at the University of Nottingham during the year, and he will also continue as editor of the *Journal of Mechanics and Physics of Solids*.

Prof. G. G. Hall

Dr. George Garfield Hall, lecturer in mathematics in the Imperial College of Science and Technology, London, has been appointed to succeed Prof. Hill. Dr. Hall graduated with first-class honours in both mathematical physics and mathematics at the Queen's University, Belfast, in 1946, and a year later gained a third degree with first-class honours in physics. From Belfast he went to Cambridge and began research on the orbital theory of chemical valency, for which he was awarded the degree of Ph.D. in 1951. In 1950 he was appointed assistant in research in theoretical chemistry and three years later was elected to a Fellowship in St. John's College, Cambridge. He was appointed to his present position in 1955, and was granted leave of absence to spend the session 1957–58 in the University of Uppsala on research. In 1961 he spent two months at the Institute of Theoretical Physics, Naples, and this year was in Florida for a shorter period. The principal field of Dr. Hall's recent research has been that of quantum mechanics as applied to problems of atomic, molecular and crystal structure, but he has also published papers on programming for an electronic computer and in the field of theoretical biology.

Expenditure by Colleges of Advanced Technology in Britain

In a written answer in the House of Commons on May 21, the Minister of Education, Sir David Eccles, estimated the total amount of tuition and residential fees receivable in 1962–63 from students at the colleges of advanced technology to be £660,000. He estimated net recurrent expenditure after allowing for income receivable from fees and sources other than Ministry grant was £7,532,107, besides capital expenditure on new buildings and equipment, towards which a grant from the Ministry was estimated to be about £2 million. For the individual colleges the figures are: Battersea College of Technology, £753,116; Birmingham College of Advanced Technology, £1,274,790; Bradford Institute of Technology, £1,040,060; Bristol College of Science and Technology, £440,205; Brunel College, Acton, £523,205; Chelsea College of Science and Technology, £526,160; Loughborough College of Technology, £501,307; Northampton College of Advanced Technology, London, £735,392; Royal College of Advanced Technology, Salford, £1,147,270; Welsh College of Advanced Technology, Cardiff, £590,702.

Power from Gas-cooled Reactor Stations

In a written answer in the House of Commons on May 21, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that, assuming a 20-year life, 75 per cent load factor and 6 per cent interest charge, the Atomic Energy Authority estimated that an advanced gas-cooled reactor station of two 500-MW. reactors would produce electricity at rather less than 0·5*d*. per unit, excluding the recovery of development expenditure. This should fall to 0·45*d*. per unit and probably lower as the reactor system is developed further and larger reactors are built. If the load factor is taken as 85 per cent, the unit cost would be reduced by about 0·04*d*. and in addition a station life of 30 years would give a further reduction of 0·03*d*. These estimates assume that the overheads of industry and the Authority in connexion with these reactors are spread over a series of stations.

British and American Industrial Research

The industrial pattern of research and development expenditure is strikingly similar in Britain and America. In both countries, the same group of industries—mainly capital goods and chemicals—account for more than nine-tenths of research expenditure although they employ less than half the total labour force engaged in manufacturing industry. The story is very different, however, when total and relative expenditures are compared. A comparative study of the subject published in the May issue of *Economic Review* shows that after allowing for differences in salaries and other factors, the American effort is more than five times as big as in Britain. In terms of real expenditure per employee, it is about three times as big and as a percentage of output about twice as high. The economy of the United States benefits from more research per worker as from more capital per worker and both contribute to high productivity. There is a marked correlation between research and growth in both countries and this appears to have a common cause. Fundamental discoveries in science and technology dictate the areas in which research and development are likely to be fruitful. Though more expenditure on research will not necessarily yield faster growth in any particular industry, yet it seems certain that any programme for faster growth in Britain must include provision for more research expenditure, especially as growth in Britain is inexorably linked with exporting. British industry cannot hope to rival American industry in the absolute volume of industrial research, but *Economic Review* suggests that to some extent British industrialists might be able to compensate for their smaller total resources by greater concentration of effort. Copies of *Economic Review* are obtainable from the National Institute of Economic and Social Research, 2 Dean Trench Street, Smith Square, London, at 12*s*. 6*d*. each.

Management Training Techniques

The management training techniques used by different organizations vary widely, and, over the past three years, the Management Development Schemes Sub-Committee of the British Institute of Management has been examining the training philosophy and management selection and development policy in a large number of commercial and industrial concerns. At a conference held in London last year under the chairmanship of Mr. C. G. Simpson, director-general of staff, National Coal Board, people individually concerned with four particular training techniques were brought together and asked to explain them. The papers they gave, together with the summing up by Mr. A. R. N. Roberts, are reprinted in *Management Training Techniques* (Pp. 68. London: British Institute of Management, 1962. 9*s*. 6*d*.). The four principal papers given were: Dr. W. D. Hargreaves (Richard Thomas and Baldwins Ltd.), "Case Studies and Projects"; Mr. B. Eldon (Shell International Petroleum Co. Ltd.), "The Business Game"; Mr. K. D. O. Cole (Westminster Bank Ltd.), "Role Playing"; and Mr. R. J. Hacon (Joseph Lucas Ltd.), "Group Dynamics".

Physics Letters

Similar in aim, size and format to *Physical Review Letters*, published by the American Institute of Physics, is the new international letter journal, *Physics Letters*, which is issued bi-monthly commencing with the April number (1, No. 1. Pp. 32. Subscription price per vol., 36 guilders; 62*s*.; 10 dollars; 40 D.M. Amsterdam: North-Holland Publishing Co.). The editors are G. E. Brown (Copenhagen) and D. Ter Haar (Oxford). Members of the advisory editorial board are drawn from twenty-five countries, including Australia, Canada, France, Great Britain, India, Japan, the United States, the U.S.S.R. and Yugoslavia, but not China. Contributions can be written in English, French or German. The April number contains thirteen letters, two in French and the others in English, which bear dates of communication between January 28 and March 13. They include letters from CERN on a new measurement of the anomalous magnetic moment of the muon; and from CISE (Milan) on an application of silicon detectors to measurements of monoenergetic neutron beams, and on (n, α) reactions in heavy elements.

Photochemistry and Photobiology

A revival of interest in photobiology in the past few years has resulted in the birth of a new periodical, *Photochemistry and Photobiology*, the first issue of which has recently appeared (1, January–March 1962. Approx. 70 pages. Published quarterly by Pergamon Press, Ltd., Oxford, New York. Annual subscription for personal use 70*s*., or 10 dollars). The object of this international journal is the publication, in English, French or German, of "papers concerning all aspects of photochemistry and photobiology but will be primarily concerned with articles of an analytical nature dealing with the photochemistry of organic substances and the action of infra-red, visible and ultra-violet light on biological systems". Papers dealing with ionizing radiations are specifically excluded. Members of the editorial board are Dr. E. J. Bowen, University of Oxford; Prof. S. Claesson, Institute for Physical Chemistry, Uppsala; Dr. A. Hollaender, Oak Ridge National Laboratory, United States; Prof. A. O. McLaren (executive editor), University of California; and Prof. D. Shugar, Institute of Biochemistry and Biophysics, Warsaw. These are supported by an international group of leading workers forming an editorial advisory board of thirty-three. Although duplicating, to some extent, the aims of *Radiation Research*, the institution of a separate journal devoted to the physical, chemical and biological aspects of non-ionizing radiations seems justifiable at the present time. The contents of the first issue (mainly concerned with nucleic acids and related substances) show a high standard of editorial policy; in particular, citations of recent literature in the field—as a regular feature—should be of great value to research workers involved.

Large-Scale Mach-Zehnder Interferometer

The new large-scale Mach-Zehnder interferometer recently announced by the Carl-Zeiss Foundation of Oberkochen, Western Germany, is a further development of the well-known single-mirror focusing model, and incorporates optical systems and focusing mechanisms of very high standard. The total deviation from parallelism and straightness in the interference fringes is less than one-tenth of a fringe width throughout, even though the light beam is reflected four times and passes through two glass plates before the measurements are made. The need for two cameras has been obviated. By shortening the focal length of the objective it has been possible to unite the cameras and, whereas previously the former intermediate image was barely 1 cm. in diameter, it is now of a size to be directly photographed. The interference image is very bright and the exposure time required for a photographic record is extremely short. By means of a large 13 × 18 cm. ground-glass screen it is possible for the interference fringes to be viewed simultaneously by several viewers. The agents in the United Kingdom are Degenhardt and Co., Ltd., 6 Cavendish Square, London, W.1, from whom a coloured illustrated booklet can be obtained in which is given full details of the interferometer, associated camera and other accessories for various applications in the fields of aerodynamics, fluid dynamics, thermodynamics and ballistics.

Solvents in Dry Cleaning

Risks of fire and explosion from the use of solvents in dry cleaning processes, and the precautions that should be taken to reduce such hazards, are discussed in *Dry Cleaning Plant: Precautions Against Solvent Risks*, a booklet in the Safety, Health and Welfare, New Series, published by the Ministry of Labour (No. 15. Pp. 20. London: H.M.S.O. 1*s.* 3*d.* net). Solvents in general use are divided into two categories—flammable and non-flammable. The former are white spirit and petroleum distillate, and the latter mainly chlorinated hydrocarbons, such as trichloroethylene and perchloroethylene. When dry cleaning was first introduced, highly flammable solvents such as benzine or petrol were used. Fires and explosions causing serious injury and damage resulted. The introduction, in 1949, of the Dry Cleaning Regulations, which restricted the use of petrol and other liquids with a flash-point below 90° F., has greatly reduced risks from fire and explosions in the washing and hydro-extraction stages. There is still a risk of serious fires and explosions in tumblers used for drying articles cleaned in white spirit or petroleum distillate. Flammable solvents now being used have a flash-point of about 100° F. At atmospheric temperatures these do not give off vapour in concentrations high enough to form flammable or explosive mixtures. In some processes, such as tumbling, temperatures of about 150° F. are usual, giving rise to explosive concentrations of solvent vapours unless adequate precautions are taken.

Hydrology in Great Britain

Because of the ever-increasing demand for water there is increasing need for information about the quantity and quality of supplies available. Data on the flow of many rivers in Britain are given annually in *The Surface Water Year-Book of Great Britain*, issued by the Ministry of Housing and Local Government and the Scottish Office. In the latest issue, that for 1959–60, for the first time graphs are given which show, for selected stations, the frequency of discharges above given values (*Hydrometric Statistics for British Rivers, together with Related Rainfalls, for the year ended 30th September, 1960.* Pp. xiv+113 (2 maps). London: H.M.S.O., 1961. 22*s.* 6*d.* net). Information of a different kind is collected in the *Selected Bibliography of Hydrology*, prepared on behalf of the International Association of Scientific Hydrology of the International Union of Geodesy and Geophysics by the Hydrology Sub-Committee of the British National Committee for Geodesy and Geophysics (*Selected Bibliography of Hydrology (with abstracts), United Kingdom, for the years 1955–59 inclusive.* Pp. 52. London: The Royal Society, 1961). This contains references, with brief abstracts, of publications which appeared between 1955 and 1959 on many aspects of hydrology of both surface and ground waters. References to overseas work are included if it has been published in the United Kingdom. It is hoped that further bibliographies, dealing with later years, will be published periodically.

Geological Atlas of British Guiana

Few regions in the world are more inhospitable to the geological surveyor than is British Guiana. Throughout about nine-tenths of the country access is greatly hindered by swamps and forest; and over much of the remainder exposures are scanty, the bedrock being hidden by deep tropical weathering and by Tertiary to Quaternary sands and clays. But despite these adverse field conditions, the post-war years have seen a great expansion in the work of the local Geological Survey, financed largely by Colonial Development and Welfare grants from the British Government; and there is now a staff of fifteen field geologists engaged in exploration, aided by a team of specialists centred in Georgetown. In 1957 a new programme of geological mapping was initiated, with the objective of covering the whole of British Guiana in seven years; and from this effort there has recently resulted a new series of black-and-white geological maps on a scale of 1 : 200,000, each sheet covering one-quarter of a degree square and bearing about 1,500 words of explanatory text. Twenty-one sheets were published in 1961 and about three-quarters of the country will be covered by this series by the end of 1962. Although the maps are necessarily only of a reconnaissance character, the rapidity of production has been remarkable and the series should do much to guide and promote the future economic development of British Guiana. Copies of the Survey maps are available from the Geological Survey of British Guiana, Georgetown.

Progress in Botany

THOSE who were present at the ninth International Botanical Congress, held in Montreal in 1959, know that it was a largely attended and well-managed affair. It was the occasion for a great outpouring of new scientific work on virtually every aspect of plant life. This, too, was well ordered in that the main work of the Congress had been organized into a comprehensive system of symposia, each of vital contemporary interest. There were also special lectures on selected topics given by distinguished exponents. All this material has now been made available in two large volumes, amounting to 1,766 pages (*Recent Advances in Botany.* Vol. 1: Pp. xvi + 1–948. Vol. 2: Pp. xiv + 949–1766. Toronto: University of Toronto Press; London: Oxford University Press, 1961. 320*s.* net the set).

There can, of course, be no question of 'reviewing' these volumes for, in a sense, they are in themselves reviews. But it should be said that they constitute unusually valuable and stimulating works of reference for research workers and teachers, providing as they do representative and coherent treatments of major topics by those most actively engaged in their investigation. The two volumes will certainly repay the general botanical reader who takes the time and trouble to browse through them; as for the research worker in any particular field, they seem likely to give him the assurance that he is at least reasonably *au fait* with what has been achieved to date. They are, indeed, the kind of books that might well be made available in departmental libraries for general reference and perusal. As to the title *Recent Advances*, what appears to emerge from this large collection of original papers is that contemporary botanical science has not only important salients but is also advancing on a broad front.

Publications of the Organization for European Co-operation and Development

THE Organization for European Co-operation and Development (O.E.C.D.) has issued a general catalogue which covers all works published since its inception. Publications have been classified under two main headings: "Economics" and "Technology and Organization", with many sub-headings. Under each heading there is an introductory note showing the connexion between the works published and the activities of the Organization in the sector concerned. Most of the publications of the European Productivity Agency will be found under the heading "Technology and Organization". O.E.C.D. publications are printed in the two official languages of the Organization. Each work therefore appears as two separate editions, one in English and the other in French. Certain works, however—statistical publications, glossaries, bibliographies—are produced in a single bilingual edition, which can be used equally well by either English or French readers. In order to give the fullest possible information, the catalogue also lists the documents which can be obtained on request, free of charge, or consulted at the Organization, as distinct from publications offered for sale. Applications for copies should be made to the Distribution and Sales Section of the O.E.C.D., 2 Rue André Pascal, Paris.

The Linnean Society of London

AT the anniversary meeting of the Linnean Society of London, held on May 24, the following were elected officers for the session 1962–63: *President*, Prof. T. M. Harris; *Treasurer*, The Earl of Cranbrook; *Secretaries*, Prof. C. T. Ingold, Dr. H. G. Vevers, Dr. J. Smart (editorial); *Vice-Presidents*, The Earl of Cranbrook, Dr. C. R. Metcalfe, Dr. Sydney Smith and Mr. P. R. Bell. *New Members of Council*, Dr. Eric W. Baxter, Dr. A. McClean Bidder, Prof. C. T. Ingold, Mr. N. W. Simmonds and Dr. E. I. White. Prof. George Ledyard Stebbins and Kurt Wein were elected Foreign Members.

The Institution of Metallurgists

AT the eighteenth annual general meeting of the Institution of Metallurgists the following officers were elected: *President*, Dr. E. G. West, technical director, Aluminium Development Association; *Senior Vice-President*, Dr. L. Northcott, principal superintendent, Materials Division, Armament Research and Development Establishment, Fort Halstead, War Office; *Vice-Presidents*, Prof. J. G. Ball, head of the Department of Metallurgy and professor of physical metallurgy, Imperial College of Science and Technology, and L. Rotherham, member for research, Central Electricity Generating Board; *Honorary Treasurer*, L. W. Derry, head of the Department of Metallurgy, Battersea College of Technology; *Ordinary Members of Council*, G. T. Harris, assistant managing director, Jessop-Saville, Ltd., J. M. Mowat, director, Wm. Beardmore and Co., Ltd., Parkhead Steelworks, Glasgow, and G. R. Morton, senior lecturer in metallurgy, Wolverhampton and Staffordshire College of Technology.

Alfred P. Sloan Awards

THE first Alfred P. Sloan Awards in cancer research have been conferred on: Dr. Oscar Bodansky, chairman, Department of Biochemistry, Memorial Hospital for Cancer and Allied Diseases, for his contributions to the advancement of biochemistry and its applications to the problems of patients with serious disease, especially cancer; Dr. Felix Wróblewski, associate of the Memorial Hospital for Cancer and Allied Diseases, for his contributions to the knowledge of enzymes in the blood tissues and their usefulness in medical diagnosis; Dr. Charlotte Friend, associate member of the Sloan-Kettering Institute for Cancer Research, for her contributions to cancer research, particularly through her discovery of and continuing studies on a virus which will induce leukæmia in mature mice; Dr. Lloyd J. Old, associate of the Sloan-Kettering Institute for Cancer Research, for his work on the role of specific and non-specific immunological factors in cancer development and growth.

Postgraduate Course in Nuclear Technology (Chemical)

APPLICATIONS are invited from graduates in chemical engineering, chemistry or metallurgy, or persons with equivalent qualifications, for a one-year course leading to the D.I.C. at the Department of Chemical

Engineering and Chemical Technology, Imperial College of Science and Technology. The course includes lectures in fundamental and applied radiochemistry and radiation chemistry; process chemistry and technology of reactor fuels; nuclear criticality and plant design; reactor fuel element technology; and radiological protection. Practical work includes participation in a design study or in a research programme. Further information can be obtained from the Registrar, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

University News :

Birmingham

THE following titles have been conferred: that of reader in mathematical physics on Dr. G. V. Chester, senior lecturer in the Department of Mathematical Physics; that of reader in chemical engineering on Dr. C. W. Nutt, senior lecturer in the Department of Chemical Engineering; that of deputy director of the Institute for Engineering Production on Mr. F. Edmondson, assistant director of the Institute; that of head of computing services on Mr. K. A. Redish, lecturer in computing in the Department of Mathematical Physics; that of reader in medicine on Dr. J. M. Bishop, senior lecturer and first assistant in the Department of Medicine; that of University Research Fellow on Dr. P. Hele, during the tenure of her appointment in the Department of Physiology. The following appointments are announced: Prof. T. Ganea, to be Senior Research Fellow in the Department of Pure Mathematics; Mr. C. E. Gough, to be Research Fellow in the Department of Physics; Dr. M. P. Osborne, to be Research Fellow in the Department of Comparative Physiology.

Edinburgh

THE following appointments have been announced: *Senior Lecturer*, Dr. G. R. Hervey (physiology); *Lecturers*, Dr. J. E. Dale (botany), A. J. Tulett (botany), Miss J. Marrian (organization of industry and commerce), Dr. D. S. Saunders (zoology).

London

PROF. P. R. OWEN, professor in the mechanics of fluids in the University of Manchester, has been appointed to the Zaharoff chair of aviation, tenable at the Imperial College of Science and Technology, from January 1, 1963. The title of reader in experimental physiology has been conferred on Dr. A. F. Cobbold, and that of reader in biology on Dr. A. G. Hamilton, in respect of their posts at St. Thomas's Hospital Medical School; the title of reader in biochemistry has been conferred on Dr. A. E. Kellie, in respect of his post at the Middlesex Hospital Medical School. Dr. J. F. Fowler has been appointed to the readership in physics tenable at St. Bartholomew's Hospital Medical College.

Sussex

DR. T. BARNA, assistant director of the National Institute of Economic and Social Research, London, has been appointed to the chair of economics, and Mr. T. H. Elkins, lecturer in geography at King's College, London, has been appointed to the chair of geography. The following appointments have also been announced: *Reader*, Dr. W. Ledermann (mathematics); *Senior Lecturers*, Dr. D. F. Brewer (experimental physics), Dr. J. P. Elliott (theoretical physics); *Lecturers*, Dr. E. Mathieson (experimental physics), Dr. W. D. Hamilton (experimental physics), Dr. L. Allen (experimental physics), Dr. R. E. Turner (theoretical physics), Dr. C. N. Banwell (chemistry), Dr. E. O. Bishop (chemistry), Dr. R. A. Jackson (chemistry), Dr. J. D. Smith (chemistry), Dr. Ruth M. Rogers (mathematics), Mr. G. B. Trustrum (mathematics).

Announcements

THE Drummond Managing Trustees have awarded a Drummond Senior Fellowship in nutrition for the year 1962–63 to Mr. E. R. Humphreys, of the Royal Free Hospital School of Medicine. The Fellowship, valued at £1,100, is tenable for two years.

THE Trustees of the Plastics Industry Education Fund are offering a limited number of training grants towards the cost of full-time study on an approved course leading to a degree or other equivalent diploma in science, technology or industrial design. Grants are usually made for periods of one year at a time, but applications for the renewal of grants are considered provided the candidate is making satisfactory progress. Further information can be obtained from the Secretary, Plastics Industry Education Fund, 6 Mandeville Place, London, W.1.

THE 1962 international conference on "Precision Electromagnetic Measurements", sponsored by the Radio Standards Laboratory of the National Bureau of Standards, the Institute of Radio Engineers and the American Institute of Electrical Engineers, will be held in Boulder, Colorado, during August 14–16. Further information can be obtained from James F. Brockman, National Bureau of Standards, Boulder, Colorado.

THE second annual meeting of the Plant Phenolics Group of North America, under the auspices of the American Institute of Biological Sciences, will be held in Oregon State University during August 24–25. The programme will include a symposium on "Plant Phenolics and their Industrial Significance". Further information can be obtained from Dr. V. C. Runeckles, Imperial Tobacco Company of Canada, Ltd., P.O. Box 6500, Montreal, Quebec.

THE eleventh Canadian High Polymer Forum, sponsored by the National Research Council of Canada together with the Chemical Institute of Canada, is to be held at Essex College, Assumption University, Windsor, Ontario, during September 5–7. Further information can be obtained from the secretary-treasurer, Dr. L. Breitman, Research and Development Division, Polymer Corporation, Ltd., Sarnia, Ontario.

THE eighth international congress for microbiology, organized by the Canadian Society of Microbiologists under the auspices of the International Association of Microbiological Societies, will be held in Montreal during August 19–25. The programme will be divided into five sections, two or more symposia being arranged in each section: structure and function; agricultural microbiology; industrial microbiology; medical and veterinary microbiology; virology. Further information can be obtained from Dr. N. E. Gibbons, Eighth International Congress for Microbiology, National Research Council, Ottawa, 2.

ERRATUM. In the communication entitled "Coccolith Formation" (*Nature*, 193, 1094; 1962), by E. Paasche, the second half of the first sentence in the third paragraph should read: ". . ., it was found that the calcium content of one *Coccolithus huxleyi* coccolith is $5 \cdot 5 \times 10^{-13}$ gm. calcium".

INHIBITION OF GASEOUS FREE RADICAL REACTIONS

AN informal discussion on the inhibition of gaseous free radical chain reactions was held during April 12 and 13 in the Department of Physical Chemistry, University of Cambridge (by kind permission of Prof. R. G. W. Norrish), under the auspices of the Faraday Society. About 120 were present. On the afternoon of April 12, six papers were presented and discussed, under the chairmanship of Prof. F. S. Dainton (Leeds).

The first paper, on the "Mechanism of Inhibitor Action for Chain Processes", by H. Eyring, T. Ree and K. Yang (University of Utah), was presented by Prof. H. Eyring. The authors considered the classical theories of inhibition, where inhibitory action is explained by consumption of inhibitor (I). As consumption of I is often not observed, it is proposed that I destroys the chain carriers and is also regenerated. Denoting by β the radical which reacts bimolecularly in the chain propagation steps of the Rice–Herzfeld mechanism and by μ the radical which reacts unimolecularly, the complexes βI and μI formed regenerate I, either by reaction with the substrate *M* or by direct dissociation. Thus, a hyperbolic function is obtained for the variation of rate with inhibitor pressure. Good agreement is observed between this function and the literature data for the effect of nitric oxide and propylene on the decomposition-rates of hydrocarbons, aldehydes and ethers. It must be noted that this agreement is dependent for the nitric oxide inhibition of propane decomposition on the occurrence of such equilibria as:

$$CH_3NO \rightleftarrows CH_3 + NO$$

(forward reaction = 'saloon effect', backward reaction = 'chaperon effect'); or the participation of bimolecular reactions such as:

$$CH_3NO + C_3H_8 \rightarrow CH_4 + C_3H_7 + NO$$

Such reactions were questioned by some.

Mr. D. R. Blackmore (Oxford) presented a paper entitled "Inhibition and Acceleration by Nitric Oxide of the Decomposition of a Series of Paraffins". It is considered that, in inhibition, both the chain-carrying radicals combine with nitric oxide and, on the basis of the mechanism employed, the individual rate constants of some of the free radical reactions involved are evaluated. Three quantities are measured, namely: (*a*) the degree of inhibition by nitric oxide as a function of paraffin pressure; (*b*) the rate of the induced decomposition at much higher pressures of nitric oxide; (*c*) the rate of the uninhibited chain reaction. It is shown (from (*b*)) that the induced reaction is first order in both paraffin and nitric oxide, approximately equal molar quantities of nitric oxide and paraffin being consumed. Therefore, the following mechanism is proposed:

$$RH + NO \rightarrow R + HNO \text{ (slow, rate determining)}$$
$$R + NO \rightarrow \text{products (fast)}$$

Six rate constants are required to specify three experimentally observed quantities: thus the numerical values of three of these being assumed, the remaining three are evaluated. In discussion, some attention was directed to the wide differences between these rate constants and those obtained by other workers.

The next paper was given by Dr. B. W. Wojciechowski and Prof. K. J. Laidler (Ottawa) under the title "The Inhibition of Organic Decompositions", in which the effect of inhibitors in pyrolytic mechanisms is considered to be the establishment of chain processes, in which the inhibitor is involved in both initiation and termination, rather than the suppression of all chain processes. Thus, in the case of the pyrolysis of ethane and other hydrocarbons, of aldehydes and ethers, a hydrogen atom is abstracted by nitric oxide:

$$RH + NO \rightarrow R + HNO$$

Chain termination is achieved by reaction between the most plentiful chain carrier and either HNO or nitric oxide, depending on whether or not hydrogen is a chain carrier. In the particular case of ethane pyrolysis, it is proposed that the two equilibria:

$$C_2H_6 + NO \rightleftarrows C_2H_5 + HNO$$

and

$$HNO + M \rightleftarrows H + NO + M$$

participate, that is, nitric oxide catalyses the equilibrium:

$$C_2H_6 \rightleftarrows C_2H_5 + H$$

It is proposed that this hydrogen abstraction is general for any inhibitor (for example, propylene). and also for surface sites. Similar mechanisms are proposed for the decomposition of alkyl halides and, for the ethyl bromide pyrolysis in the presence of nitric oxide, the important reactions are:

$$C_2H_5Br + NO \rightarrow C_2H_5 + NOBr \text{ (initiation)}$$
$$C_2H_5 + NOBr \rightarrow C_2H_5Br + NO \text{ (termination)}$$

Surface control (that is, initiation and termination at the surface) in the pyrolysis of isopropylbromide is discussed on the similar basis:

$$Pr^iBr + S \rightarrow Pr^i + SBr$$
$$SBr \rightleftarrows S + Br$$

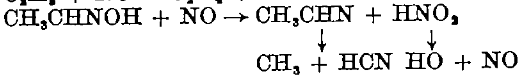
followed by chains initiated by bromine atoms and terminated by reactions of the radical having the odd electron on the terminal carbon atom.

Dr. G. L. Pratt (Cambridge) presented his paper (with Dr. J. H. Purnell) on "The Thermal Decomposition of Lead Tetraethyl in the Presence of Nitric Oxide". In the temperature-range, 235°–265° C. with excess nitric oxide, the pyrolysis-rate is reduced by a factor of 3–4, *n*-butane formation is entirely suppressed, ethane and ethylene still appeared in the products, and the predominant products formed are methyl cyanide and water. The latter products are explained by the sequence:

$$C_2H_5 + NO \rightarrow C_2H_5NO \rightarrow CH_3CH{=}NOH \xrightarrow{\text{surface}} CH_3CN + H_2O$$

No evidence for the addition of more than one nitric oxide molecule to each ethyl radical is obtained, although some reduction of nitric oxide to nitrous oxide by reaction with lead is observed. The small amounts of C_2 hydrocarbons may be explained by reactions such as:

$$C_2H_5 + NO \rightarrow C_2H_4 + HNO$$



$$\begin{array}{lll} CH_3CHNOH + NO \rightarrow & CH_3CHN & + \; HNO_2 \\ & \downarrow & \quad\;\; \downarrow \\ & CH_3 + HCN & \;\; HO + NO \end{array}$$

The acetaldoxime is decomposed very rapidly.

Dr. Margaret I. Christie (Oxford) then discussed her paper (with Mr. J. S. Frost) on "Kinetics of the Association of Alkyl Radicals with Nitric Oxide and of Subsequent Reactions", the relative rates of the reactions:

$$R + NO \rightarrow RNO$$
$$R + I_2 \rightarrow RI + I$$

being obtained from quantum yields of iodine produced in the gas phase photolyses of alkyl iodides in presence of varying concentrations of nitric oxide. It is shown that for R = Et, Pr^n, Pr^i, the ratio is about 0·1, independent of pressure, and the second order character of the association is thus demonstrated. In addition, reaction of an initial photolysis product (presumably RNO) with nitric oxide to form a brown substance Y is observed and $\mathrm{d}Y/\mathrm{d}t = k(R\mathrm{NO})^1(\mathrm{NO})^2$. At 25° C., it is shown that k increases in the order Me < Et < Pr^i < Pr^n, and, from the temperature dependence, negative activation energies ($-2{\cdot}8 \leftrightarrow -5{\cdot}9$ kcal. mole^{-1}) are measured. The product Y is decomposed on condensation and re-heating. In the absence of excess nitric oxide, the rate of disappearance of the monomer due to:

$$2RNO \rightarrow (RNO)_2$$

is measured, the activation energy being about 5 kcal. mole^{-1} and the steric factor about 10^{-8}. Discussion centred on the nature of Y and the negative activation energy. It was suggested that this explained the failure of this reaction to participate at higher temperatures. A suggested mechanism was:

$$RNO + NO \rightleftarrows R(NO)_2 \xrightarrow{NO} R(NO)_3$$

The paper "Free Radical Scavengers in Gaseous Radiolysis Systems" was given by Dr. L. J. Stief (Sheffield). It is shown that four criteria for ideal scavenging in any particular system are available, namely: (*a*) lack of appreciable isotopic mixing in the presence of scavengers; (*b*) independence of non-scavengable or 'molecular' yields of products with temperature, pressure, or particular scavenger used; (*c*) identification of the products of the inhibition reaction and comparison of these product yields with the yields of radiolysis products believed to have a particular free radical precursor; (*d*) the accuracy of rate constants, pre-exponential factors, and activation energies for free radical reactions obtained from radiolysis systems, when corrections for molecular contributions, based on scavenger experiments, are applied.

On the morning of April 13, five papers were presented and discussed, under the chairmanship of Prof. K. J. Laidler (Ottawa). The first paper in this session was "Inhibition by Nitric Oxide of some Reactions of Nitrogen Dioxide", by Dr. P. G. Ashmore (Cambridge). For the reaction:

$$H_2 + NO_2 \rightarrow H_2O + NO$$

nitric oxide acts as an inhibitor, probably due to the reaction:

$$NO + OH + M \rightarrow HNO_2 + M$$

the hydroxyl radical being a chain carrier. The reaction is also catalysed by nitric acid and nitrosyl chloride and both these reactions are inhibited by nitric oxide. It is likely that nitrosyl chloride initiates chains by the sequence:

$$NOCl + M \rightarrow NO + Cl + M$$
$$Cl + H_2 \rightarrow HCl + H \text{ (chain initiated)}$$

and that nitric oxide has little effect on this. The fast reversible reaction:

$$NO_2 + NOCl \rightleftarrows NO_2Cl + NO$$

followed by:

$$NO_2Cl + M \rightarrow NO_2 + Cl + M$$

is mainly responsible for the catalysis (nitryl chloride dissociation is faster than nitrosyl chloride). Reaction is inhibited by nitric oxide because of the reverse reaction and diminished nitryl chloride concentration in addition to the normal inhibition reaction with hydroxyl radicals.

Dr. P. J. Thomas (Imperial Chemical Industries, Ltd., Widnes) then discussed "The Effect of Nitric Oxide and of Olefinic Inhibitors on the Free Radical Chain Decompositions of the Lower Aliphatic Alcohols". It is shown that nitric oxide can both inhibit the thermal decomposition of alcohols (500°–600° C.) and also induce decomposition. Thus the rate/inhibitor concentration curves pass through a shallow minimum. This minimum rate is observed to be independent of nitric oxide concentration over a limited range only, and this range is varied in extent and concentration for different alcohols. When either propylene or isobutene are used as inhibitors (0–300 mm. added), the reaction rates are reduced to well-defined limits, which for sec-propanol and tert-amyl alcohol coincide for the minimum rates observed with nitric oxide addition. However, for ethanol, *n*-propanol, *n*- and iso-butanol the limiting rates observed with propylene and iso-butene are two- to three-fold greater than the minimum nitric oxide rate. The limiting rates observed with propylene are reduced by the addition of nitric oxide and therefore must be predominantly free radical chain reactions. *Cyclo*pentadiene is intermediate in inhibiting power to nitric oxide and propylene, and is similar to nitric oxide in that a marked increase in overall rate is observed at the high inhibitor concentrations. The results are interpreted in terms of concurrent inhibition and induced decomposition reactions: at maximum inhibition it is suggested that in addition to molecular decomposition modes, a limited amount of free radical decomposition of the alcohol also occurs. Discussion centred largely on the meaningfulness of the assumption of the molecular modes of decomposition, and on the determination of the orders of the reactions at all stages of addition of inhibitor.

Mr. R. A. Ross (University College, London) presented his paper (with Dr. A. Maccoll) on "Inhibition and the Unimolecular Pyrolysis of Organic Bromides". The homogeneous gas phase elimination reactions of organic bromides and chlorides are divided into two groups: (i) those that are inhibited by propylene and *cyclo*hexane, and (ii) those which show no inhibition. Group (i) are characterized by (*a*) induction periods, (*b*) 1½ order kinetics (in some cases), (*c*) difficulty of obtaining reproducible results, (*d*) acceleration by stimulants, and (*e*) the ability to produce chains in a substrate that can sustain them. Group (ii) are characterized by (*a*) first order behaviour and 'fall-off', (*b*) no acceleration by stimulants (in some cases), and (*c*) no ability to induce chains in a substrate that can support them. In support of the assumption of complete inhibition, it is shown for both group (ii) and the maximally inhibited group (i) that (1) decompositions have all the characteristics of unimolecular reactions, and that (2) the effects of structural variations are comprehensible within the framework of present-day chemical theory. Discussion centred on the effect of surfaces, and the variations between the experimental fall-off characteristics and those predicted by Slater and Kassel theories.

The paper "The Inhibition of *n*-Butane Pyrolysis by Propylene" by Mr. C. P. Quinn and Dr. J. H. Purnell (Cambridge) was presented by the former. The kinetics of the formation of the products of the pyrolysis of *n*-butane in the presence and absence of propylene are studied by means of high-speed gas chromatography. It is shown that the product ratio $C_2H_4 : C_2H_6$ is greater than unity, a function of temperature and pressure, and increases linearly with inhibitor pressure. It is concluded that this is incompatible with the hypothesis of two concurrent processes, one of which (free radical) is halted by the inhibitor and the other of which (molecular) is unaffected. It is therefore proposed that the fully inhibited reaction proceeds by a modification of the free radical chain mechanism which is responsible for the whole of the uninhibited reaction. Competition between the two reactions:

$$C_2H_5 + C_4H_{10} \rightarrow C_2H_6 + C_4H_9$$
$$C_2H_5 \rightarrow C_2H_4 + H$$

is found to play an important part in both the uninhibited and the fully inhibited processes. The increase in the activation energy for *n*-butane pyrolysis at low pressures is thus explained, and the classification of paraffins on the basis of changes with pressure of the order and activation energy of the fully inhibited pyrolysis (due to Peard, Stubbs and Hinshelwood) is interpreted in terms of the classes of hydrocarbons which do and do not give the competitive reactions listed above.

The final paper on "The Effect of Surface and Nitric Oxide on the Pyrolysis of Isopentane" was given by Prof. W. A. Bryce (University of British Columbia, Vancouver). The effect of surface : volume ratio and nitric oxide pressure on the decomposition-rate and product distribution of isopentane is studied. It is shown that alteration in the surface : volume ratio alters the rate at any fixed pressure, and the rate/nitric oxide pressure curves for the inhibited reaction. The main products of pyrolysis are methane, propylene and iso-butylene; the latter is unaltered by increase in pressure of nitric oxide, whereas the other two products are increased. Ethylene and ethane are minor products and the ratio increases with nitric oxide pressure, and with decrease in surface : volume ratio. The observations are interpreted on the basis of heterogeneous initiation of the pyrolysis. It is shown that *n*-pentane and neopentane behave differently from isopentane, and that di-allyl sensitizes the rate of decomposition of *n*-pentane, presumably by the reaction:

$$C_3H_5 + n\text{-}C_5H_{12} \rightarrow C_3H_6 + C_5H_{11}$$

The discussion covered many issues and there was no general agreement on inhibition mechanisms. The following brief summary from the discussion indicates some of the important questions to which answers should be sought in any examination of the effect of inhibitors on gas phase decomposition reactions: (1) the order of the reaction in both substrate and inhibitor; (2) consumption (or otherwise) of inhibitor; (3) the nature of the reaction(s) between radical and inhibitor, and substrate and inhibitor; (4) subsequent reactions of the products of these reactions; (5) the effect of surface; (6) the effect of different inhibitors; (7) the effect of adding a second inhibitor to a maximally inhibited reaction; (8) the assumption of molecular decomposition mechanisms; (9) comparison of product analysis/time curves with pressure/time curves; (10) the effect of temperature variation on reaction possibilities.

It is hoped that this discussion, by revealing the scope of the many kinetic problems involved in an understanding of inhibition mechanisms, will lead to an advance in our understanding of a theme that can be as complex as oxidation. B. G. GOWENLOCK

CELL DIFFERENTIATION AND INTERACTION

THE annual research conference of the Biology Division, Oak Ridge National Laboratory, was held at Gatlinburg, Tennessee, during April 9–12. The subject was "Specificity of Cell Differentiation and Interaction", and a mixed gathering of bacteriologists, biochemical cytologists, embryologists, geneticists and immunologists, including a small number from Europe and Sir Macfarlane Burnet from Australia, attended.

After introductory remarks by Dr. A. Hollaender, head of the Biology Division, Oak Ridge National Laboratory, and Dr. A. Weinberg, director of the Oak Ridge National Laboratories, Dr. J. Brachet (Université Libre de Bruxelles) gave the opening talk in which he outlined results of measurements of deoxyribonucleic acid and ribonucleic acid on amphibian oocytes and embryos. New methods have confirmed that there is little or no DNA synthesis before the late blastula stage in the embryo, so that pre-existent cytoplasmic DNA evidently contributes to the nuclei during cleavage. This probability was used by Dr. J. A. Moore (Columbia University, New York), the second speaker, to explain the results of interspecific nuclear transfers in amphibian embryos that he reviewed, in which the nuclei seem to undergo more or less permanent genetic change after dividing a few times in foreign cytoplasm. In discussion, Dr. R. W. Briggs (Indiana University) mentioned that chromosomal translocations have been detected in diploid nuclei after transplantations of this kind. Brachet described nuclei with various staining peculiarities, suggestive of abnormal DNA and RNA content, that arise in some lethal hybrid embryos. He also mentioned new work on *Acetabularia*, including indications that some low-molecular forms of DNA may be synthesized in the cytoplasm in the absence of the nucleus.

Cell-interaction during embryonic differentiation was discussed from various angles by Drs. T. Yamada (Oak Ridge), C. Grobstein (Stanford University) and A. A. Moscona (University of Chicago). Dr. Yamada had achieved the conversion of explanted amphibian gastrula ectoderm into mesoderm, after contact for 3 hr. with a protein fraction from guinea-pig bone marrow. He was studying the mechanism of this conversion, and also of the regeneration of lens from iris cells, by autoradiography, fluorescent antibody techniques and electron microscopy. Dr. Grobstein pleaded for a distinction between 'heterotypic' and 'homotypic' control of cell differentiation, to interpret the dependence of pancreatic epithelium not only on the presence of mesenchyme but also on integrity of reasonably large epithelial pieces, for good differentiation of acini. Dr. Moscona, describing the ability of mixed cultures of dissociated mouse and chick tissues to form composite organs whereas cells of different tissue types from the same animal do not combine, pointed out that after an initial non-selective phase of reaggregation the cells sort out into distinct tissue types by a process which he believes may depend on a molecular fit between protein and carbohydrate at the cell surfaces. The problem he raised of how embryonic cells recognize each other needs linking up with theories about the development of immune mechanisms in embryos, but in an evening round-table discussion on this topic the cellular embryologists failed to get to grips with the immunologists. Immunologists who spoke at the conference were Dr. T. Makinodan (Oak Ridge), who emphasized the importance of plasma-cell differentiation during immune response, and Dr. G. Möller (Karolinska Institutet), who described brilliantly the use of tumour-cell strains to study tissue antigenicity and pointed out discrepancies that had arisen in dating the earliest appearance of transplantation antigens in developing animals by different methods. Dr. R. E. Billingham (The Wistar Institute, Philadelphia) brought together genetical and immunological considerations in reviewing the different abilities of bone marrow and lymph-node tissues to induce tolerance of skin grafts, when different strain crosses of rats and mice were used.

Another aspect of cell differentiation was dealt with by Prof. C. H. Waddington (University of Edinburgh), who showed electron micrographs of developing amphibian notochord cells, and of ommatidia of normal and eye-mutant strains of *Drosophila*. He also showed a film illustrating the different behaviour of myoblast and neural cells in culture, even before they are structurally distinguishable. Any theory of differentiation controlled by genes has to account for the regional differentiation within cells that is now visible in electron micrographs, as well as how such differences are established when the cell-contents are in constant commotion as the ciné pictures show.

In his final summary of the conference, Sir Macfarlane Burnet (The Walter and Eliza Hall Institute of Medical Research, Melbourne) reiterated that genetic information must control all types of differentiation, and he called for an intensified study of gene-controlled immunological anomalies, such as human a-gammaglobulinæmia. He also pointed out our complete ignorance of whether systemic immune mechanisms exist in invertebrates, which do not appear to possess gamma-globulins.

Other speakers at the conference were Drs. L. Herzenberg (Stanford University) and A. J. Riker (University of Wisconsin), who described differentiation in cultures of mouse lymphoma, and gall-infected plant tissues, respectively, and Dr. V. Hamburger (Washington University, St. Louis), who reviewed evidence for cell-specificity in the development of the nervous system. The full proceedings, including discussions, will be published as a supplement to the *Journal of Cellular and Comparative Physiology* later this year.

E. M. DEUCHAR

GRAZING IN TERRESTRIAL, FRESHWATER AND MARINE ENVIRONMENTS

THE fourth symposium to be arranged by the British Ecological Society was held at the University College of North Wales, Bangor, during April 11–14. The subject was treated in the broadest sense, and included not only pasture and range grazing by terrestrial herbivores but also planktonic grazing and other grazing-like phenomena of freshwater and marine environments.

In the first contribution to the symposium, Mr. A. Macfadyen, Zoology Department, University of Swansea, classified the main routes through which matter and energy can flow in a model ecosystem, and discussed the magnitudes of the main trophic components in several contrasting systems, including grassland and plankton. The relationship between stock and productivity can vary over several orders of magnitude, according to the biology of the organisms concerned. Thus the standing crop of plant material in the marine environment is very small compared with that in terrestrial environments, although the energy-flow is of similar magnitude. Grazing yields can be reduced by energy-flow into unproductive pathways, such as ungrazed organic matter by plants, and inefficient utilization of plant material by herbivores. It is therefore clearly important to have a framework within which to compare present performances of ecosystems with potential yields.

Mr. M. B. Alcock, Department of Agriculture. University College of North Wales, Bangor, discussed the problem of achieving maximum output of animal products from grassland. The responses of the plant to defoliation may be due to changes in root growth and uptake of minerals, to utilization of carbohydrate reserves, or to changes in the interception of light energy.

Dr. J. D. Ovington, Nature Conservancy, St. Ives. Huntingdonshire, described work carried out at the University of Minnesota in which he compared the productivity of prairie, savanna and oakwood. The presence of woody plants greatly increases the nutrient circulation and utilization of the site, with the result that the primary productivity of the oakwood almost reaches that of intensively managed fields of maize nearby.

Prof. L. B. Slobodkin, University of Michigan. discussed grazing phenomena in fresh-water, and emphasized that the limited extent and uniformity of most freshwater environments leads to unstable relationships between planktonic species and, in consequence, to wide fluctuations in population numbers.

Dr. Cushing, of the Fisheries Laboratory, Lowestoft, summarized existing knowledge bearing on one of the economically important food chains in the North Sea. He compared the efficient utilization of the planktonic herbivore *Calanus* by the herring with the apparently wasteful feeding on diatoms by *Calanus* itself. In certain years at least, zooplankton grazing limits the growth of phytoplankton. More detailed information on the nutrition and reproduction of *Calanus* obtained from laboratory work was given in a later contribution by Dr. Marshall and Dr. Orr, of the Marine Station, Millport. Dr. D. T. Gauld, Zoology Department, University of Aberdeen, described a second mode of feeding by means of which certain copepods, including possibly *Calanus*, can augment their diet. The maxillæ can be used by the larger copepods not only as passive filtering organs but also as an active 'sweep net' to catch the larger diatoms, dinoflagellates and even animal food.

A further session was devoted to examples of the activities of organisms which occupy a significant place as 'grazers' in various marine habitats. Fortunately, the discussion provoked by the fascinating problems which the speakers introduced took precedence over any consideration of what phenomena should properly be regarded as 'grazing'. Dr. D. J. Crisp, Marine Biology Station, University College of North Wales, considered how the food requirements of barnacles, as measured from growth-rates and feeding-rates, can be met from the amount of suspended matter in sea-water, and concluded that growth is usually limited by the availability of food. Mr. P. R. Walne, of the Fisheries Laboratory, Conway, emphasized the importance of the quality and concentration of phytoplankton cells constituting the food of marine bivalve larvæ. In the laboratory, good growth necessitates a higher concentration of cells than normally occurs in the sea, and it is possible therefore that some other source is available to naturally occurring larvæ. Grazing by limpets was reviewed in the contribution from Dr. A. J. Southward, of the Laboratory, Plymouth. The grazing-rate of the common limpet was measured by observations on algae-covered aquarium surfaces; however, under natural conditions, limpets remove not merely algae but also considerable quantities of the softer rocks such as chalk and limestone. Dr. T. E. Thompson, Department of Zoology, University College, Cardiff, directed attention to the very specialized diet of many nudibranchs; it is possible that the veliger larvæ of some nudibranchs can recognize the presence of their prey and respond by settling on it. Nudibranchs which pass through several generations in a season are prodigal feeders of transitory prey, whereas those with annual life-cycles browse more sparingly on stable and abundant populations of food organisms. Mr. P. J. S. Boaden, Department of Zoology, Queen's University, Belfast, dealt with food relationships within sand and gravel. The interstitial fauna consumes dead organic matter, bacteria, and autotrophs such as diatoms, sometimes removing them individually from grains of sand. The number of animals found in the interstitial spaces indicates that coarse sands contain a fairly rich source of food for the large indiscriminate sand grazers.

Several papers were devoted to the influence of grazing animals, both indigenous and introduced, on terrestrial vegetation. Dr. D. F. Costello, of the U.S. Department of Agriculture, Portland, Oregon, described how ecological research has led to improved practices in range management, especially with regard to re-vegetation, eradication of harmful species, prevention of land erosion and controlled grazing. Dr. J. W. Marr, of the Institute of Arctic and Alpine Research, University of Colorado, and Dr. D. R. Flook, of the Canadian Wildlife Service, Edmonton, Alberta, described the soil conditions, the natural vegetation and the dominant herbivores of the Alpine tundra of the Front Range, Colorado, and of the Banff and Jasper Natural Parks, Alberta. Mrs. D. J. Carr, Department of Botany, Queen's University, Belfast, described degenerative changes and the early stages of accelerated erosion in certain areas of the Bogong High Plains, Victoria, which are believed to be a consequence of their use for summer grazing by the European settlers. Changes extending over a much longer time-scale were described by Dr. R. L. Dix for the North American grassland. The original grassland was traced from its formation in the central Great Plains area during the late Oligocene or Miocene to the present century.

Prof. F. A. Pitelka, and Dr. A. M. Schultz, of the University of California, Berkeley, introduced evidence obtained at the Arctic Research Laboratory, Barrow, Alaska, on the well-known cyclic fluctuations in the populations of the brown lemming, *Lemmus trimucronatus*. These regular changes can be explained on the basis of a simple interaction between herbivore and vegetation with a three-year nutrient cycle. Following overgrazing in a peak year, the nutrients are only slowly recovered and made available again by the third year, and by this time the plant cover has reached a sufficient quantity and quality to support another rapid population increase. Cycles in plant production and in soil nutrient-level were found to correlate with population changes in the lemmings.

The last session of the symposium dealt with the effects of animal behaviour on grazing. Dr. G. W. Arnold, of the Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, Canberra, discussed the importance of the animal's behaviour in selecting and harvesting its food to its nutrition and to the productivity of the system. Decreasing the availability of suitable pasture, though at first compensated by an increase in the time of grazing, eventually leads to a reduction in the total intake of food. Dr. R. Elfyn Hughes, of the Nature Conservancy, Bangor, described experiments on productivity in which the sheep were allowed to distribute themselves voluntarily between richer and poorer mountain pasture.

A technique for analysing sheep diet from epidermal plant fragments identified from fæces, which has a distinct advantage over fistula sampling methods, was described by Dr. D. J. Martin, West of Scotland Agricultural College. A surprising observation, which was confirmed by others taking part in the discussion, was reported by Dr. R. F. Hunter, of the Hill Farming Research Organization. Sheep do not appear to roam freely, but form family groups which restrict themselves to certain territories differing in altitude, aspect and vegetation. This finding is of obvious importance to management of hill sheep. It may also vitiate attempts at breeding by simple selection if the desirable characteristics are attributable more to the home range environment of the sub-flock than to genetic differences.

The proceedings of the symposium will be published and will represent the fourth of the series published by the Society. D. J. Crisp

DEVELOPMENT OF RESEARCH IN NORTHERN AUSTRALIA

AN important advance in the development of northern Australia occurred recently when the Minister-in-Charge of the Commonwealth Scientific and Industrial Research Organization, Senator Gorton, opened a new research laboratory at the C.S.I.R.O.'s Coastal Plains Research Station. The Station is situated forty miles east of Darwin, close to Humpty Doo.

The aim of the research workers in this laboratory, in collaboration with Northern Territory authorities, is to develop agricultural procedures for the development of the coastal plains in the Northern Territory. The crop plant receiving major attention is tropical rice. The breeding of special rice varieties, the determination of planting, harvesting and fertilizer techniques to meet the particular conditions of the area, form an important part of this work. The possibility of forms of agricultural development other than rice are also to be examined.

Work commenced at the Station in 1959 when the Commonwealth Government, through the Northern Territory Administration, provided £124,000 for its capital development and £50,000 per annum for running costs. Since that time it has been possible to provide accommodation and special facilities to enable research workers to carry out their programme under tropical conditions. These facilities have attracted a staff of fifteen officers to the Station.

The agricultural development which has so far taken place in Australia has mainly evolved from European agricultural practices although Australian scientists have solved many of the problems peculiar to the Australian environment, particularly in tropical Queensland. The essential European temperate agricultural background has resulted in greater development of the southern areas of Australia, and only within the past decade or two has Australia been able to divert its scientific effort to the less-developed areas of the northern environment.

More than half a million square miles of northern Australia have now been surveyed and mapped by C.S.I.R.O. scientists. Large areas have also been examined for possible mineral development by the Bureau of Mineral Resources.

The development which is now occurring at the Coastal Plains Research Station is a further step forward in the essential advances of this vast area. The station is one of a number of C.S.I.R.O. units working on agricultural problems of the north. Others are located at the Ord River in the Kimberley region of Western Australia, at Katherine and Alice Springs in the Northern Territory, and at Mareeba, Ingham, Rockhampton, Brisbane and Cunnamulla in Queensland.

The proposed establishment of a new pasture research laboratory at Townsville also indicates the strong desire of the Government to provide a sound scientific basis for the development of these areas.

All these, in conjunction with the activities of the Northern Territory Administration Technical Services, will contribute to the eventual development of a vast region, the potential of which has scarcely been touched.

THE ARTHUR D. LITTLE RESEARCH INSTITUTE

THE annual report of the Arthur D. Little Research Institute for 1961 * also briefly reviews the first five years achievements of the Institute, and, besides a list of senior staff, includes a list of the Acorn Lectures delivered since January 1958. Lists of publications are included under the several sections. The Biochemistry and Biophysics Section is now well established, and during the year centred on isolated wheat gluten. Starch-gel electrophoresis has shown that there are at least 26 protein components and paper chromatography that the phospholipids of flour are present in the same complexity in isolated gluten. Equipment has been designed to study the fundamental properties of gluten as an elastomeric gel.

The Organic Chemistry Section has prepared a range of stable true sodium sucrates up to the hepta derivative and studied their reactions with more than 50 chloro compounds, particularly the reactivity of penta-sodium sucrate under mild conditions in the presence of dimethoxyethane. Other work has been concerned with the polymerization of glucose and its derivatives, the preparation of carbohydrate polyamides with viscosities comparable with that of nylon by interfacial polycondensation, polyglucoses and the chemical modification of wood, for example, its phosphorylation, the dimensional stability of wood, the preparation of chlorinated phenyl derivatives of cellulose and the emission of corrosive volatiles from wood, while early in 1960 the study of the chemistry of ethylene sulphide was begun.

In physical chemistry, the polymer group has been concerned with the mechanism and kinetics of formation of 'isotactic' polymers and with certain of their bulk properties with the view of a better understanding of the effect of structural and environmental parameters. The kinetics of the reaction between molten aluminium and water vapour at about 1,000° C. have also been studied, while the Metallurgy Section has attempted to establish relations between the effectiveness as corrosion inhibitor of an additive in aqueous solution and its molecular structure. Although 470 inhibitors were tested, no clearly defined relation was established between threshold concentration and molecular structure. A study was also made of the influence of additives on the relation between applied current and metal potential for both cathodic oxygen reduction and anodic metal dissolution. Investigations have commenced on the hydrogen embrittlement of very strong steels with the view of devising a procedure for acid pickling which avoids subsequent hydrogen embrittlement.

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* Arthur D. Little Research Institute. Annual Report for 1961. Pp. 46. (5th Anniversary Report.) (Inveresk, Midlothian: Arthur D. Little Research Institute.)

SCIENTIFIC AND TECHNICAL EDUCATION IN SCOTLAND

THE Staff Association of Paisley Technical College is to be commended for the way it has made a critical examination of the Scottish system of education with particular reference to advanced technical education. A memorandum submitted to the Robbins Committee on Higher Education is concerned mainly with the present system and the possible future structure of higher education in relation to the technological and technical training provided in Scottish Central Institutions. The Staff Association considers that there is no general lack of opportunity for higher education in science and technology as there appear to be only rare cases of qualified students not being accepted for the course of their choice. On the other hand, there is a distinct lack of information of the kind which would enable a potential student to make the most suitable choice.

The present courses in scientific and technological subjects are suitable, on the whole, for those attending them. If better guidance were available for students there would be fewer instances of students attending courses not suited to their abilities and inclinations. The ordinary B.Sc. in pure science, awarded by the Scottish universities, does not give a satisfactory qualification for teaching science, except at lower levels, nor does it provide a suitable qualification for any other career in science. Many students who are at present directed into pure science may have more aptitude and likelihood of success in a branch of applied science. There is insufficient opportunity for those who have demonstrated abilities above their chosen level to change to a higher level of education. The admission requirements and the exemptions allowed for entry to higher courses and professional institutions are unrealistic in some cases and should be rationalized.

The Association considers that there should be more opportunities for people who have already gained higher education qualifications to add to these; many older people who have not received any higher education could successfully complete courses in higher education if given the opportunity. Lack of information and guidance often prevent suitable people from starting on a new career. The various forms of membership offered by professional institutions safeguard the standards within the professions but, like educational institutions making external awards, they are slow to change syllabuses, examination procedures and admission qualifications to suit changing conditions.

There is sufficient freedom of academic and administrative policies at lower levels, but many higher educational establishments would function more efficiently if they had a greater degree of autonomy at the higher planning levels. This would encourage some much-needed integrative planning among higher education institutions and would lead naturally to over-all integration of the higher education system.

Copies of the memorandum are available from the Secretary, Staff Association, Technical College, Paisley.

WORLD-WIDE MICROSEISMIC STUDY

By DR. H. M. IYER*

Meteorological Office, Bracknell, Berks.

EVEN though the over-emphasized importance of microseisms for meteorological and oceanographical investigations has waned fast over the past decade the geophysical implications still remain interesting. The following are some interesting possibilities:

(1) The energy in microseisms is predominantly contained in the 0·25–0·1 c.p.s. band[1], with a peak in the neighbourhood of 0·15 c.p.s. Considering that microseisms contain an appreciable amount of surface waves of the Rayleigh type travelling in one or more directions[2] and in the frequency-range covered by them earthquake surface waves are seldom observed, it seems logical to use microseismic data to supplement investigations of earthquakes.

(2) The well-known idiosyncrasy of certain sources (usually intense storms) in the ocean giving entirely different microseismic intensities at two seismograph stations on the same coastline suggests strong refraction effects. When this observation is taken in conjunction with the characteristic peaking of the microseismic spectrum, it makes sense to postulate that the Rayleigh waves in microseisms are strongly affected by the transition from the ocean to the continent. The dispersion relation for such transition is very well known[3] even though its application to the examination of propagation of microseisms is often overlooked. I have suggested that the continental margin acts as a low-pass filter for microseisms[4]. The direct verification of this has to wait until a seismograph station is established in the deep ocean. However, a preliminary Russian experiment of recording microseisms on the floor of the Black Sea[5] and comparing it with simultaneous recording on land showed that 2-sec. microseisms were twenty times stronger under the sea than on land.

(3) The third interesting fact about microseisms is that they seem to be always present everywhere. In island stations like the United Kingdom, the basic background of microseisms is of the order of 2–3μ peak to peak, the activity rising to several times this value under certain meteorological conditions. On the other hand, there are places like California where the background of the order of 1-μ movement, with the usual spectral peak, occurs day after day with no specific source to which this could be attributed. The interesting possibility of the Earth being filled with 'seismic noise' cannot be over-ruled.

(4) For the very practically minded, the immediate problem of detecting low-level seismic signals calls for a knowledge of the directional spectrum of 'seismic noise'.

(5) Last, but not the least in importance, is the classical problem of the origin of microseisms. Several well-known theories of origin are in existence;

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* Gassiot Fellow in Seismology.

but no one completely accounts for all the observations. However, I believe that postulation of new theories and modification of old ones are only of academic interest unless preceded by consistent detailed observation and analysis over several points of the globe for an appreciable length of time.

Hence, it appears that the war with microseisms is not over, and only considerable research spread over space and time can provide answers to all the questions.

During the International Geophysical Year a valuable amount of microseismic data was collected on this basis. But I contend that such information obtained from conventional earthquake seismographs with widely different characteristics is only of statistical interest. The present is the most opportune moment to think about a programme of international study of microseisms in line with the general renaissance in seismology and particularly because a world-wide network of standardized seismographs is coming into existence, for the first time in the history of seismology.

The aim of this article is to call for incorporating certain essential instruments with all good seismograph stations so that a detailed examination of microseisms on an international level can be made possible. The cost of such instrumentation is negligible compared with the overall expenditure incurred in establishing the station.

Theory of Microseisms

The first question is to decide what information is required from each seismograph station, and for this it is essential to understand some basic ideas on the nature of microseisms.

As in any geophysical time series, let us assume that microseisms consist of signal and noise. Very useful directional results have been obtained in the past[6] by assuming the signal to consist of Rayleigh waves coming from certain coherent direction or directions and noise confined mainly to the horizontal components and isotropic in nature. Based on this model, in the simple case of a source making an angle θ with the east–west axis, we can write for the three orthogonal components:

$$\text{east–west } x(t) = R(t) \cos\theta + \text{noise} \qquad (1)$$

$$\text{north–south } y(t) = R(t) \sin\theta + \text{noise} \qquad (2)$$

$$\text{vertical } z(t) = kR(t) \qquad (3)$$

where $R(t)$ is the Rayleigh wave time series and k is the polarization factor for Rayleigh waves (assumed to be independent of frequency over the range of interest). The problem is to estimate:

(1) Spectra of $x(t)$, $y(t)$, $z(t)$

(2) Spectrum of $R(t)$/noise

(3) θ and k.

As in any signal-to-noise problem, the solution is to make spectral and cross-spectral (cross correlation) estimates of the components at the same station and between different stations. For a single source of microseisms, assuming the vertical component to contain pure Rayleigh waves, by correlating each of the horizontal components in turn with the vertical, the Rayleigh wave part could be completely defined. The operation can be represented as follows:

$$\overset{(\tau)}{C}xz = \int_T x(t)z(t+\tau)\,dt \qquad (4)$$

$$\overset{(\tau)}{C}yz = \int_T y(t)z(t+\tau)\,dt \qquad (5)$$

$$\overset{(\tau)}{C}xy = \int_T x(t)y(t+\tau)\,dt \qquad (6)$$

where C is the correlation function, $\int_T$ represents integration over a time-interval T and τ is the usual time lag used in estimating the correlation function.

Let us confine our attention to a narrow band of mean frequency f_0 c.p.s. Since, for Rayleigh waves, the vertical component is 'in quadrature' with the horizontal components, maxima of $\overset{(\tau)}{C}xz$ and $\overset{(\tau)}{C}yz$ occur every time $\tau = \pm\,(2n+1)\,\frac{\pi}{2}\,\frac{1}{f_0}$ where $n = 0$, $+1$, $+2$, etc.

From equations 1, 2, 3 we can thus write:

$$C_{xz}^{\max} \alpha k\overline{R^2(t)} \cos\theta \qquad (7)$$

and:

$$C_{yz}^{\max} \alpha k\overline{R^2(t)} \sin\theta \qquad (8)$$

Therefore:

$$\tan\theta = \frac{C_{xz}^{\max}}{C_{yz}^{\max}} \qquad (9)$$

Once θ is estimated the other unknowns can be evaluated from a knowledge of the spectra of $x(t)$, $y(t)$ and $z(t)$.

Techniques

(1) The most obvious way of evaluating the parameters already mentioned is to start from the analogue records produced by the instruments and analyse them either directly or after digitization, depending on available resources. In any event, a good record is a prerequisite. Unfortunately, the drum recorders used in most seismograph stations do not provide sufficient resolution either in time or in amplitude to perform this analysis. Since microseismic samples need be taken only for half an hour or so for twice a day, the ideal thing would be to incorporate a sensitive three-channel strip chart recorder at the output of the seismographs. Most of the long-period seismographs in use nowadays have a sensitivity of more than 25 μv./μ movement near the microseismic peak frequency and it is not difficult to visualize a suitable recorder with least resolution of about 0·5μ and dynamic range of 100.

Even at a speed of recording of 1 mm./sec., in half an hour it will produce only 6 ft. of record. Such a system should not cost more than 2 per cent of the cost of overall equipment layout. The records thus collected could be sent to a few experimental centres where reading facilities and computer time are available for spectral and cross-spectral studies. In certain areas, a few neighbouring stations can join together and produce time-synchronized records leading to very valuable studies of spacial distribution of microseismic sources and propagation problems. This type of collection of data takes off the limitation of the simple model outlined so far and lends itself to more general analysis.

(2) For certain stations (isolated island stations in hurricane belts) immediate knowledge of the direction of arrival of microseisms is of great interest. In any event, a quick and easy way of directly obtaining this information from seismograph signals is desirable rather than waiting for the results of detailed analyses of the type outlined in the previous paragraph. The most practical way of handling this problem is to

estimate $C_{xz}^{\max}$ and $C_{yz}^{\max}$ (equations 7 and 8) or their nearest approximations using a simple analogue 'in line' correlator. A method already used[7] employs simple contact galvanometers at seismograph outputs to estimate correlation co-efficients using Tomoda's zero crossing principle. The contact galvanometers are used to examine at successive intervals of time whether the two outputs used are both deviated on the same side or opposite sides of zero. From this 'in phase' and 'out of phase' information, taken over a sufficient length of time, the correlation coefficient (maximum) between the two outputs can be computed.

A much more elegant method will be to compute $C_{xz}^{\max}$ and $C_{yz}^{\max}$ directly and feed them simultaneously to the two deflexion systems of an X–Y plotter. The position of the point vectorially represents the direction of arrival θ. If such an instrument is in operation during a 'microseismic storm' the scatter diagram of θ on the X–Y plotter should indicate the average direction of the source. A type of automatic direction finder as above can be easily made by subjecting suitably amplified and filtered signals from seismographs to addition, squaring, integration and plotting. Squaring can be easily carried out using a simple thermal square law unit and integration by a low leakage capacitor of the plastic film type. In fact, the instrumentation could be made very compact, cheap and simple.

Another basis for designing a direction-finder is to use a technique similar to that used by Jensen[8]. Since Rayleigh waves in the vertical are in quadrature to those in the horizontal components, it is seen that every time the vertical crosses zero the Rayleigh waves in the horizontal should reach a theoretical maximum in the positive or negative direction. Thus by estimating the deflexion of the east–west and north–south components at the zero crossing times of the vertical, for a sufficient length of time, it is possible to make a statistical estimate of the ratio of the north–south to east–west component of Rayleigh waves, which is $\tan\theta$. It could be easily arranged so that every time the vertical component crosses zero, a simple unit can be made to generate a pulse and this pulse could cause the instantaneous values of east–west and north–south deflexions to be plotted on an X–Y plotter. The series of points thus obtained should indicate the mean value of θ.

Conclusion

In conclusion, it is proposed that all modern seismograph stations take an active interest in microseismic study as well, and:

(1) Produce a good microseismic record with sufficient amplitude and time resolution for detailed study, twice a day.

(2) That groups of interested workers join together to analyse such records in places where analogue or digital analysers are available.

(3) Simple direction finding instruments be incorporated in the seismograph stations to provide scientific and utilitarian data on the relation between storms and microseisms.

The foregoing programme, or a better alternative, if carried out at least over a selected number of stations for an experimental period of time, should provide solutions to the many unsolved problems in microseismic science and prove a valuable tool in geophysics and in the meteorology of remote island stations.

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RADICAL PRODUCTION AND ENERGY TRANSFER IN RADIATION CHEMISTRY AND IN PHOTOCHEMISTRY

By DR. GISELA KALLMANN OSTER and PROF. HARTMUT P. KALLMANN

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ONE of the most interesting questions in the radiation chemistry of organic substances is which of the excited states of the solvent is involved in radical production and in energy transfer. It has been demonstrated with organic scintillators[1] that up to 5 per cent of the absorbed ionizing radiation energy (γ-radiation) appears in the first excited singlet electronic state, either due to direct excitation to this state or due to cascading down from higher states. Radical yields in benzene–carbon tetrachloride mixtures are in excess of those expected from direct absorption by the constituents which should be proportional to their mass ratios[2]. This excess radical production has been attributed to energy transfer from the benzene to the carbon tetrachloride molecule[3]. We have determined which excited states of benzene are involved in this process by comparing radical production by optical excitation and by ionizing excitation particularly in the crucial region of low carbon tetrachloride concentration.

The radical concentration was determined by scavenging the radicals with the stable purple free radical diphenyl picrylhydrazyl (DPPH). The disappearance of the scavenger was followed by measuring the decrease in optical density at 546 mμ where the absorption of the yellow scavenger product is insignificant. All samples were thoroughly degassed by five-time freezing and thawing under high vacuum. The ionizing radiation source was a tungsten target X-ray tube operated at 250 kV. and 15 m.amp. with an output at the sample of 30,000 r./hr. as measured with a calibrated ionization chamber. The photochemical reactions were carried out using an 8-W. mercury resonance lamp producing mainly

254 mμ, the 185 mμ line being filtered out. The quartz reaction cell (1 cm.^{2} cross-section) which was fitted for degassing was placed at a distance of 30 cm. from the lamp, where it received 2·45 × 10^{-8} einsteins/min./c.c. as shown by uranyl oxalate actinometry. Care was taken to ensure that the overwhelming absorption was due to benzene by making the concentration of benzene so large that its optical density at 254 mμ was at least ten times higher than the sum of the optical densities of all other constituents. In separate experiments it was established that DPPH in carefully degassed solutions is not photolysed by 254 mμ radiation. In order to avoid local depletion of the scavenger at the front surface of the cell, vigorous stirring by means of a magnetic stirrer was utilized. Without stirring it was noticed that a slow disappearance of the scavenger occurred after illumination. This dark reaction, which does not occur when the system is stirred, is attributed to the reaction of DPPH with chlorine produced from chlorine radicals when scavenging is incomplete. All rates of radical production are initial rates in order to assure that DPPH was always present in excess. The rates only decreased somewhat after 70 per cent of the scavenger has been consumed.

We have found that free radicals are also produced when benzene is irradiated with 254 mμ light in the presence of small amounts of carbon tetrachloride. This radical production can only be caused by energy transfer from the first excited state of benzene to a carbon tetrachloride molecule. Trivial re-absorption is ruled out since benzene is only weakly fluorescent, yet the quantum yield of radical production may be as high as 0·66. On comparing the formation of radicals by ionizing radiation and by 254 mμ radiation, we notice some significant differences. In Fig. 1, the excess radical formation with ionizing radiation due to energy transfer from benzene to carbon tetrachloride is computed by deducting from the observed rate of radical production the rates of radical production due to primary decomposition of either benzene or carbon tetrachloride. These latter rates were determined by measuring the rates of radical production in the pure solvents and assuming that in a mixture these rates are proportional to their relative masses when equal amounts of energy are absorbed.

It is seen that in the ionizing radiation case (Fig. 1) considerably higher concentrations of carbon tetrachloride are required to reach a plateau as compared with the rapid rise of curve *A* of Fig. 2. This demonstrates that shorter lifetimes of excited states, presumably higher electronic states, are involved in the ionizing radiation case. When certain substances, such as phenylbiphenylyloxadiazole, *o*-terphenyl or fluoranthene, which are known to deactivate the first excited state of benzene and the fluorescence of which is not quenched by carbon tetrachloride[3] are added to the benzene–carbon tetrachloride mixtures, they markedly reduce the rate of radical production under ultra-violet excitation but have absolutely no effect under ionizing radiation excitation. The concentration of these substances was of the order of 0·1 *M* corresponding to their maximum solubility, and the carbon tetrachloride concentration was varied from 0·1 *M* to 0·005 *M*. This finding further substantiates our conclusion that only higher excited electronic states of benzene contribute to radical production under high-energy excitation.

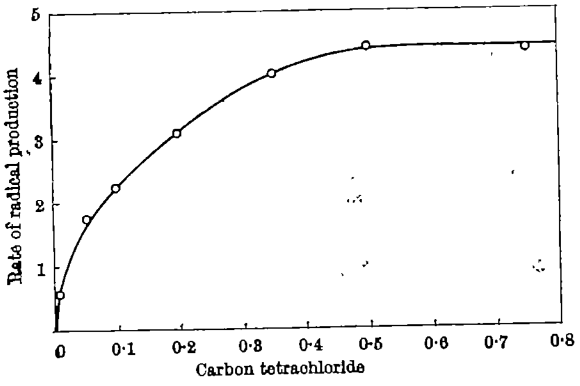

Fig. 1. Rate of excess radical production (moles/l. min. × 10^{6}) as a function of carbon tetrachloride concentration (moles/l.) using ionizing radiation (30,000 r./hr.). Initial DPPH concentration 5 × 10^{-4} moles/l.

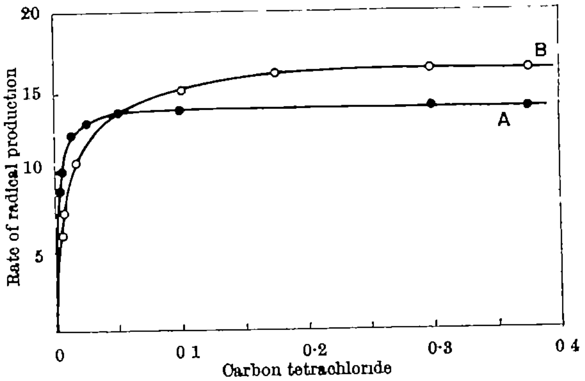

Fig. 2. Rate of radical production (moles/l. min. × 10^{6}) as a function of carbon tetrachloride concentration (moles/l.) using 254 mμ source (2·45 × 10^{-8} einsteins/c.c./min.). Initial DPPH concentration 2 × 10^{-4} moles/l. Curve *A*, solvent pure benzene, curve *B*, solvent *cyclo*hexane/benzene (5 × 10^{-3} moles of benzene/l.)

The region above 1 *M* carbon tetrachloride (approximately 0·1 mole fraction), corresponding to the condition where each benzene molecule is in contact with at least one carbon tetrachloride molecule, is just the region where we see the curve of Fig. 1 approaching a plateau. Apparently for ionizing radiation only those excited benzene molecules contribute to radical production which have a carbon tetrachloride molecule as a nearest neighbour. The concentrations used in the aromatic quencher experiments make it unlikely that any excited benzene is being simultaneously surrounded by both carbon tetrachloride and aromatic quencher; hence no competition will occur between quencher and carbon tetrachloride. This situation is completely different from the case of light excitation where energy migration is involved. With 254 mμ radiation only the lower state of benzene is excited the energy of which migrates because of the relatively long lifetime of this state and reaches in this manner the carbon tetrachloride molecule.

The difference of curves *A* and *B* of Fig. 2 at low carbon tetrachloride concentrations can be attributed to energy migration which would only occur to a significant extent in pure benzene (curve *A*). In curve *B* the energy reaches the carbon tetrachloride molecule only by material diffusion, which is not as fast as energy migration and therefore requires larger carbon tetrachloride concentrations. The difference of curves *A* and *B* at high carbon tetrachloride concentrations may be ascribed to two different reactions between carbon tetrachloride and excited benzene molecules, one leading to the formation of radicals, the other just to a deactivation of excited benzene. The relative extent to which these two reactions occur may be affected by the solvent. We have evidence from other experiments for the existence of these two types of reactions. It may be further noted that the relatively high quantum yield of radical production (0·66 for the plateau of curve *B*) indicates that the excitation energy of benzene is relatively easily transferred to the atomic vibrations of the carbon tetrachloride molecule, where it results in dissociation into free radicals.

Under excitation by ionizing radiation the first electronic state of benzene is also excited, but since this excitation amounts to not more than 3 per cent[1], its contribution to radical production is insignificant compared to that by higher excited states.

Grateful acknowledgment is made of the support of this investigation by the United States Army Signal Corps, under contract No. *DA* 36–039 *SC*–78056 and by the Aeronautical Research Laboratory, under contract No. *AF* 33(616)–8317.

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VERY LOW COERCIVE FORCE IN NICKEL–IRON FILMS

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THIN films of evaporated 80–20 nickel–iron alloy, 1000 Å. thick, normally exhibit domain wall coercivities of 1·5–2·5 oersteds, depending on the experimental conditions. I have recently prepared laminated films, with a total nickel–iron thickness of 1000 Å., with coercitivities of about 0·2 oersted[1].

Fig. 1 shows the magnetization curves, obtained with a 500 c./s. loop tracer, of a normal film in the easy and difficult directions (H_C = 2·0, H_K = 3·5 oersteds), and underneath are shown the corresponding magnetization curves for a composite, laminated film prepared in the same deposition (H_C = 0·2, H_K = 3·5 oersteds). There is, therefore, a 10 : 1 change in domain wall coercivity, whereas the hard direction magnetization curve is unaltered.

The low coercivity film consists of ten laminations of nickel–iron, each 100 Å. thick, interleaved with silicon monoxide layers of comparable thickness. The silicon monoxide and nickel–iron layers were deposited alternately in the same vacuum, using a differential shutter, starting and finishing with the silicon monoxide. The pressure in the vacuum system was about 1 × 10^{-6} mm. mercury during the deposition of nickel–iron, and the substrates were glass microscope slides heated to 320° C.

The coercivity of a magnetic material can be reduced by relief of stress, or by the reduction of the impurity content. The isotropic stress in the laminated films may have been reduced in the manner described by Blois[2]. By laminating nickel–iron, which has a thermal expansion coefficient larger than that of his glass substrates, with a material of low thermal expansion, he could reduce the stress in his layers. However, in the present experiments, laminated layers of silicon monoxide and nickel–iron deposited on copper substrates had the same low coercivity as layers made in the same deposition on glass.

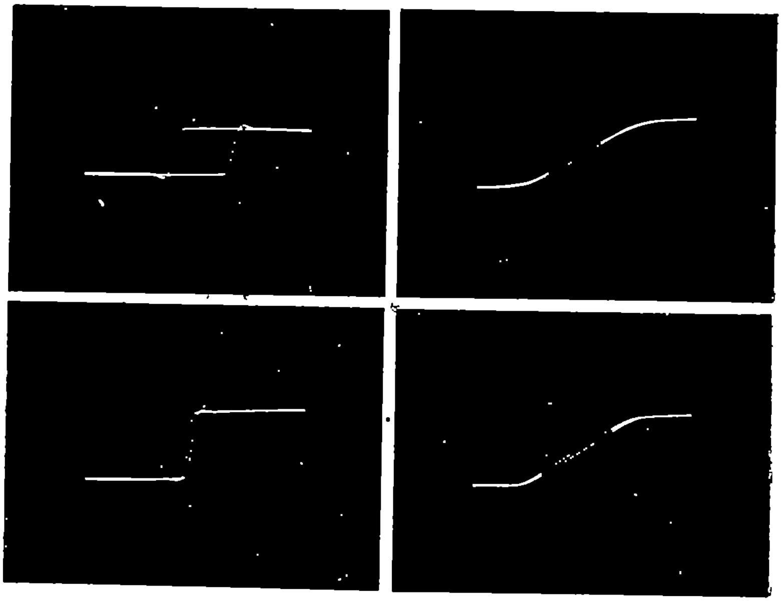

Fig. 1. Top left, longitudinal *I–H* loop 1,000 Å. solid nickel-iron film; top right, transverse *I–H* loop 1,000 Å. solid nickel-iron film; bottom left, longitudinal *I–H* loop 1,000 Å. composite nickel-iron film; bottom right, transverse *I–H* loop 1,000 Å. composite nickel-iron film

Further, it seems unlikely that laminating a nickel–iron film with silicon monoxide could improve its purity, especially as nickel–iron laminated with calcium fluoride also has low coercivities. Again, a single lamination of nickel–iron does not have a low coercivity whereas several laminations do produce this effect, as described.

A more likely interpretation of the effect may be that the reduction of coercivity is due to a reduction of the domain wall energy, γ. The coercivity of a thin film may depend on either the domain wall energy itself or on its variation with wall position, $\frac{d\gamma}{dx}$ (ref. 3). To account for a reduction of coercivity in this way it is then necessary to assume that $\frac{d\gamma}{dx}$ is itself dependent on γ.

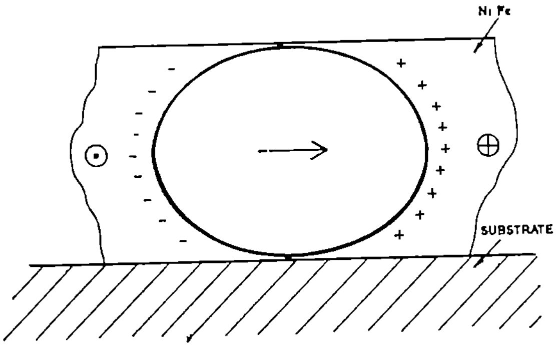

Fig. 2. Néel wall

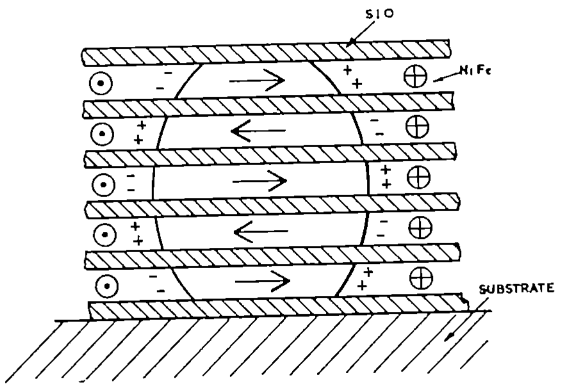

Fig. 3. Proposed wall structure in composite film

Fig. 2 shows diagrammatically a cross-section through a Néel wall in a thin film. (The cross-section is here shown as elliptical, but the actual shape is unimportant.) Exchange interaction causes the whole of the magnetization inside the wall to lie in essentially the same direction, as shown by the arrow in Fig. 2, giving rise to a large free-pole energy.

In a laminated film there can be no exchange interaction through the silicon monoxide separating neighbouring nickel–iron layers. Hence there appears to be no reason why the magnetization within the Néel wall should not reverse its direction at every lamination, thus reducing the magnetostatic energy, as shown in Fig. 3. The surface energy density γ of such a configuration would be much smaller than that of a conventional Néel wall, and this reduction may account for the very low coercivities observed here.

Using a dried colloid technique, domain walls were readily observed on solid nickel–iron layers 1000 Å. thick covered with silicon monoxide. However, no domain walls were seen on laminated layers with a total nickel–iron thickness of 1000 Å. This is at least an indication that there are smaller stray fields associated with domain walls in laminated layers than there are with those in solid layers, as would be expected from the above model.

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KARST MORPHOLOGY IN AUSTRALIAN NEW GUINEA

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THE increasing interest in recent years in the climatic morphology of limestone[1-3] has been chiefly directed at discerning the different karst styles associated with horizontal climatic variation. Fewer attempts[4] have been made as yet to see what happens to limestone land-forms as they are traced through height, though the principle that morphoclimatic systems are storied is well established[5]. In a further approach to this question during 1960 and 1961, we made reconnaissance studies of a number of karst areas in Australian New Guinea. These range in height from sea-level to well above 3,000 m. and are developed in limestones which vary considerably in age and in lithology (Fig. 1).

(*a*) *Low levels* (cf. *tierra caliente*). In the lower Kikori area (Gulf District) at heights mainly below 200 m., Lower Miocene limestones subject to 5,000–7,500 mm. annual rainfall give rise over wide areas to typical 'Kegelkarst' of Lehmann's Goeneng Sewoe type[6]. The relief consists of a sea of roughly hemispherical hills with enclosed star-shaped depressions —the 'cockpits' of tropical karst[7]. There is some orientation, apparently joint-controlled. This 'Kegelkarst' is developed in different lithologies—in white chalky limestone, cream algal limestone and brown crystalline limestone.

Additionally, there is what the oil geologists call 'crevice' country; this is level or gently undulating in overall relief but intricately cut up in detail by irregular fields of close-set solution corridors. It resembles most closely the 'giant grikeland' phase of

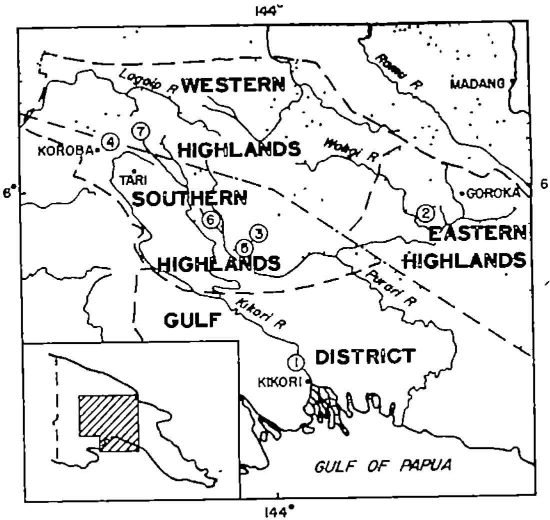

Fig. 1. Location of the Karst areas discussed. 1, Lower Kikori R.; 2, Yandimi; 3, Tualamara; 4, Paru R., Kambe Ck., and Tabaiya Mabu; 5, Sugu R.; 6, Nembi R.; 7, Mt. Kaijendi. Stippling denotes mountainous country

the tropical semi-arid karst of the Limestone Ranges of West Kimberley in Australia[8], though lacking its larger dimensions and greater rectilinearity through joint control.

Since the two sorts of terrain can be found on the same limestone and since 'crevicing' can occur within 'Kegelkarst', an explanation in terms of lithology for the two does not seem possible. In some cases crevice karst appears to be formed in flat erosion surfaces along major valleys, perhaps formerly alluviated, but this association is not invariable.

(*b*) *Intermediate levels* (cf. *tierra templada*). In some areas doline karst prevails, that is, the relief is dominated by enclosed depressions of moderate dimensions and rounded contour, ranging from conical to dish shape. Such karst around Tualamara (Southern Highlands District) about 1,580 m. high and with approximately 3,100 mm. rainfall occurs on an impure Miocene limestone formation with interbedded siltstones, whereas in the neighbourhood of Yandimi (Eastern Highlands District) it is found on the pure Chimbu Limestone of Upper Cretaceous–Eocene age (height 1,500 m., annual rainfall 2,200 mm. with a short dry season). Around Korenda and Tabaiya Mabu (Southern Highlands District) doline karst seems to be located synclinally where Orubadi Mudstone has recently been stripped from Darai Limestone.

Equally widespread at the intermediate levels, however, is limestone terrain dominated by projecting residual hills, which nevertheless overlook conical or bowl-shaped dolines. The hills assume either a blunted pyramidal form with slopes about 40° or else a tower form, conical at the top but with vertical or nearly vertical sides. Both appear to be fashioned by the deepening and inosculation of the dolines. The towers seem to have evolved from the pyramids by the development of high free faces. Examples of such kinds of karst are: (1) Sugu R. area, height 1,400–1,650 m., rainfall 3,100 mm.; (2) Nembi R. area, height 1,400–1,750 m., rainfall 2,700 mm.; (3) Paru R.–Kambi R. area, height 1,600–2,000 m., rainfall 2,400 mm. All these are developed in the pure Darai Limestone of Miocene age in the Southern Highlands District. Within them occur large enclosed depressions elongated along the strike, the floors of which consist of fields of dolines, for example, Pewa depression west of the Nembi valley.

(*c*) *High levels* (cf. *tierra fria*). In the one high-lying area examined, between the Porgera–Lagaip drainage and the Tari Basin at elevations of 2,600–3,700 m., three karst types were found in limestones probably Upper Cretaceous–Eocene in age. On the Nia alpine grassland (3,100 m.) is a limited area of doline karst associated with pure limestone being stripped of a siltstone cover. This is surrounded by higher forested karst of the pyramid-and-doline type. The third type consists of vertically walled, knife-edged arêtes and pinnacles of bare limestone standing like battlements around dolines covered by montane forest of mossy aspect. This occurs on the south flank of Mt. Kaijende (Fig. 2) and in two other areas southwards towards the Tari basin.

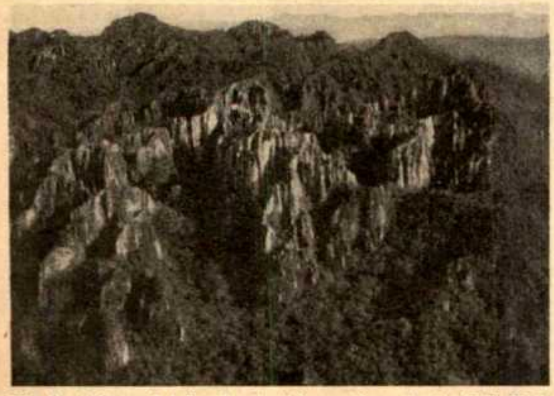

Fig. 2. Arête-and-doline karst of the southern flank of Mt. Kaijende, Western Highlands District, Australian New Guinea

Though these observations pose many questions as yet unanswerable, they bear significantly on some of the issues much discussed recently by students of karst.

(1) In the humid tropics, 'Kegelkarst' has been related to pure limestones, whereas doline karst has there been thought to be associated with impure calcareous formations[7,9]. Although no doline karst at low levels has yet been encountered in Australian New Guinea, doline karst at intermediate levels is definitely not restricted to impure calcareous facies.

(2) Lehmann[10] has shown how geomorphologists were confused by established terminology based on classical studies of the Yugoslavian karst when they first came to consider tropical humid karst. Observers such as Daněs[11] failed to recognize some of the significant differences between temperate dolines and tropical cockpits, between temperate hums and tropical residual limestone hills. Through their transference without much qualification of the classical karst terminology to these different tropical land-forms, these early authors who described tropical humid karst misled theorists such as Grund in Europe[12]. The latter consequently regarded the tropical 'Kegelkarst' as a more advanced stage of a single karst cycle, representing the further evolution of doline karst. Largely as a result of Lehmann's and von Wissmann's work in the 1930's, the misconceptions were recognized and the idea that tropical 'Kegelkarst' developed in a separate manner altogether was established.

However, though the New Guinea low-level observations seem to sustain the modern point of view, the data from intermediate and high levels call for an elaboration of it. Much of the karst here is dominated by fields of residual limestone hills, but these are genetically associated with dolines clearly of European type. In fact the relief resembles most closely, not tropical 'Kegelkarst' as described more precisely by modern workers, but the false picture Alfred Grund conceived of it in his attempt to put tropical and temperate forms into one evolutionary sequence. His theoretical block diagram[12] sketches hollow-chested, horn-like peaks rising between inosculating, flat-floored dolines. These imagined residual projections correspond in large measure with the pyramidal hills of much of the karst at intermediate levels in New Guinea. However, the dolines associated with the latter have not developed significant flat floors. The Davisian deductive approach has been adversely criticized recently, but certain virtues may be demonstrated in that something very close to Grund's mature karst, which was at least as much the product of his theoretical deduction as of Daněs's observations, occurs in New Guinea.

(3) It is to be noted, however, that the pyramid— or tower-and-doline—karsts occur at higher levels in climates resembling European temperate climates more closely than do the full tropical conditions of the lower Kikori 'Kegelkarst'. At higher levels still the Mt. Kaijende karst departs even more completely from 'Kegelkarst', the residual hills being hollowed

out on their flanks until they are often reduced to arêtes with gendarmes. Thus, there seems to be an altitudinal climatic zonation of karst in New Guinea, though at each level the picture is not a simple one, complicated as it is by the cross-cutting influences of lithology, structure and evolutionary history (cf. Verstappen[13]).

It has been found that the periglacial phenomena of high tropical mountains do not match completely those of sub-polar regions but have their own character. It is apparent from the data presented here that the same lack of full parallelism between the effects of latitudinal and of altitudinal climatic gradients applies also to karst.

(4) Within tropical 'Kegelkarst', two major categories have been recognized by many authors, characterized by the hemispherical residual hill ('Kugelkarst') and by the tower ('Turmkarst'). Lehmann[14] and others have associated the latter with lateral solutional undercutting in association with the development of alluvial covers at some local rest-level of ground-water or else close to sea-level. The same factor, though locally operative at intermediate levels in New Guinea, for example, near Nanje in the lower Amia valley (Southern Highlands District), does not generally explain the differentiation of pyramids and towers there. The very steep walls of these towers usually rise from footslopes which correspond in angle and nature to the slopes of the pyramids without any intervention of lateral solutional undercutting or association with horizontal surfaces at all.

(5) Coleman and Balchin[15] have recently produced a strong case for regarding all the dolines of the Mendip Hills as stages in the evolution of collapse dolines and for rejecting the idea of solution dolines entirely for that area. They extend this finding to maintain that collapse dolines and their derivatives alone enter into 'the' karst cycle, which from their examples is implicitly regarded as applying to tropical as well as to temperate regions. This extension does not seem justifiable. In New Guinea, fields of evenly scattered dolines, both in normal doline country and in association with pyramids and towers, cover large areas, on divides as well as in valleys. It seems unlikely that all these dolines could be due to regular collapse patterns into underground river systems. Surface solution must be dominant here.

(6) Finally, a problem of great controversy has been the relative rate of limestone solution and of karst development in different climatic conditions. Lehmann, Bögli and Birot[1–3], among others, have maintained that in humid tropical lowlands conditions are optimal for these processes, relying chiefly on the *a priori* reasoning that chemical reactions will proceed fastest in accordance with Arrhenius's equation and that water going underground will have the greatest amounts of carbon dioxide in solution through the high rate of vegetation growth and decay, and of microbiological activity in the soil.

Corbel[16], on the other hand, has argued that cold temperate zones with high precipitation are the loci of most rapid limestone solution and land-form evolution. This is on the grounds of the higher saturation equilibria of carbon dioxide in water at these lower temperatures, of the high carbon dioxide content of the air in snow and so of meltwater. He has further[17] produced empirical evidence in support of his thesis, notably in a detailed comparison of the actual amounts of calcium carbonate carried out in solution from a catchment in Florida and one in Alaska, a comparison greatly in favour of the latter.

He has also maintained that tropical limestone streams are frequently saturated at low bicarbonate contents. Determinations in New Guinea show lower contents from the lower Kikori and its tributaries than in rivers at higher levels there; but the lowland streams remain very aggressive and discharges are enormous because of the amount and intensity of the rainfall.

Moreover, the rate of removal of limestone in solution from a limestone area must not be equated with the rate of limestone solution or of karst landform development[18,19,8]. In New Guinea re-deposition within the limestone areas of tufa along the rivers has already been noted[20] and our own observations suggest it is a widespread phenomenon.

Part of Corbel's thesis has been that cold temperate karsts have in most cases had little time for their development because of the interruptions of Pleistocene glaciations, whereas tropical 'Kegelkarsts' have had a much longer evolution. New Guinea can add many examples, however, to the tally of 'Kegelkarsts' which have been elaborated in a very short space of time. In the lower Kikori, Pliocene rocks are folded and the karst must have developed within the Pleistocene. There the major relief is tectonic; but in the Southern Highlands, though folding and uplift may be older (probably Pliocene), there is relief inversion and much of the highly developed karst lies in synclines.

Nevertheless, this controversy can as yet scarcely be said to be resolved. New Guinea suggests a complication to this question. The vigour of relief of the arête-and-doline karst at high levels is remarkable, surpassing in intensity even the 'biscuit-cutting' effects[21] of glacial cirque erosion, with which it has no genetic connexion however. The orogenic movements which raised these highlands are probably Pliocene. So this karst is a young one in terms of age, though not in geomorphological stage. The possibility needs to be considered therefore whether, at certain levels, high tropical mountains may not provide the optimal conditions for karst evolution, combining as they do lower temperatures, hence higher saturation equilibria for carbon dioxide, with a fairly high and an effective precipitation and with the prolific vegetation growth of mossy montane forest throughout the year.

One of us (J. N. J.) thanks Oil Search, Ltd. for providing transport and other facilities for a visit to their area of operations.

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CELL DIFFERENTIATION AND CARCINOGENESIS

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THE mechanisms which determine the biological functions of the cell and govern the regulation of its activities are gradually being elucidated by various investigators. It is the purpose of this article to present some suggestions which might be related to cellular differentiation, dedifferentiation and carcinogenesis.

On the basis of the role of ribonucleic acids in protein synthesis and from the work on enzyme induction and antimetabolites in micro-organisms, it has been postulated that in the regulation of cellular activities, the activity of any enzyme in a metabolic sequence may be controlled through the stimulation or inhibition of the enzyme-forming systems (that is, the RNA 'templates') by substrate, metabolic intermediate or end-product[1]. Therefore, it is conceivable that in the multicellular organism, the activities of any one cell are influenced by the products of metabolism of the surrounding cells, and by alterations in the supply of nutrients, and in the rate of diffusion of its own end-products due to its environment of surrounding cells. Indeed, the literature on cell and tissue culture is well documented with the influences of environmental conditions on both the morphology and metabolic activities of mammalian cells in culture[2].

Consider a simple model system where a single cell forms a linear chain of cells: at the two-cell stage, the activities of either daughter cell will be altered by being in contact with the other. At the four-cell stage it might be expected that the inner two cells will have metabolic functions differing from the two outer cells, since in the former case each cell is bounded on two sides by other cells, whereas in the latter case, the outside cells are each adjacent to only one cell. Further, each of the inner cells will be bounded by two cells the activities of which are not alike, thereby allowing the possibility for even greater alterations in function as compared with the original cell. Whether such processes can lead to permanent changes, for example, differentiation or the formation of a blastula from a fertilized egg, is not known, although it is conceivable that when cytoplasmic activities have been altered for a long enough period of time this might occur. There are several examples in the literature regarding permanent changes which occur in cells in culture, for example, the production of cancer cells from long-term culture of cells derived from normal tissue, and the production of cells with altered numbers and morphological appearance of the chromosomes.

Such permanent alterations may be the result of changes in the pattern of activities of the ribonucleoproteins alone, or may reflect nuclear changes as indicated by the latter example. The possibility must not be ignored that the regulation of enzymatic activities of the cell is not confined to a closed cycle concerning only the ribonucleic acid enzyme-forming system alone, but might also involve an initial effect of cytoplasm on nuclear function, and then nuclear function on the enzyme-forming system. That is, in cellular regulation, the spectrum of metabolic activities of the deoxyribonucleoprotein molecules may fluctuate, perhaps by slight alterations in the spacial orientation of the molecules or by partial breakdown and re-synthesis. Long-term changes in environment might, therefore, lead to subtle and permanent alterations in the DNA.

It is of interest that recent work has suggested the possibility that different parts of the gene may have similar metabolic functions[3]. In this respect, it is easier to conceive of variations in combinations of activities as a result of the twisting or partial breakdown of the DNA molecules, when several polynucleotide groups have similar responsibilities.

Little is known of what is involved in the ultimate decision as to when and how a partly differentiated cell will differentiate. In the replacement of a dying differentiated cell, it might be considered that the final process in differentiation may depend on factors released from the dying cell. It is also possible that, in similar circumstances, the factors released may enter the recipient cell and upset the balance of its metabolic activities in such a manner as to give rise to a cancer cell. While there are no definitive data to support the above suggestions, the results of experiments being carried out in this laboratory are in accord with the above ideas. Several workers have shown that ribonucleic acid preparations are capable of influencing mitosis, morphology, and the re-aggregation of mammalian cells in culture. In this laboratory, saline extracts of fast-growing tissue (for example, chick embryo) have been shown to stimulate mitosis in polynucleated, amitotic cells of a hamster ascites population. Preparations of DNA and RNA obtained from chick embryonic tissue are also active in this respect. However, the influence of DNA is not transitory as compared with the action of the RNA and is suggestive of the transformations observed with micro-organisms. Similar activities of salt extracts are obtained from regenerating, but not from normal, rat liver, indicating that the amounts of active salt extractables vary with the metabolic state of the tissue[4].

In order to study further the possibility that mammalian cells are susceptible to alteration (for example, transformation) by materials from other cells, investigations were continued, using a HeLa cell strain sensitive to the growth inhibitory influences of small amounts of certain steroids, and steroid resistant sub-lines selected from it[5]. The sensitive strain was found to exhibit a stem-line containing 68 chromosomes, whereas the steroid resistant sub-lines showed a 74-chromosome stem-line[6]. Short-term culture of the sensitive strain in the presence of cell-free salt extracts obtained from the resistant lines resulted, in some cases, in significant increases in resistancy to the inhibitory action of the steroid. Further, after such treatment the cultures exhibited not only the 68-chromosome stem-line, but also a 74-stem-line characteristic of the steroid resistant sub-lines (Fig. 1). Such alterations are not

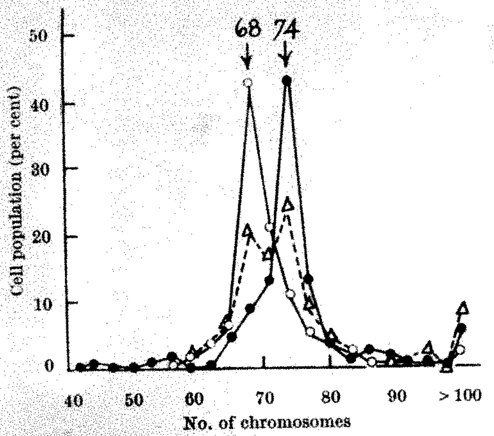


Fig. 1. Chromosome stem-lines of HeLa strain and sub-lines (a minimum of 200 metaphase cells counted in each case)
○, Steroid-sensitive strain; ●, steroid-resistant sub-line; Δ, altered HeLa line

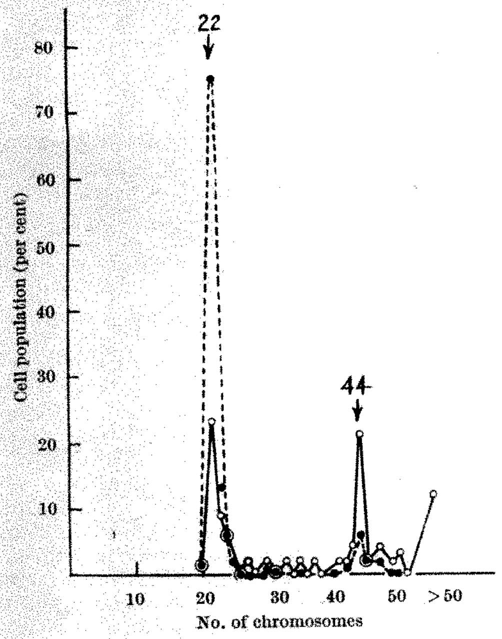


Fig. 2. Chromosomes of stable, classic diploid cells (*FAF* strain) and altered line (a minimum of 200 metaphase cells counted in each case)
●, Untreated *FAF* control; ○, altered *FAF* line

temporary; the bimodal character of the stem-line appears to be permanent, while increased resistancy to steroid lasted two to three months in actively growing cultures. It should be pointed out that neither of these characteristics is obtained when cell-free extracts of the steroid-sensitive strain itself is used. To obtain some information as to whether it is indeed possible to alter the metabolic and chromosomal characteristics of cells by materials obtained from identical cells, a normal cell strain with a more stable diploid character, and containing smaller numbers of chromosomes than the HeLa lines, was investigated (Chinese hamster cells, strain *FAF*, 22-chromosome classic diploid[7]). Fig. 2 shows the chromosome numbers in the normal *FAF* strain, and the *FAF* strain cultured for 4 weeks in rapidly growing culture after a short treatment with sterile-filtered salt extracts of its own cells; tetraploidy and polyploidy are greatly increased. Not only do the numbers of chromosomes change, but there are also significant changes in the idiograms (that is, the ratios of the various morphological species of chromosomes) within 80 per cent of the cells containing the same total number of chromosomes. Alterations in chromosome morphology also occur. That such changes are reflected by alterations in the metabolic characteristics of the cells has been indicated by variations in the growth-rates of the treated cultures as compared with untreated controls.

It has been well established that in some instances extracts of tumour cells passed through a bacterial filter have the capacity to produce tumours in normal tissue. The possibility that filtrates of normal cells themselves might be capable of producing alterations in the metabolic interrelationships of a similar normal cell, in such a manner as to produce a tumour cell, was tested. Normal untreated *FAF* cells and the normal *FAF* cells treated with sterile-filtered salt extracts of the *FAF* cells themselves (Fig. 2) were injected into the cheek-pouch of Chinese hamsters. Even though the opportunity for tumour-takes was negligible owing to the fact that the *FAF* strain is an F_1 hybrid derivative, and injections were made into adult, untreated hosts which are lacking in histocompatibility, the results in Fig. 3, nevertheless, demonstrate that the treated cells did not evoke a normal, immediate, antigenic response. This should not be confused with the delayed response observed with the Syrian hamster, and indeed is characteristic of the longer lag periods exhibited when cancer cells are injected. Similar studies are being undertaken with highly inbred cells and hosts.

In order to test this hypothesis further, and under *in vivo* conditions, the following experiment was carried out. Of 6 male adult rats, 5 were partially hepatectomized and the remaining lobes immediately injected with either saline (0·9 per cent) or sterile-filtered cell-free saline extracts of the removed lobe. Two rats received saline and three received cell

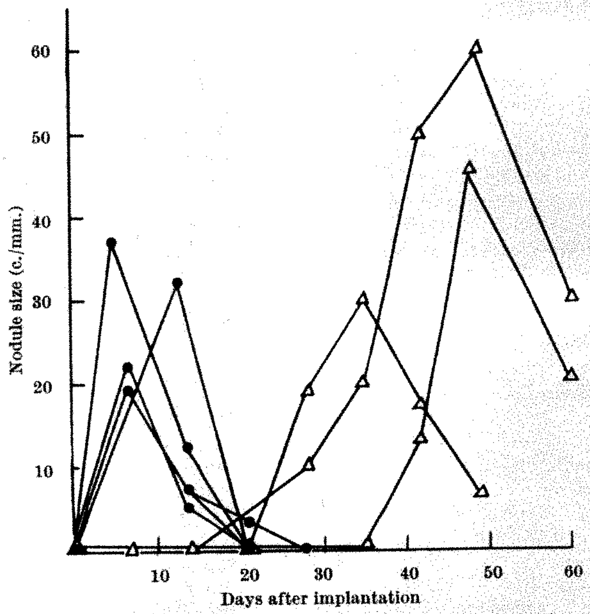


Fig. 3. Nodule formation in hamster cheek pouch after injection of *FAF* and altered *FAF* cells. (In some experiments 3×10^5 control and altered cells were injected; in other experiments 1×10^4 cells were used)
●, Normal *FAF* cells; Δ, altered *FAF* cells

extracts. A total of 1·0 ml. was used per animal, the injections being given in 0·1-ml. doses at ten different sites, and approximately two months later the rats were killed. Gross examination did not reveal any obvious signs of hepatoma, although it was noted that the livers from the liver-extract injected animals had greyish areas which were not seen in the saline-treated rats. Several samples of the liver tissue were taken from each animal and fixed in both absolute alcohol and formalin for histological study.

All histological and photographic procedures were identical for normal, saline control, and experimental animals. The photographs are × 970. Fig. 4 shows a section of a normal, untreated rat liver. The regenerated rat liver which received saline exhibits, in some areas (Fig. 5), degenerative and necrobiotic changes. In comparison, each of the livers injected with the liver extracts show remarkable alterations not associated with necrosis, as indicated in Figs. 6, 7, 8 and 9. Between the normal lobular arrangement of the cords of cells were present nodular-like areas exhibiting irregularities in structure. The epithelial cells of such areas have well-defined cytoplasmic membranes, are larger in size than the normal cells or the saline-treated controls, and show a granular cytoplasm containing a relatively large vesicular nucleus containing 1 to 3 large basophilic nucleoli. On each of these points, such cells bear a striking resemblance to hepatic tumour cells and are often

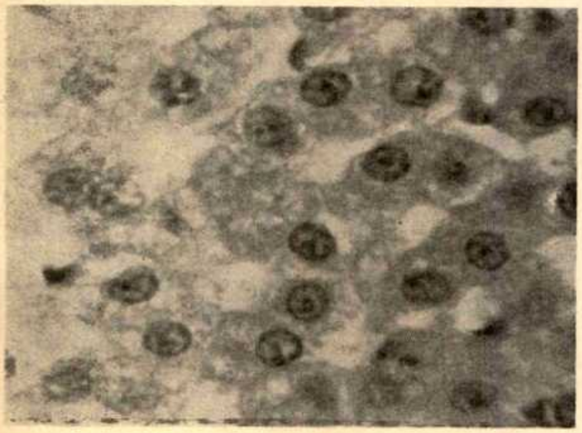


Fig. 4. Normal rat liver (× 970)

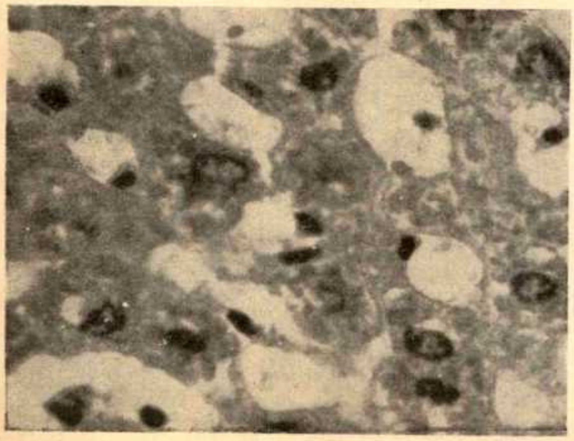
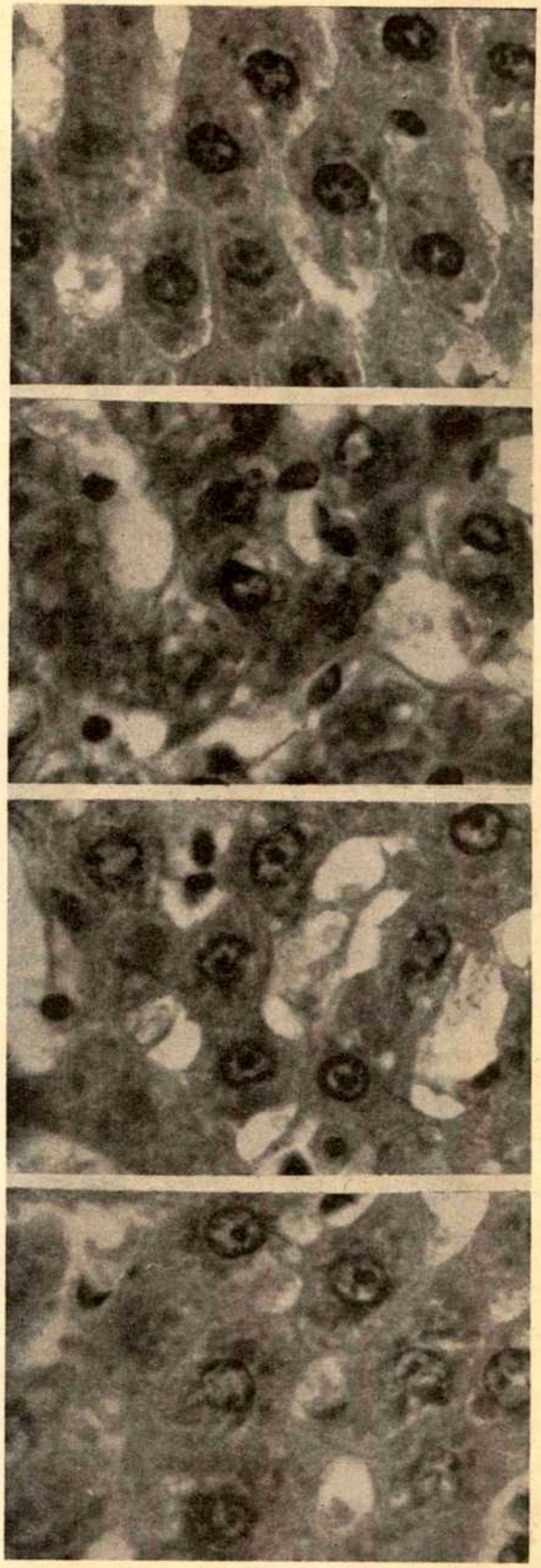


Fig. 5. Regenerated rat liver injected with sterile saline (× 970)



Figs. 6–9. Top to bottom, regenerated rat liver injected with sterile cell-free saline extracts of rat liver (× 970)

seen in acinar structures, similar to the glandular structures which occur not only in well-established tumours but also in hyperplastic atypical parenchymal nodules which appear to represent early neoplasm. In this respect, histological examination also indicates that in the livers treated with the test material, but not the saline, there are greater numbers of cells containing more than normal amounts of glycogen.

While no definite conclusions can be drawn from the foregoing results, they nevertheless are of interest with respect to possible mechanisms involved in differentiation and carcinogenesis.

I thank Prof. Y. S. Kang (Department of Zoology, Seoul National University, South Korea) for his kind co-operation with the chromosome studies, Dr. Likar (Mason Research Institute, Worcester, Mass.) for the histology, and to Dr. G. Yerganian (Children's Cancer Research Foundation, Boston, Mass.) for suggestions and for supplying both the cell strain and Chinese hamsters used in these investigations. This work was financially supported by the U.S. Public Health Services, the National Science Foundation, and the American Cancer Society (Mass. Division).

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FUNCTION OF POLYURIDYLIC ACID AND RIBONUCLEIC ACID IN PROTEIN BIOSYNTHESIS BY RIBOSOMES FROM MAMMALIAN RETICULOCYTES

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THE importance of ribosomes to protein biosynthesis in both bacterial and animal cells is now well established[1]. In cell-free systems, consisting essentially of ribosomes, amino-acid activating enzymes, transfer ribonucleic acid (RNA) and an energy source, the incorporation of labelled amino-acids into polypeptide linkage has been studied in detail and experiments on the biosynthesis of hæmoglobin by ribosomes from rabbit reticulocytes[2] and of albumin by liver microsomes[3] have demonstrated the formation of specific proteins in such cell-free preparations.

The function of ribonucleic acid in protein biosynthesis has recently been clarified by experiments with similar cell-free systems from bacteria, particularly *Escherichia coli*. In addition to the relatively low-molecular-weight 'soluble' RNA which is involved in the transfer of activated amino-acids to ribosomes[4], a ribonucleic acid of higher molecular weight has been implicated in the polymerization of the amino-acids. This so-called messenger RNA is distinct from the bulk of the ribosomal RNA in nucleotide composition[5], rate of turnover[6] and metabolic activity[7].

The most striking demonstration of the role of a messenger in protein synthesis is, however, the discovery by Nirenberg and Matthaei that the addition of polyuridylic acid to *E. coli* ribosomes specifically brings about the biosynthesis of polyphenylalanine[7] and the extension of this work to synthetic co-polymers of uridylic, cytidylic, adenylic and guanylic acids which has resulted in the elucidation of a code for most of the 20 amino-acids which commonly occur in proteins[8-10]. Viral RNA also markedly stimulates incorporation of amino-acid and thus appears to function as messenger RNA[7,11].

No comparable evidence is, however, available concerning the function of ribonucleic acid in animal cells. It is also not known whether the genetic code as determined by the experiments with synthetic polynucleotides and *E. coli* ribosomes is generally applicable to other organisms.

In the work reported here, the function of ribosomes and their RNA in the biosynthesis of a specific protein, hæmoglobin, by cell-free preparations from reticulocytes has been investigated. It has been reported that, in cell-free preparations of reticulocytes from different animal species, the specificity of the hæmoglobin synthesized is determined not only by the ribosomes but also by the cell sap[12] or by the '*p*H 5 enzyme' fraction[13] after removal of the ribosomes by ultracentrifugation. We have not been able to confirm these observations. Using mixed systems from lysed rabbit and guinea pig reticulocytes, we have found that the specificity of synthesis of hæmoglobin is associated only with the ribosome fraction (Table 1), in agreement with the results obtained by Bishop, Favelukes, Schweet and Russell[14] with mixed cell-free preparations from rabbit and mouse reticulocytes. Our experiments

Table 1. SPECIFICITY OF HÆMOGLOBIN SYNTHESIS BY CELL-FREE PREPARATIONS FROM RABBIT AND GUINEA PIG RETICULOCYTES

	Origin of cellular components		Specific radioactivity (mμc./gm.)		
Exp. No.	*p*H 5 enzymes	Cell sap	Ribosomes	Rabbit hæmoglobin	Guinea pig hæmoglobin
1	Rabbit	None	930	601	47
			1,300	635	85
	Guinea pig	None	1,540	368	79
			1,410	516	124
2	Rabbit	Rabbit	1,150	1,005	114
	Guinea pig	Guinea pig	1,220	370	126
	Rabbit	Rabbit	1,000	1,040	94
	Rabbit	Guinea pig	1,060	900	92

Each tube contained (in 2·15 ml.) 400 μmoles sucrose, 40 μmoles potassium chloride, 13 μmoles magnesium chloride, 50 μmoles *tris*, *p*H 7·6, 5 μmoles glutathione, 20 μmoles phosphoenolpyruvate, 1 μmole adenosine triphosphate, 0·25 μmoles GTP, 150 μgm. pyruvate kinase, 0·3 ml. of an amino-acid mixture (ref. 2) containing L-[^{14}C] valine (1 μc., 15 μgm.) and L-[^{14}C] leucine (1 μc., 24 μgm.), 7·8 mgm. of *p*H 5 enzymes and 8·8 mgm. of rabbit reticulocyte ribosomes (ref. 2). Rabbit or guinea pig reticulocyte cell sap after removal of *p*H 5 enzymes (28 mgm. and 26 mgm., respectively) was added where shown. After incubation at 37° for 1 hr., the corresponding amounts of either rabbit or guinea pig cell sap, or both, were added as carrier, the ribosomes were removed by centrifuging at 100,000*g* for 1 hr. and the hæmoglobins were separated by Pevikon block electrophoresis (cf. ref. 13), precipitated by trichloroacetic acid, washed and counted.

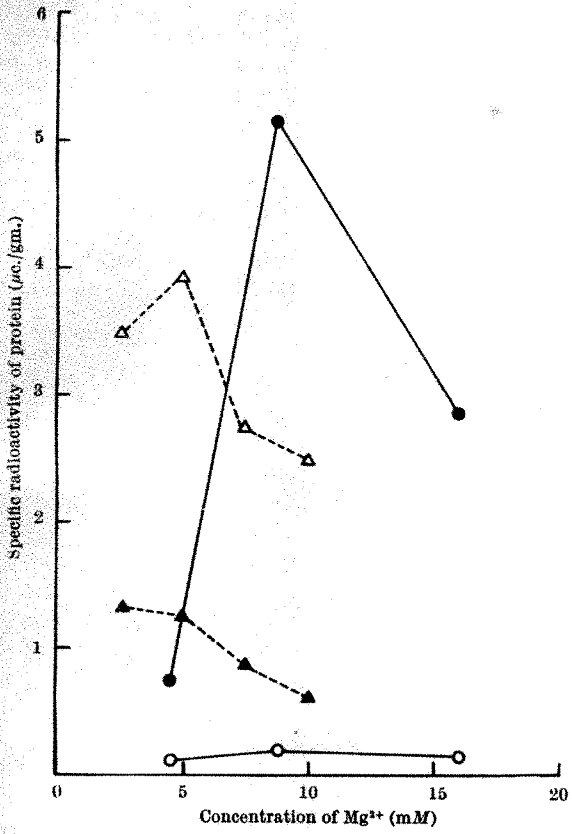

Fig. 1. Effect of magnesium on the incorporation of amino-acids by cell-free systems from rabbit reticulocytes. The cell-free preparation was similar to that of Schweet *et al.* (ref. 2). Pre-incubation and subsequent dialysis of the ribosomes (Exp. No. 2) were carried out essentially as described by Nirenberg and Matthaei (ref. 7). Exp. 1. Incorporation of L-[^{14}C]valine by fresh ribosomes. △, ribosome protein; ▲, soluble protein (hæmoglobin). Exp. 2. Incorporation of L-[^{14}C]phenylalanine by pre-incubated ribosomes into total protein. ○, in the absence of polyuridylic acid; ●, in the presence of polyuridylic acid (24 μgm./mgm. of ribosome)

suggest that the 'messenger' is already attached to the ribosomes and may be removed or inactivated either by pre-incubation or by mild treatment with ribonuclease. These treatments destroy the ability of reticulocyte ribosomes to incorporate amino-acids; but neither impairs the structural integrity of the ribosomes[15] nor leads to degradation of the ribosomal RNA, as judged by its sedimentation in the ultracentrifuge[16]. During treatment with the enzyme a relatively small proportion of ultra-violet-absorbing material is released, indicating the preferential destruction of a minor, but functionally important, component of the RNA[15]. Though this fraction may correspond to 'messenger' RNA in the bacterial system, it has not yet been possible to restore amino-acid incorporation by addition of high molecular weight RNA, which was extracted from reticulocyte ribosomes by either the phenol[17] or the guanidine[18] method. We have found, however, that rabbit reticulocyte ribosomes, like those from *E. coli*[7,9], can be induced to synthesize polyphenylalanine by the addition of polyuridylic acid (Table 2). The utilization of a mixture of 16 amino-acids labelled with carbon-14 and unlabelled phenylalanine was not affected by polyuridylic acid, demonstrating the specificity of polyuridylic acid for incorporation of phenylalanine. This result also shows that polyuridylic acid does not replace or inactivate existing 'messenger' RNA on the ribosomes since this would be expected to result in an inhibition in the incorporation of amino-acids other than phenylalanine.

Table 2. SPECIFICITY OF THE STIMULATION OF PHENYLALANINE INCORPORATION BY POLYURIDYLIC ACID

Exp. No.	Radioactive precursor	Additions	Specific radioactivity of protein (mμc./gm.)
1	Phenylalanine*	None	246
	Phenylalanine*	Polyuridylic acid (40 μgm.)	6,260
	Phenylalanine*	Polyuridylic acid (40 μgm.)	55†
2	Amino-acid mixture‡	None	1,422
	Amino-acid mixture‡	Polyuridylic acid (40 μgm.)	1,515

Ribosomes from rabbit reticulocytes were incubated (ref. 7), dialysed (ref. 7) and re-isolated by centrifuging. After suspension, they were incubated with fresh pH 5 enzymes, phosphoenolpyruvate, ATP and GTP, and either labelled phenylalanine and a mixture of unlabelled amino-acids (ref. 2) (Exp. No. 1) or unlabelled phenylalanine and a mixture of 16 labelled amino-acids (Exp. No. 2).

* 0·5 μc. of L-[^{14}C]phenylalanine.

† Phosphoenolpyruvate, ATP and GTP omitted.

‡ 0·5 μc. of each of the following ^{14}C-labelled amino-acids: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-proline, L-serine, L-threonine, DL-tryptophan, L-tyrosine, and L-valine.

The effect of polyuridylic acid is highly dependent on magnesium, about 0·01 *M* being the optimum concentration (Fig. 1). It is of interest that this concentration is considerably higher than that required for the maximum incorporation of valine into ribosomes and soluble protein in the absence of polynucleotide.

The effect of polyuridylic acid on polyphenylalanine synthesis was observed with freshly isolated ribosomes and with ribosomes which had been inactivated by either pre-incubation (cf. ref. 7) or mild treatment with ribonuclease[15]. Under comparable conditions, the stimulation of phenylalanine incorporation by polyuridylic acid was proportional to the incorporation of this amino-acid in the absence of polynucleotide (Fig. 2). It is concluded, therefore, that only active ribosomes can be induced to synthesize polyphenylalanine. The ratio of about 30 : 1 in incorporation of phenylalanine is consistent with the polyuridylic acid interacting to the same total length as a hæmoglobin messenger RNA.

In conclusion, our results indicate that the specificity of protein synthesis by the reticulocyte is determined by a fraction of RNA in the ribosomes. This

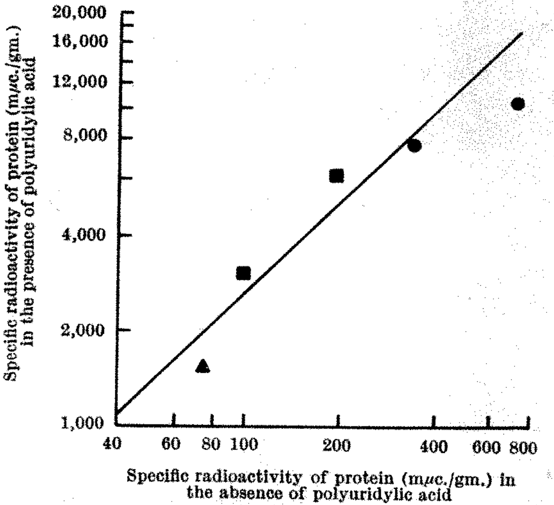

Fig. 2. Stimulation of phenylalanine incorporation by rabbit reticulocyte ribosomes in the presence of polyuridylic acid. ●, fresh ribosomes; ▲, ribosomes treated with ribonuclease (ref. 15); ■, pre-incubated ribosomes

RNA is readily destroyed by traces of ribonuclease and is not replaceable by added RNA, although we may not have achieved optimum conditions for the interaction of RNA and ribosomes. Polyuridylic acid is active as a specific 'messenger' for the biosynthesis of polyphenylalanine, but functions only in the presence of ribosomes which are active in protein synthesis. The polynucleotide and 'messenger' RNA apparently occupy different sites, both of which are equally susceptible to ribonuclease and pre-incubation, or alternatively occupy the same site consecutively.

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TITRATION OF SOME COMMON COLD VIRUSES (RHINOVIRUSES) AND THEIR ANTISERA BY A PLAQUE METHOD

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TYRRELL and Parsons[1] have described the conditions under which they were able to propagate in tissue culture cytopathic viruses from some persons with common colds. Certain agents multiplied in monkey kidney cell cultures and were designated *M* strains; others, known as *H* strains, failed to grow in monkey kidney cells but multiplied and produced a cytopathic effect in cultures of human embryonic kidney cells[2]. The general term 'rhinovirus' was suggested by Andrewes *et al.*[3] and has been used by workers at the Common Cold Research Unit, Salisbury, to include *M* and *H* strains which are clearly distinct from certain other viruses known to be capable of causing common colds[4]. The assay method devised by Parsons and Tyrrell[5] depended on the enumeration of small foci of degenerating cells seen when infected cultures were examined under the microscope. This approach has enabled the Salisbury group and others to examine many different rhinoviruses, but it has two major disadvantages. The first is that it is somewhat laborious involving the examination of the entire cell sheet of a number of replicate tubes for every specimen being tested. The second is that the end-point changes with time as virus released from cells infected initially enters further cells and gives rise to secondary foci. This difficulty is avoided by the use of an agar overlay, as first applied to animal viruses by Dulbecco[6], but Parsons and Tyrrell[5] were unsuccessful in their attempts to produce plaques under agar. This report describes the successful application of an agar overlay technique to rhinoviruses resulting in the development of plaques which can be counted by the naked eye and the use of this method in virus neutralization tests.

Preliminary experiments were carried out with *HGP* virus using monkey kidney cell monolayers. Some plaques were obtained, particularly in cultures overlaid with thin layers of agar, indicating that in these cells a high degree of oxygenation was necessary, as has been found with a number of other viruses[7]. Different batches of monkey kidney cells varied considerably in their sensitivity, using macroplaques as an indication of virus growth, and replicate cultures with a single batch of monkey kidney cells gave inconsistent results, there being no constant relationship between virus dose and plaque count. Attention was therefore directed to the use of cultures derived from human embryonic lung cells which were known to be capable of supporting the multiplication of certain common cold viruses[8]. Earlier difficulties over the availability of human embryonic material were overcome by the use of cells frozen in the presence of dimethyl sulphoxide[9]. The experiments described here were carried out with cultures derived from two ampoules each containing approximately 4×10^6 cells initially obtained from different embryos; both strains appeared to be equally sensitive to *HGP* virus, although most tests were carried out with one series of cultures.

The medium contained 0·25 per cent lactalbumin hydrolysate, 0·25 per cent peptone (Difco proteose peptone) and 10 per cent pre-colostral calf serum (obtained from new-born calves before suckling) in Gey's buffered salt solution containing 0·11 per cent sodium bicarbonate and 0·0025 *M tris* (hydroxymethyl)aminomethane. After thawing from the frozen state cells were diluted 1 : 10 in growth medium and distributed into 1-oz. bottles in 3-ml. volumes. These were incubated at 37° C. until a confluent cell sheet had formed; this took about ten days during which time the medium was changed twice. The growth medium was discarded, the cells were rinsed quickly with 0·25 per cent Difco trypsin, which was discarded, and the bottles were incubated without medium for 5–10 min. until the cells loosened[10]. Double the original volume of medium was added, the cells were dispersed by gentle pipetting and were then divided into equal parts in two new bottles. As the cells multiplied, stock cultures were passaged every 3–5 days using 10 ml. of medium in 4-oz. bottles. The yield of one 4-oz. bottle (20 ml.) was later divided into two 4-oz. bottles, six 1-oz. bottles or five Petri dishes. Bottles or Petri dishes for plaque tests were used when densely packed monolayers had formed; this took about five days, but commonly the medium was changed at this time and the cells were incubated for a further two or three days. Prior to infection, cells were washed once with diluent, which consisted of growth medium without calf serum, and buffered with *tris* only. Virus dilutions in this fluid were allowed to absorb for 2 hr. at 32° C. after which the cultures were rinsed once before overlaying. The overlay medium contained 0·25 per cent lactalbumin hydrolysate, 0·25 per cent peptone, 5 per cent pre-colostral calf serum in Gey's solution containing 0·11 per cent sodium bicarbonate without *tris* in bottles, or 0·0025 *M tris* without bicarbonate in Petri dishes. Noble agar was used at a final concentration of 1·5 per cent in bottles and 0·7 per cent in Petri dishes. The volume of the overlay was normally 2·5 ml. in 1-oz. bottles and 4 ml. in Petri dishes. Neutral red was added after 48 hr. incubation at 32° C. as 1·0 ml. of a 1 : 10 dilution of stock neutral red (1 : 1,000 in distilled water) in the peptone lactalbumin hydrolysate diluent.

Preliminary experiments were carried out using stock *HGP* virus in the form of monkey kidney cell culture fluid received from Dr. D. A. J. Tyrrell. Human embryo lung cells at the third passage-level were infected with serial dilutions of virus, one bottle being incubated with liquid medium while others were overlaid with agar. On the third day the liquid from one infected bottle was removed and frozen at − 70° C. for future experiments; no clear cytopathic effect was evident at this stage. By the fifth day small plaques were developing in bottles infected with undiluted and 10^{-1} dilutions of monkey kidney fluid inoculum, and by the twelfth day clearly defined plaques up to 3 mm. in diameter were present at 10^{-2} dilution of virus (Table 1).

Table 1. PLAQUES WHICH DEVELOPED IN HUMAN EMBRYO LUNG CULTURES INFECTED WITH MONKEY KIDNEY PASSAGED *HGP* VIRUS

Virus dilution	Day 5	7	12	15	21
10^{0}	??	?	confluent	confluent	lysed
10^{-1}	0	J	a50	> 50	> 50
10^{-2}	0	0	9	14	18
10^{-3}	0	0	0	2	2
Control	0	0	0	0	0

Later experiments, using the supernatant fluid from this experiment, showed that plaques were well developed at 48 hr. although they increase in size, and to some extent also in number, on further incubation (Fig. 1). When the titre of this material had been established, a neutralization test was set up as follows: to serial four-fold dilutions of a rabbit immune serum (kindly supplied by Dr. D. Taylor-Robinson) and to 1 ml. of a 1 : 50 dilution of a normal rabbit serum in 1 ml. volumes was added 1 ml. of a dilution of *HGP* estimated to contain about 200 plaque-forming units. Control tubes contained diluent mixed with the same virus dose, and with the virus diluted 1 : 4, 1 : 16 and 1 : 64. The mixtures were kept at bench temperature for 1 hr. and were then added in 1 ml. volumes to bottle cultures of human embryo lung cells. After adsorption for 2 hr., the cultures were washed twice, overlaid with agar and incubated at 32° C. Neutral red was added on the second day and plaques were counted on different days with the results given in Table 2. The virus control contained more plaques than could be counted, but on the basis of the 1 : 4 and 1 : 16 dilutions of virus, the challenge contained between 100 and 200 plaque-forming units of virus. From these results it is clear that the 50 per cent plaque neutralization titre is 1 : 3,200. The same serum gave almost complete neutralization at a dilution of 1 : 500 when examined by a generally similar technique but using microplaques as evidence of virus activity[11]. A final assessment of the sensitivities of the two methods must await more detailed comparative studies; these preliminary results indicate that the macroplaque technique is certainly not less sensitive than the microplaque method.

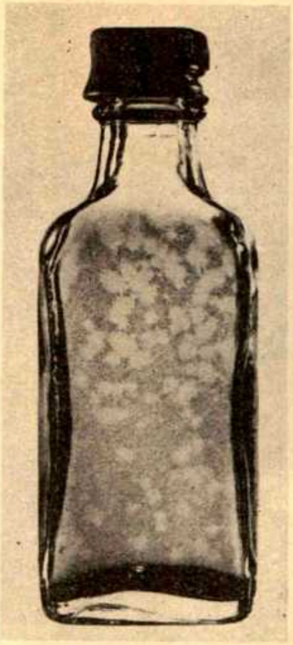

Fig. 1. Human embryonic lung fibroblasts infected with a 10^{-2} dilution of *HGP* virus. Photograph taken after six days incubation at 32° C.

Experiments were next carried out using the plaque inhibition test in which fish spine beads dipped in sera are applied to the surface of the agar covering virus-infected cells[12]. A zone of plaque inhibition 18 mm. in diameter was produced around a bead containing a 1 : 2 dilution of *HGP* immune serum. Later tests, using serial dilutions of *HGP* virus and a 1 : 5 dilution of *HGP* serum, are illustrated in Fig. 2. At a 10^{-1} dilution of virus, representing about 3,200 plaque-forming units of virus, the inhibition zone measured 16 mm. in diameter. It was slightly larger (18 mm.) with the next dilution of virus, estimated to contain 320 plaque-forming units, and the 10^{-3}

Table 2. PLAQUES DEVELOPING IN BOTTLE CULTURES OF HUMAN EMBRYONIC CELLS AFTER INCUBATION WITH MIXTURES OF *HGP* VIRUS AND HOMOLOGOUS RABBIT IMMUNE SERUM

Inoculum	Day 3	4	5	12
Virus and serum 1 : 50	0	0	0	2
Virus and serum 1 : 200	0	0	1	1
Virus and serum 1 : 800	0	2	6	6
Virus and serum 1 : 3,200	20	20	20+	20+
Virus and normal serum	++	+++	+++	+++
Virus 1/4	7	11	12	28
Virus 1/16	2	5	9	14
Virus 1/64	0	0	1	3
Cell control	0	0	0	0

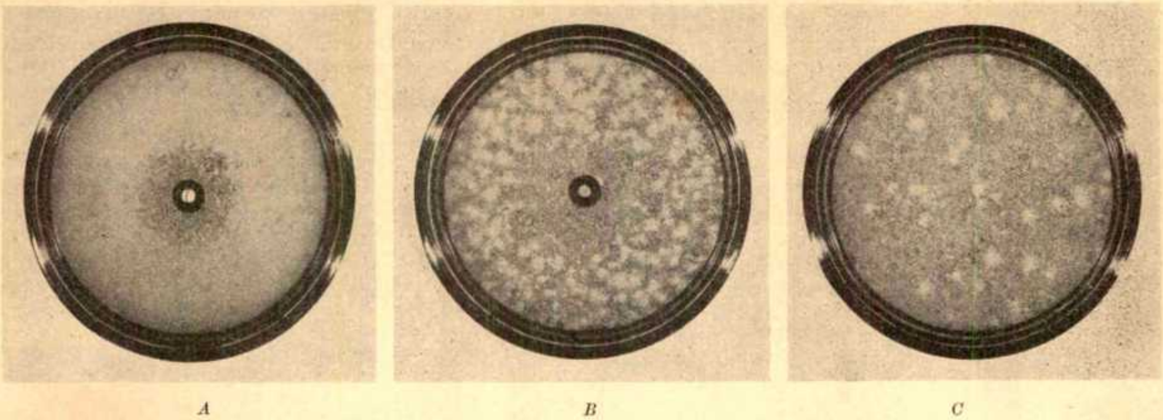

Fig. 2. The plaque inhibition test applied to *HGP* virus and homologous rabbit immune serum. *A*, virus 10^{-1} plus immune serum in central bead; *B*, virus 10^{-2} plus immune serum in central bead; *C*, virus 10^{-3}. Photographs taken after 6 days incubation at 32° C.

dilution gave 32 plaques. An immune serum prepared against another rhinovirus *B*632 gave no inhibition at 1 : 5 dilution of serum, although this material was slightly cytotoxic when used undiluted.

Less-extensive tests with two other rhinoviruses have also given positive results. An ampoule of a second *M* strain, *B*632, said by Dr. Tyrrell to contain about 1,000 microplaque-forming units per ml., produced 10 plaques in human embryo lung cultures infected with a 10^{-3} dilution of stock monkey kidney virus. These plaques were not clearly visible until the twelfth day and remained small and feathery in appearance, quite unlike the large clear plaques which developed with *HGP* virus but resembling those described by Ellem and Colter[13] produced by Mengo virus in human embryonic lung cultures. One *H* strain 'Norman', also produced small feathery plaques after an incubation period of fifteen days. Whether these strains will produce larger and clearer plaques more rapidly on further passage in human embryo lung cells is still to be determined.

The present article describes a technique for titrating the infectivity of some rhinoviruses based on the counting of macroscopically visible plaques of degenerated cells in a monolayer of human embryo lung fibroblasts covered by an agar overlay. The number of plaques bears a direct linear relationship to the dose of virus applied. Two *M* strains of rhinovirus gave plaque counts of the same order as those obtained by a microplaque technique in monkey kidney cells incubated under optimal conditions. The development of discrete plaques enables virus stocks to be purified by passage from single plaques. It also enables experiments involving infectivity titrations to be carried out with greater precision than has been possible so far. Virus plaques were inhibited by specific immune sera prepared against two different viruses before they had been passaged in human embryonic lung cells, thus excluding the possibility that a contaminating virus was responsible for the development of the plaques observed. Although the number of cells required to prepare one bottle for plaque titrations would prepare three tubes for microplaque studies, it is probable that the amount of information to be derived from the use of the former is greater.

A number of points of technique appeared to be important. It was essential to use densely packed monolayers, otherwise small areas of glass not covered by cells, or having only a very thin sheet of cells, might be mistaken for plaques. The medium described here gave better results than were obtained with Parker's solution 199 containing 10 per cent calf serum. Plaques developed when cells were overlaid with a peptone lactalbumin hydrolysate mixture without calf serum, although they were smaller than in comparable cells overlaid with the complete medium containing 5 per cent pre-colostral calf serum. Serum obtained from older calves inhibited plaque formation when incorporated in the overlay. It appeared that one passage of *HGP* virus in human embryo lung cells modified the virus so that it grew more readily in these cells than did monkey kidney stock material. This adapted virus gave approximately the same plaque count in two different human embryonic lung strains which had been preserved by freezing, the passage level in these tests varying from third to twenty-first. A third source of human embryonic lung fibroblasts was examined in secondary cultures prepared without freezing; plaques again appeared to a dilution of 10^{-3} of the stock virus, although they were less well defined than in cells which had been passaged regularly, probably because the cell sheet was more irregular.

Human embryo lung cells overlaid with agar appear to survive in bottles for two to three weeks without difficulty. In preparations containing two or three plaques only, these have increased in size to 8–12 mm. after incubation for 21 days at 32° C. In contradistinction to the first results obtained in monkey kidney cells, varying the volume of agar from 1 to 4 ml. did not decrease the plaque count; on the contrary, bottles containing the larger volume showed better survival of the cell sheets and at least as many plaques developed with 4 ml. as with 2 or 1 ml. of overlay.

The bead method of plaque inhibition has proved to be a convenient and economical method for carrying out serological typing of arthropod-borne viruses[14] and for detecting the presence of neutralizing antibodies in human sera following infection with arthropod-borne viruses[15]. The work reported here suggests that the method may have similar applications in the study of rhinoviruses.

I thank Drs. D. A. J. Tyrrell and D. Taylor-Robinson for the stocks of common cold viruses and antisera provided. The passage history of these strains was as follows: *HGP*, 6 passages in human embryo kidney followed by 12 passages in monkey kidney; *B*632, one passage in human embryo kidney followed by 6 passages in monkey kidney; 'Norman', 11 passages in human embryo kidney and one passage in human embryo lung cells.

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ISOMERIC BILE PIGMENTS AS PRODUCTS OF THE *IN VITRO* FISSION OF HÆMIN

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WHEN hæmoglobin is catabolized to biliverdin, the overall change is the removal of an iron atom from each of the four prosthetic hæm groups and the oxidative removal of one methene group from each of the resulting protoporphyrin IX molecules. IXα, IXβ, IXγ and IXδ bile pigments with different orders of their side chains will theoretically be formed according to whether the α, β, γ, or δ methene bridges are lost. The IXα and IXγ pigments may be differentiated by oxidation with permanganate, when they form respectively the αα′-pyrrole dicarboxylic acids (I) and (II) which can arise only from the two central pyrrole rings (Fig. 1). The other bile pigment isomers must give rise to mixtures of both these acids. The naturally occurring bile pigments have thus been shown to possess only the IXα structure[1]. No detailed examination of the bile pigments obtained in the *in vitro* oxidation of hæmin has yet been published. Lemberg[2] claimed that the dimethylester of the biliverdin formed by the coupled oxidation of protoporphyrin and ascorbic acid was wholly IXα in structure but esters of the 'green hæmins' formed by treatment of aqueous pyridine hæmochromogen with hydrazine sulphate had lower melting points than those of the natural bile pigments. Since much material remained uncrystallized in these experiments and no degradation investigations were made, Lemberg admitted that his product and mother liquors might contain other than IXα isomers. We now report evidence for the formation of such isomers under similar conditions.

In our experiments oxygen was passed through a solution of 0·5 gm. hæmin in 2 : 5 (v/v) pyridine/water at 50°–60°; 1·3 gm. hydrazine sulphate in 33 ml. 0·5 *N* sodium hydroxide was added, the temperature being maintained for about 5 min. The mixture was cooled and kept at 0° for 20 min. The aqueous pyridine hæmochromogen before oxidation absorbed maximally at 420 and 525 mμ. At the completion of oxidation a strong maximum at 650 mμ due to biliverdin had appeared. The pigment was then isolated by Engel's method[3]. Pigments were first extracted into ether, the extract showing maxima at 395 and 535 mμ with a particularly large maximum at 650 mμ. On continued washing with water these maxima changed to 380, 525 and 675 mμ, the aqueous washings acquiring maxima at 410 and 535 mμ due to the removal of residual hæmochromogen. Extraction of the ethereal solution with 5 per cent w/v hydrochloric acid caused almost complete transfer of the maximum at 675 mμ. The green pigment, now in the aqueous phase, was purified by partition five times between hydrochloric acid and ether, neutralizing the acid on each occasion with sodium acetate until the ratio of absorption 380 and 675 mμ in the ethereal solution became constant at 2·2. The ether solution then had the spectrum of pure biliverdin (Fig. 2) with no indication of the presence of porphyrin or of hæmin.

36 mgm. of porphyrin-free biliverdin from six such preparations was oxidized with alkaline permanganate and the resulting pyrrole dicarboxylic acids were analysed chromatographically by the method of Nicolaus[4]. Authentic acids (I) and (II) were used as chromatographic markers. Corresponding spots were obtained in all experiments and when these acids were included in the experimental chromatographic solutions they failed to produce duplet spots. The results are summarized in Table 1.

The formation of the acids (I) and (II) on permanganate oxidation of the biliverdin fraction

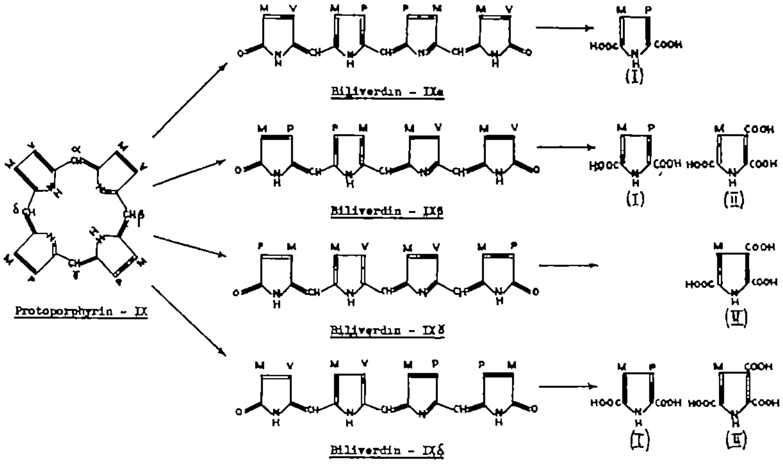

Fig. 1. The four isomeric verdins theoretically formed by fission of protoporphyrin IX at the α, β, γ and δ methene bridge and the pyrrolic acids expected from their oxidation. $M = CH_3$; $V = CH = CH_2$; $P = CH_2CH_2COOH$

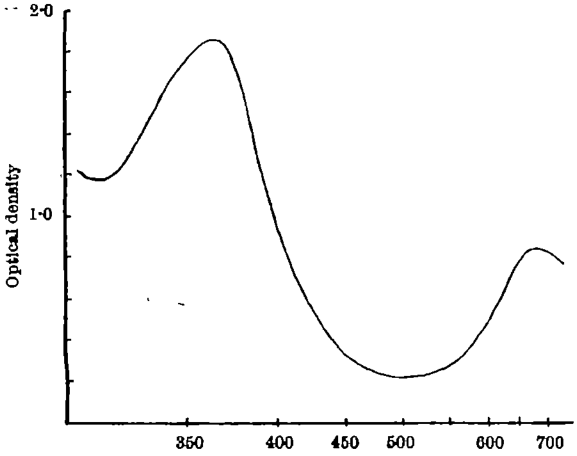

Fig. 2. Absorption in ether of biliverdin obtained by the *in vitro* fission of hæmin

Table 1. R_F VALUES FOR MAIN OXIDATION PRODUCTS FROM BILIVERDIN OBTAINED BY *in vitro* FISSION OF HÆMIN AND FOR AUTHENTIC 3-METHYL-4-CARBOXYETHYLPYRROLE-2 : 5-DICARBOXYLIC ACID (I) AND 3-METHYLPYRROLE-2 : 4 : 5-TRICARBOXYLIC ACID (II)

(Descending chromatography on Whatman No. 1 paper)

Substance	Solvent systems Ethanol/ammonia/water =20 : 1 : 4 (v/v/v)	 Butanol/acetic acid: water =4 : 1 : 5 (v/v/v)
Oxidation products from verdinoid fraction	0·04–0·06*	0·85–0·88*
	0·21–0·28	0·47–0·49
(I)	0·05–0·07	0·84–0·86
(II)	0·20–0·25	0·48–0·51

* Minor quantities of other, unidentified products were also present.

indicates that it contains not only IXα isomers but also other isomers. Thus the *in vitro* fission of the protoporphyrin of the hæmin under the conditions described must involve the loss of methene bridges other than that at the α position. During hæm catabolism the exclusive rupture of the protoporphyrin ring at the α methene bridge has been attributed to the electron density at that position being slightly higher than at other similar groups in the molecule[5]. The differences in electronegativities of the four bridge methene groups of protoporphyrin is small, however, and our present results suggest this is not the only factor responsible for the exclusive α fission which occurs under physiological conditions. The recently isolated enzyme catalysing the natural fission of protoporphyrin IX (ref. 6) may be more important and may specifically activate the α methene bridge.

We thank Dr. Aleksandra Kulczycka for a gift of samples of 3-methyl-4-carboxyethylpyrrole-2 : 5-dicarboxylic acid and 3-methylpyrrole-2 : 4 : 5-tricarboxylic acid.

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EFFECT OF ENZYMES ON BLOOD GROUP ANTIGENS

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IN 1946, Pickles[1] reported that treatment of Rho(D) erythrocytes with a cholera culture filtrate, presumably containing various enzymes, induced agglutination by an incomplete type of anti-D antibody in saline. Since then, it has been reported that treatment with a number of enzymes, such as trypsin[2], papain[3], ficin[4] and bromelin[5], markedly increased erythrocyte agglutinability. These observations apparently were not due to an enhanced non-specific agglutinability of erythrocytes against the corresponding antibody often observed following prolonged incubation, and it has been suggested that an alteration of the blood group substances had occurred.

Recently, Hackel *et al.*[6,7] reported that the hæmagglutination with anti-Rh and anti-Lutheran sera was specifically inhibited by a number of ribonucleic acid derivatives such as cytidine sulphate, adenylic acid, uridylic acid and cytidylic acid. These authors suggested that the Rh and Lutheran antigens contained nucleotide moieties as part of their antigenic structure. In another communication, Hackel *et al.*[8] then described the effects of ribonuclease on the Rh and Lutheran antigens of human erythrocytes. They reported that the agglutinability of the enzyme-treated erythrocytes had decreased considerably when compared with the untreated controls. Mäkelä *et al.*[9] reported that trypsin destroyed M and N antigens and released *N*-acetylneuraminic acid (NANA) and hexosamine from the erythrocytes. They further stated that both the M and the N antigens were also destroyed by neuraminidase. A specific inhibition of anti-Rho(D) by the addition of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid to their test system was reported by Dodd *et al.*[10] and by Johnson *et al.*[11].

Some of the experiments already mentioned were repeated in this laboratory, and we would like to report our observations on the effects of trypsin, ribonuclease, neuraminidase, cellulase, hyaluronidase and lysozyme on the ABO, MN and Rh antigens of human erythrocytes.

Anti-A and anti-B sera were obtained from normal individuals of group B and group A, anti-M (human and rabbit) and anti-N sera (rabbit) from the Ortho Research Foundation, and both complete and incomplete types of anti-Rho(D) sera were given to us by the Ortho Research Foundation and Knickerbocker Biologicals, Inc.

Blood was obtained from normal individuals and was used immediately and also after 4 days storage at 5° C. Samples of blood were centrifuged and the erythrocytes washed 3 times with 20 volumes of 0·15 M sodium chloride solution. An aliquot of 1·0 ml. of washed, packed erythrocytes was then added to the following mixtures of 0·2 ml. each: (1) trypsin (Fisher Scientific Co.), 10 mgm. per ml.

Table 1. TITRATION SCORE OF TREATED ERYTHROCYTES WITH ENZYMES

Antiserum	Titration score: Original	Treated with trypsin	Un-treated control	Enzyme concentration (mgm./ml.)	Time for treatment
Anti-A (IHS)	55	78	52	10	4 hr.
		102	50		24 hr.
Anti-B (IHS)	32	64	30	10	4 hr.
		96	28		24 hr.
Anti-M (IHS)	36	0	36	10	1 hr.
		0	33		4 hr.
		0	16		24 hr.
Anti-N (IRS)	19	19	18	10	1 hr.
		24	16		4 hr.
		26	15		24 hr.
Anti-D* (IHS)	21	22	20	10	1 hr.
		23	17		4 hr.
		23	13		24 hr.

Antiserum	Original	Treated with neuraminidase	Un-treated control	Enzyme concentration (units/ml.)	Time for treatment
Anti-A (IHS)	65	64	65	100	30 min.
		62	62		3 hr.
		59	60		6 hr.
		55	57		24 hr.
Anti-B (NHS)	34	35	36	100	30 min.
		33	33		3 hr.
		30	31		6 hr.
		27	28		24 hr.
Anti-M (IHS)	48	5	43	100	1 hr.
		1	42		4 hr.
		0	27		24 hr.
Anti-N (IRS)	24	22	22	100	1 hr.
		20	20		4 hr.
		18	21		24 hr.
Anti-D* (IHS)	33	30	29	100	30 min.
		25	25		3 hr.
		21	21		6 hr.
		18	19		24 hr.

IHS, immune human serum; IRS, immune rabbit serum; NHS, normal human serum.

* Complete antibody which may contain a trace of incomplete antibody.

of 0·1 M phosphate buffer pH 7·7; (2) ribonuclease (Mann Research Laboratories), 4 or 10 mgm. per ml. of 0·1 M phosphate buffer pH 7·5; (3) cellulase (Worthington Biochemical Corp.), 10 mgm. per ml. of 0·1 M phosphate buffer pH 5·6; (4) hyaluronidase (Worthington Biochemical Corp.), 10 mgm. per ml. of 0·1 M phosphate buffer, pH 6·8; (5) lysozyme, 10 mgm. per ml. of 0·1 M phosphate buffer pH 6·5; (6) 0·35 ml. of neuraminidase solution (Behringwerke Marburg-Lahn), 100 units per ml., adjusted to pH 6·0.

All mixtures were made isotonic by the addition of the required amounts of sodium chloride solution. The mixtures were incubated at 37° C. for periods of 30 min.–24 hr. At the end of the incubation period the erythrocytes were washed with saline at 0° C. and a 2 per cent suspension of erythrocytes in saline was prepared. The control samples were prepared by incubation of the erythrocytes with the corresponding buffers.

Erythrocyte suspensions in saline were added to two-fold serial dilutions of anti-sera. For the determination of erythrocyte agglutinability against the incomplete Rh antibody, a 20 per cent bovine albumin solution was substituted for saline. Agglutination titres for A, B, M and N antigens were determined after incubation at 25° C. for 1 hr. Agglutination titres for Rho(D) antigen were read following incubation at 37° C. The agglutinability of erythrocytes was scored on the modified scale of Dunsford and Bowley[12] following centrifugation at $200g$ for 1 min.

The score for the agglutinability of A and B antigens against anti-A and anti-B sera, so-called naturally occurring agglutinins of the complete type of antibody, was not significantly altered by any treatment. As shown in Table 1, the titres of the immune human anti-A and anti-B sera were enhanced by the use of trypsin-treated erythrocytes. The agglutinability of M antigen, however, decreased rapidly after treatment, as compared with the controls. N antigen, in contrast, increased slightly by treatment with this enzyme. Ribonuclease did not affect either the M or the N antigen. With neuraminidase, a gradual decrease of the agglutinability against the M antigen was observed. A similar effect of neuraminidase on the N antigen was not detected.

Following the treatment of erythrocytes with trypsin for more than 1 hr., the non-specific agglutination with anti-Rho(D) sera containing an incomplete type of antibody was observed. This phenomenon was not detected with the complete type of anti-Rho(D) antibody. Erythrocytes treated with ribonuclease, neuraminidase, cellulase, hyaluronidase and lysozyme showed lower agglutinability against anti-Rho(D) sera after prolonged incubation. The score for treated erythrocytes was similar to that obtained for erythrocytes incubated in the absence of enzymes.

The amount of neuraminic acid released after incubation with various enzymes was determined by the method of Warren[13]. Following incubation with neuraminidase for 3 and 6 hr. respectively, between 0·348 and 0·381 μM of *N*-acetylneuraminic acid were released from a sample of 1·0 ml. of packed erythrocytes. Both Rho(D) positive and negative erythrocytes released similar amounts of neuraminic acid when incubated with this enzyme. Release of neuraminic acid after treatment with trypsin, ribonuclease, cellulase, hyaluronidase and lysozyme was not observed. Incubation of erythrocytes with neuraminidase for periods up to 24 hr. failed to affect the agglutinability against anti-Rho(D) sera.

Trypsin affected only the M antigen as demonstrated in the presence of both immune human and rabbit anti-M sera. Rosenfield *et al.*[14] obtained similar results with human anti-M serum. They also observed the non-specific agglutination similar to '*T*' agglutination (Huebener-Thomsen phenomena[15]) by using rabbit anti-M and anti-N sera. Our observations contrast with the findings of Mäkelä *et al.*[9] who reported that both M and N activity were destroyed by trypsin. On the other hand, erythrocytes treated with trypsin showed a slight increase in titre in the case of immune rabbit anti-N. It is suggested that different preparations of trypsin and the particular samples of anti-serum used may affect the results of such studies. The activity observed with neuraminidase appeared to be specific for the M antigen.

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MITOSIS-STIMULATING PROPERTIES OF EMBRYONIC NUCLEO-PROTEIN CONSTITUENTS IN CELL CULTURE

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As previously reported, factors of high molecular weight found in chick embryo extracts (EE_{50}) stimulate growth of tissues in culture[1]. These factors have been segregated in a nucleo-protein fraction[2] and recently isolated in the protein moiety[3] of the nucleo-protein fraction, which contained most of the biological activity originally present in the parent embryo extract.

These embryonic factors were previously assayed by plasma clot culture methods[1] using increase in area of a tissue explant as an index of growth. This measure does not discriminate between the more basic growth parameters of cell size, cell number (mitotic stimulation), cell migration or extracellular deposition, all of which could be involved in tissue growth.

To determine if mitotic stimulation was involved, it was necessary to assay the effects of the embryonic fractions using quantitative cell enumeration techniques, following a direct (primary) culture of dispersed cells from the host animal. Previous experiments in another laboratory, using second-generation cell cultures, showed adult tissue nucleo-protein fraction and protein moiety to be active, but were inconclusive for embryonic fractions[4,5].

A more direct method for primary cell culture and quantitative cell enumeration was developed as described here, and comparisons were made of biological effects of the parent embryonic extract, embryo nucleo-protein fraction, embryo protein moiety and background medium.

All procedures are performed under sterile conditions in this method. Trypsin (Nutritional Biochemicals) and chicken serum (Microbiological Associates) solutions are made in Puck's saline[6]. Twelve- to fourteen-day chick embryo thighs are removed into 1 per cent chicken serum. Bone and skin are removed and the muscle tissue is transferred into a 12 ml. centrifuge cone. 2·2 ml. of 0·1 per cent trypsin per ml. of tissue are added. After incubating for 15 min. at 37° C. on a platform rotating at 70 r.p.m., most of the muscle cells will have settled. The supernatant containing some muscle cells, debris and red blood cells is aspirated off. An equal volume of fresh trypsin is added to the residue, which is incubated as before for 30 min.

The trypsinization is stopped by adding an equal volume of 5 per cent chicken serum. The cells are collected by centrifuging for 9 min. at 600 r.p.m. and the supernatant is discarded. The cells are re-suspended by gentle tapping in 5 ml. of fresh 5 per cent chicken serum and poured through a double layer of sterile gauze. An extra 5 ml. of fresh 5 per cent chicken serum is used for rinsing the tube and gauze and combined with the suspension. Hæmocytometer counts on the suspension from 2 ml. original tissue average between 1–5 million/ml. Aliquots are diluted to 75,000–100,000 cells/ml. using the basal nutrient medium. In this medium, penicillin–streptomycin–glucose contains 10 mgm. phenol red, 75 mgm. dihydrostreptomycin (Pfizer), 90 mgm. sodium penicillin (Merck) and 0·12 gm. glucose in 50 ml. of Earle's solution. $EE_{50}UC$ is 13-day chick embryo extract (EE_{50}) clarified by spinning for 1 hr. at 105,000*g*, and sterilized using ultra-fine filters. The composition by volume of the basal nutrient medium is: 80 per cent medium No. 199 (Microbiological Associates), 10 per cent penicillin–streptomycin–glucose, 1 per cent fœtal calf serum (Microbiological Associates) and 9 per cent Earle's solution in which growth factors ($EE_{50}UC$, nucleo-protein fraction[2], protein moiety[3]) are incorporated, on a dry weight basis.

Culture series are set up in *D*-3, 5 Carrel flasks, allowing several flasks for each count. A saline (basal medium) and an ultracentrifuged embryo extract ($EE_{50}UC$) control are incorporated in each experiment. 2 ml. of cell suspension in nutrient medium are added to each flask. Cultures are then gassed with 5 per cent carbon dioxide in air to a phenol red colour change at *p*H 7·6, closed with silicone rubber stoppers, and incubated at 37° C. The cells settle out and soon become attached to the glass surface, growing first as a monolayer culture, later becoming multi-layered.

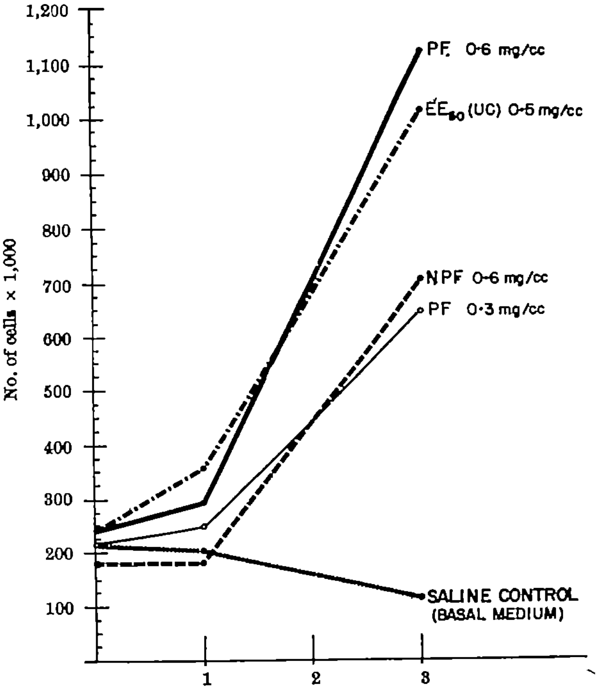

Fig. 1. Typical response of chick myoblast cells in first-generation glass substrate cultures
PF, protein moiety; *NPF*, nucleo-protein fraction; $EE_{50}(UC)$, 13-day chick embryo extract (EE_{50})

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At 24 hr. after inoculation and on succeeding alternate days, the cultures are given a 50 per cent medium change and gassed as above. Contents of three flasks are pooled and 4–8 aliquots are counted in a hæmocytometer. Three representative flasks per series are saved to be pooled for each cell count and their supernatants discarded. Each flask is rinsed with 1 ml. of Puck's saline A[6], which is also discarded. Using a capillary pipette, the cells in each flask are gently dispersed in 1 ml. of 0·1–1·0 per cent trypsin.

A typical experiment (Fig. 1) shows that: (1) the basal medium controls exhibit a third-day drop (or a static population, seen in other series); (2) all supplemented series increase rapidly during the first three days, with as much as a five-fold increase; (3) the nucleo-protein fraction is not as stimulating as the protein moiety on a dry-weight basis; (4) the protein moiety is almost as stimulating as $EE_{50}UC$ on a dry-weight basis; (5) the cell population response is proportional to the dose of material tested, within a certain range of concentration. For $EE_{50}UC$ a maximum response is obtained at the 4 per cent level.

It is noted that embryo extract (EE_{50}) prepared by low-speed centrifugation is very weakly and irregularly stimulating. After ultracentrifuging, the clear mid-fraction ($EE_{50}UC$) is uniformly very stimulating. Evidently the cell particulates or the lipids, which are both removed, interfere with the cell-growth response in this system.

In all cases the cells remain small and display a uniform non-granular spindled appearance typical of chick skeletal muscle fibroblasts in glass monolayer cultures. Extracellular fibre formation is usually noted in supplemented series with high populations.

It should be noted that this method uses tissues directly from the animal in contradistinction to the usual type of culture using established cell lines. The primary type of culture used in this experiment eliminates intervening mutational and adaptational effects arising during preconditioning of cultures, and makes interpretation of results in culture more applicable to events in the original host tissue in the animal. These effects may explain why the earlier experiments with the second generation cultures were inconclusive for embryonic fractions, for example, adapted cells may have lost their capacity to respond to the protein moiety.

These results indicate that there is a definite mitotic stimulation by the protein moiety and it is proportional to the dose of material. It is interesting to note that on a dry-weight basis, the ultracentrifuged EE_{50} is almost as active as its daughter product, the protein moiety. This may be due to progressive inactivation without denaturative precipitation during the long isolation procedures of the protein moiety. This could result in a large percentage of biologically inactive material in the final product which is still soluble and chemically indistinguishable from the active product.

With regard to growth mechanisms involved in the action of embryonic protein moiety, it is now possible to state that an increase in cell size does not seem to be involved in the growth response to the protein moiety, but an increase in cell number (mitotic stimulation) and extracellular deposition, that is, fibre formation, are directly involved. Cell migration may be involved, but more experiments are necessary to determine this.

Future experiments on the determination of the specific chemical groups involved in the biological activity of the protein moiety are planned. These may throw further light on the mechanism of inactivation during processing as well as the chemical basis of growth stimulation.

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CARBOHYDRATE–PEPTIDE LINKAGES IN GLYCOPROTEINS AND METHODS FOR THEIR ELUCIDATION

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CARBOHYDRATE–PROTEIN complexes with chitin, hyaluronic acid, chondroitin-sulphates and similar polysaccharides as sugar moieties have been known for a long time. In these complexes the carbohydrate is characterized by a small repeating unit and a high degree of polymerization. A more recent development is the realization that a great number of proteins, classified as 'glycoproteins', have attached to them a carbohydrate with a relatively low degree of polymerization and so far with no indication of a short unit repeating itself. Ten years ago the concept was advanced[1] that glycoproteins are best visualized as conjugated proteins consisting of a polypeptide chain (core) to which relatively small polysaccharides are attached as prosthetic groups. The carbohydrate groups of the various glycoproteins differ widely in molecular weight, being, for example, 512 in ovine submaxillary gland glycoprotein and about 3,500 in human γ-globulin. They consist invariably of two or more different sugar residues; most of them contain hexosamine, and many possess a terminal sialic acid unit. The term 'heterosaccharide' may conveniently describe this wide range of carbohydrate structures since it does not prejudice the size. The number of heterosaccharide residues per glycoprotein molecule may be as low as one, as in ovalbumin (mol. wt. 45,000) and human γ-globulin (mol. wt. 160,000) or as high as 800 in ovine submaxillary glycoprotein (mol. wt. 1×10^6).

Concerning the carbohydrate–peptide linkage in glycoproteins, the available evidence indicates that a covalent bond joins the heterosaccharide to the polypeptide chain. For a complete understanding of this linkage the following information is required: (1) the composition of the heterosaccharide; (2) the nature of the amino-acid residue acting as 'acceptor' for the heterosaccharide; (3) the functional groups of the heterosaccharide and amino-acid residues respectively involved in the linkage. The first approach to the characterization of such linkages was the preparation of short glycopeptides by enzymatic fragmentation of glycoproteins. Thus, Rosevear and Smith[2] isolated from the papain digest of γ-globulin three glycopeptides by a procedure involving ion exchange resin, ethanol precipitation and starch column electrophoresis. The glycopeptides differing in length had the common sequence Asp. Tyr. Glu. Asp. (carbohydrate), the carbohydrate containing 3 galactose, 5 mannose, 2 fucose, 8 glucosamine and 1 sialic acid residues. After exhaustive treatment of the glycopeptides with aminopeptidase and carboxypeptidase a fraction was obtained which on hydrolysis with 6 *N* hydrochloric acid yielded only aspartic acid and glucosamine, thus establishing aspartic acid as the acceptor. Johansen, Marshall and Neuberger[3] digested crystalline ovalbumin with various proteolytic enzymes in succession and submitted the digest to column chromatography and column electrophoresis. The glycopeptide Asp(carbohydrate).(Leu, Ser, Thr).Val was thus obtained. The carbohydrate consisted of 5 mannose and 3 *N*-acetylglucosamine residues Nuenke and Cunningham[4] reported very similar results with regard to the composition of a glycopeptide isolated by them from ovalbumin by similar procedures; however, even the best preparations could be fractionated further by countercurrent distribution. The sequence analysis by these authors was nearly coincident with that given by Johansen *et al.* Both groups succeeded in removing from the non-reducing glycopeptide by carboxypeptidase all amino-acids but aspartic acid, to which the carbohydrate remained attached. This achievement proved linkage of the carbohydrate to aspartic acid. The authors also concurred in the observations that the amino-group of aspartic acid was non-substituted and that on mild acid treatment 1 mole ammonia/mole glycopeptide was liberated. Hydroxylamine failed to separate the carbohydrate from the peptide. From these data an aspartylglycosylamine structure (that is, an *N*-glycoside involving the amide group of asparagine), analogous to glycinamide ribotide (*N*-glycyl-5′-phosphoribosylamine)[5], was tentatively proposed.

Over the past years, our laboratory has made a detailed study of the composition and structure of ovine submaxillary gland glycoprotein (OSM). In this glycoprotein a carbohydrate–peptide linkage different from that present in ovalbumin was discovered. Application of chemical reagents acting preferentially on this linkage rendered it possible to define with precision the nature and position of the functional groups of the sugar and amino-acid residues involved in the linkage, without the laborious preparation, purification and sequence analysis of glycopeptides.

OSM is a macromolecule of about 1 × 10⁶ particle weight. In aqueous solution the particle most probably has a random coil configuration[6]. The substance is composed of about 41 per cent carbohydrate and 59 per cent protein. The carbohydrate consists nearly exclusively of equimolar amounts of *N*-acetylneuraminic acid and *N*-acetylgalactosamine. These components are present as the disaccharide α-D-*N*-acetylneuraminyl (2 → 6) *N*-acetylgalactosamine[7]; about 800 of such prosthetic groups are distributed along the polypeptide chain[8]. 85 per cent of the terminal *N*-acetylneuraminic acid residues can be split off by *Vibrio cholerae* neuraminidase, an enzyme acting specifically on the ketosidic linkage joining a terminal sialic acid unit to an adjacent galactose or galactosamine residue[9].

For the elucidation of the type(s) of linkage joining the prosthetic groups to the polypeptide chain three independent approaches were made:

(1) OSM (4 mgm./ml.) was heated in excess 0·2 *N* sodium hydroxide at 100°. After 5 min. only 8 per cent of the total prosthetic groups were found to be still bound to the polypeptide chain. The separation of the released prosthetic groups, identified chromatographically, was effected on 'Sephadex *G* 25' columns. Assuming pseudo-monomolecular reaction mechanism, $k = 4{\cdot}9 \times 10^{-1}$ min.$^{-1}$, that is, in 0·2 *N* sodium hydroxide at 100°, 50 per cent of the disaccharide residues were released in 1 min. 25 sec.

(2) OSM (0·7 mgm./ml.) was dissolved in excess *M* hydroxylamine at *p*H 12·2 and 37° and the *p*H kept constant as measured by type *B* glass-electrode (Titrator Type *TTT* 1*b*; Radiometer Inc., Copenhagen). The first-order rate constant of decrease of protein-bound prosthetic groups was $k = 2{\cdot}3 \times 10^{-3}$ min.$^{-1}$. The action of alkali (*p*H 12·2) on its own, determined in a control experiment without hydroxylamine, gave a first-order rate constant $k_{\mathrm{OH}^-} = 0{\cdot}6 \times 10^{-3}$ min.$^{-1}$. Since it is known that the presence of a negative charge in the vicinity of a hydroxylamine-susceptible bond decreases the rate of the hydroxylamine reaction[10], in another set of experiments 97 per cent of the *N*-acetylneuraminic acid residues of OSM were split off prior to the hydroxylamine treatment. Under these conditions the respective values for k and k_{OH^-} were found to be 7×10^{-3} min.$^{-1}$ and $1{\cdot}8 \times 10^{-5}$ min.$^{-1}$. In each case the release of the prosthetic groups by hydroxylamine was accompanied by the formation of hydroxamic acids, as shown by the absorption spectrum of their ferric complexes.

(3) OSM (1·7 mgm./ml.) was refluxed in excess 0·3 *M* $\mathrm{LiBH_4}$ in tetrahydrofuran for 10 hr. At the end of the reaction the boron-intermediate complex formed was decomposed according to equation (1):

$$2\,R{\cdot}\mathrm{CO{\cdot}O}{\cdot}R' + \mathrm{LiBH_4} \rightarrow \mathrm{Li^+}\,[(R{\cdot}\mathrm{CH_2O})_2\mathrm{B}(\mathrm{O}{\cdot}R')_2]^- \xrightarrow[3\,\mathrm{CH_3OH}]{\mathrm{HCl}} 2\,R{\cdot}\mathrm{CH_2OH} + 2\,R'\mathrm{OH} + \mathrm{LiCl} + \mathrm{B(OCH_3)_3} \qquad (1)$$

Analysis of the reaction products showed that 88 per cent of the dicarboxylic acids, corresponding to 80 per cent of the prosthetic groups, were reduced (amides and free carboxyl groups are not acted on under the same conditions). The prosthetic groups released by this treatment had their reducing groups intact[11]. In the case of bovine submaxillary gland glycoprotein (BSM), which closely resembles OSM in composition and structure, 83 per cent of the total dicarboxylic acids, corresponding to 81 per cent of the prosthetic groups (α-D-sialyl (2 → 6) *N*-acetylgalactosamine[12]), were reduced by $\mathrm{LiBH_4}$. The reduced dicarboxylic acids were identified as α-amino-γ-hydroxy-*n*-butyric acid (homoserine) and α-amino-δ-hydroxy-*n*-valeric acid. Again the released disaccharides were reducing[13].

There can be little doubt that in our OSM preparation either all or nearly all prosthetic groups are identical in composition and structure. Whether the 0·85 per cent non-amino sugars (galactose, mannose and fucose) are an intrinsic component of the molecule or a contaminant[7], is difficult to decide. However, recent investigations by Tsuiki *et al.*[14] would suggest that in BSM, in addition to the aforementioned disaccharides, an oligosaccharide composed of glucosamine, galactose and fucose is a minor component of the molecule rather than a contaminant. Again, whereas in OSM the sialic acid is nearly exclusively N-acetylneuraminic acid, in BSM a mixture of various N- and O-acylated neuraminic acids is present[15].

The susceptibility of most of the disaccharide–peptide linkages in OSM to mild alkali, hydroxylamine and lithium borohydride seems to prove this linkage to be of the ester type. The recovery of the disaccharides, liberated from the boron-intermediate complex, with their reducing groups intact, would indicate that this group is engaged in the ester linkage (see equation (1)). This is also borne out by the observation that OSM and BSM do not react with Benedict's reagent or o-dinitrobenzene in weak alkali, when heated shortly. The linkage is, therefore, of the glycosidic–ester type. The configuration of the anomeric carbon atom engaged in this linkage is unknown.

Sensitivity to very mild alkaline conditions seems to be characteristic for glycosidic-ester linkages. Recently, Zenk[16] reported that 1-(indolyl-3-acetyl)-β-D-glucose, isolated from the leaves of *Colchicum neapolitanum* Ten., was saponified at room temperature by both N potassium hydroxide and ammonia. Another ester linkage between carbohydrate and peptide, susceptible to mild alkali (N barium hydroxide, 100°, 1 hr.), was found in barley albumin. From the trypsin digest of a formic acid hydrolysate of this glycoprotein the reducing and ninhydrin-positive compound 4-L-alanyl-D-xylopyranose was isolated[17]. On the other hand, it is known that an N-glycosidic linkage to the amide-group of asparagine reacts very slowly with 0·2 N sodium hydroxide at 100° ($k = 5{\cdot}5 \times 10^{-3}$ min.$^{-1}$)[18].

In contrast to the general building plan of glycoproteins of animal origin, one basal component of the rigid structure of the cell wall of Gram-positive bacteria is a polymer backbone of alternating residues of N-acetylglucosamine and N-acetylmuramic acid to which small peptides are attached. Here, the linkage between carbohydrate and peptide seems to be invariably an amide link between the carboxyl group of muramic acid and the amino group of the terminal L-alanyl unit[19]. In the crystalline glycoprotein taka-amylase an O-glycosidic linkage to serine seems to be established. Tsugita and Akabori[20] prepared from this glycoprotein, by treatment with *Streptomyces* protease, a glycopeptide which had as N-terminal a serine residue and which on shortening with carboxypeptidase yielded a serine–carbohydrate complex.

For the investigation of glycosidic-ester linkages between carbohydrate and protein the procedures described in this article offer a simple and rational approach. It is to be hoped that future research will add to the available tools for linkage determination, specific enzymes such as the *Streptomyces* amidase[19] which cleaves in bacterial glycopeptides the amide link between muramic acid and L-alanine.

Throughout the animate world Nature adheres strictly to the glycosidic type of linkage when joining sugar to sugar, and to the amide type of juncture when adding amino-acid to amino-acid. Yet, in the attachment of carbohydrate to peptide almost any of the functional groups of the latter may be involved, resulting in a variety of modes of linkage.

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DIFFUSION STUDIES OF BOVINE SERUM ALBUMIN WITH JAMIN INTERFERENCE OPTICS AND MICRO-DIFFUSION CELL

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ALTHOUGH there has been a number of reports on the diffusion studies of bovine serum albumin using different physico-chemical conditions, most of them were carried out with the help of Rayleigh or Gouy interference optics. A general account of these works has been given by Gosting[1] and more recently by Thomson and Svensson[2]. The present article gives the results of diffusion investigations on bovine serum albumin, carried out with the Jamin interference technique and a micro-diffusion cell provided in the Antweiler[3] micro-electrophoresis diffusion equipment.

With this method, different points along the height of a Tiselius type of micro-diffusion cell can be scanned in steps of 0·1 mm. The refractive increment Δn or the corresponding concentration difference Δc compared to a reference channel is obtained directly as a function of X. This leads to a curve given by the well-known diffusion equation:

$$C_x = \frac{C_0}{2}\left[1 - \frac{2}{\sqrt{\pi}}\int_0^{x/\sqrt{4Dt}} \exp(-x^2/4Dt)\,\mathrm{d}(x/\sqrt{4Dt})\right] \quad (1)$$

where C_x is the concentration at a distance x from the place where the boundary is formed at an instant $t = 0$. Equation (1) may be written as:

$$\frac{C_x}{C_0} = \frac{1}{2}\int_{x/\sqrt{4Dt}}^{\infty} \frac{2}{\sqrt{\pi}} \exp(-x^2/4Dt)\, d(x/\sqrt{4Dt}) \quad (2)$$

Since the values of the probability integral $\frac{2}{\sqrt{\pi}}\int_{hx}^{\infty} \exp(-h^2x^2)\, d(hx)$ are tabulated in standard texts for different hx, one can easily prepare a table relating $x/\sqrt{4Dt}$ with any measured value of C_x/C_0. This relation may be represented by:

$$D = \frac{x^2}{t} \cdot g(C_x/C_0) \quad (3)$$

With the help of such a previously prepared table the diffusion coefficient D may be obtained from the measured values of C_x/C_0, x and t.

Often it is more practical to have the diffusion not between the concentrations C_0 and 0 but between two protein solutions of concentrations C_0 and C_v. In this case equation (3) becomes:

$$D = \frac{x^2}{t} \cdot g\left(\frac{C_x - C_v}{C_0 - C_v}\right) \quad (4)$$

With the help of the foregoing method the diffusion coefficient of crystalline bovine plasma albumin, fraction V, obtained from L. Light and Co., Ltd., Colnbrook, England, has been measured. For this purpose the protein was dissolved in a buffer (0·18 *M* sodium chloride + 0·02 *M* sodium acetate + 0·02 *M* acetic acid) of *p*H 4·55 and ionic strength 0·2. At a time t after layering of the two solutions the whole cell, covering a length of 4 cm., was scanned with light in steps of 0·2 mm. From the dial reading the fraction C_x/C_0 or $\frac{C_x - C_v}{C_0 - C_v}$ could be obtained directly and plotted against x. A preliminary diffusion experiment carried out with 0·5 per cent solution of bovine serum albumin in distilled water, diffusing against distilled water, revealed a marked asymmetry in the final curve given by equation (1).

Afterwards, all the experiments were performed by allowing the protein solution in the above buffer to diffuse against the same protein solution of lower concentration, the difference in concentration being always the same and equal to 2×10^{-3} gm./c.c. Fig. 1 shows a set of curves obtained in this way at different times within 2–5 hr. after the layering of the solutions. From each curve one gets for different values of x a set of D values. The mean of these values of D is taken as the diffusion coefficient for that time-interval. In order to take into account the time-interval involved during the layering process, the values of diffusion coefficient D obtained for different times were plotted against $1/t$ and extrapolated to $t = 0$. This gave the diffusion coefficient D_T at the mean concentration $\bar{C} = (C_0 + C_v)/2$.

The experiments were carried out at temperature T close to 25° C. and the observed diffusion coefficients D_T were referred to 25° C. by the relation $D_{25} = 298.\frac{D_T\eta_T}{\eta_{25}T}$, where η_{25} and η_T are the viscosity coefficients of the solvent at 25° C. and T respectively.

Diffusion coefficients were measured at several mean protein concentrations. Concentrations were measured generally by the dry-weight method and checked by the ultra-violet extinction value[4] ($E^{1\%}_{1\,\text{cm.}} = 6\cdot67$ at 279 mμ). When $1/D_{25}$ was plotted against $\bar{C}$, a straight line was found to fit best the experimental points (Fig. 2). The following relation between $1/D^0_{25}$ and C was deduced from the experimental curve, $1/D^0_{25} = 4 \times 10^5\, C + 1\cdot62 \times 10^6$. This is consistent with the theoretical observation of Fujita[5] and Philip[6] that $D = (mc + a)^{-1}$ was one of the solutions of the equation $\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left[D(c)\frac{\partial c}{\partial x}\right]$, subject to the conditions $c = 0$, $x > 0$, $t = 0$ and $c = 1$, $x = 0$, $t > 0$. The extrapolated value of D at $c = 0$ was $D^0_{25} = 6\cdot173 \times 10^{-7}$ cm.²/sec.

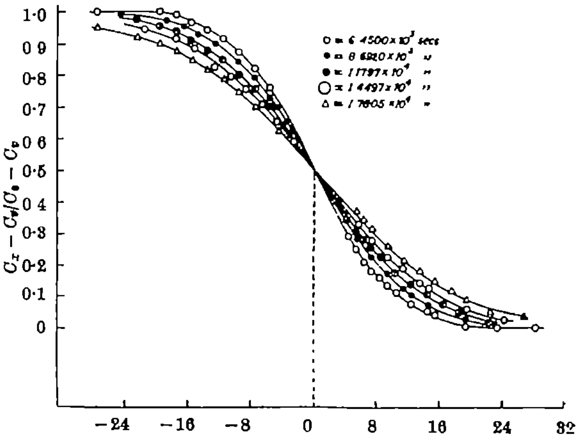

Fig. 1. Solute concentration distribution in the diffusion cell at different times in the process of diffusion

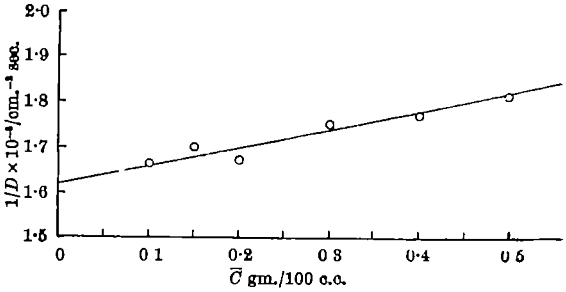

Fig. 2. Concentration dependence of the diffusion coefficients of bovine serum albumin

The measurements reported here refer to a concentration difference $\Delta C = 2 \times 10^{-3}$ gm./c.c. between the protein solutions. The influence of different values of ΔC on the nature of concentration dependence of D and also on the final value of D^0_{25} are being investigated and will be reported later.

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FORMATION OF THE REPRESSOR FOR LYTIC PHAGE DEVELOPMENT AFTER INFECTION OF *SALMONELLA TYPHIMURIUM* BY PHAGE *P22*

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AFTER adsorption of a temperate phage by a sensitive bacterium the cell can develop either a lytic or a lysogenic infection. Which reactions occur seem to depend on a 'race' between two reaction chains[1-3]. One chain leading to lysogenization seems to depend on the synthesis of a specific cytoplasmic substance, called the repressor, which is able to overcome the alternative reaction chain leading to lysis. (Since our knowledge of the chemical nature of the repressor and the steps involved in its synthesis is very meagre, we confine our definition of the repressor to a substance the synthesis of which causes the limiting factor in establishing the repression of lytic phage development.) The alternative reaction chain leading to lysis of the complex involves the synthesis of specific proteins necessary for development of vegetative phage. According to a hypothesis proposed earlier[2] the reaction chain reaching the 'winning post' 'decides' for lysis versus lysogenization of the cell.

The chance in favour of lysogenization can be increased experimentally in two different ways: (*a*) Infection of a cell with more than one temperate phage[4-7], leading to the so-called 'multiplicity effect'. (*b*) Selective inhibition of the competing reaction chain, leading to lysis by a block of the protein synthesis, for example, with chloramphenicol (CHL)[1,2,8-10]. In both cases the synthesis of the repressor seems to be favoured. The following experiments were undertaken to obtain some information about the kinetics of repressor formation and its relation to protein synthesis.

(*A*) *Kinetics of repressor formation in the presence of chloramphenicol.* With regard to the kinetics of the 'multiplicity effect', it has been shown that the chance of 'survival' for lytic infected (L) and uninfected cells (U) decreases with multiplicity of infection m (mean number of phages per bacterium), or, inversely expressed, the yield of lysogenic infections (G) within a population of bacteria increases in one-hit kinetics with multiplicity m:

$$-\frac{\mathrm{d}(L+U)}{\mathrm{d}m} = k_{\text{lyg}} \times m\,; \quad \text{integrated}$$

$$L + U = \exp(-k_{\text{lyg}} \times m)$$

Since:

$$G + L + U = 1 \text{ respectively } L + U = 1 - G,$$

we get:

$$1 - G = \exp(-k_{\text{lyg}} \times m) \qquad (1)$$

where k_{lyg} denotes the probability for lysogenization for each infecting phage which depends on genetic factors and physiological conditions.

Equation (1) shows that lysogenization is 'dominant' over lysis. Therefore the lysogenic infections increase with 'multiplicity of lysogenization' $m \times k_{\text{lyg}}$.

It seems reasonable to conclude from equation (1) that every infecting phage is able to synthesize a certain amount of repressor under the physiological conditions given. With increasing multiplicity this amount may increase proportionally. Analogous to equation (1) the probability of an infected cell yielding a lytic response (L) will decrease with increasing concentration r of the repressor:

$$-\frac{\mathrm{d}L}{\mathrm{d}r} = c \times r; \quad \text{integrated}$$

$$L = \exp(-c \times r) \text{ resp.}$$

$$1 - G = \exp(-c \times r) \qquad (2)$$

Since we regard in this formulation only infected cells the relative amount of lysogenic infections (G) equals the frequency of lysogenization; c denotes a constant describing the probability of action of the synthesized repressor.

If the expression of the multiplicity of infection in terms of repressor concentration is correct (equation 2), then it can be expected that it is unimportant by what mechanism the repressor concentration is changed. As already stated, lysogenization with respect to repressor formation is favoured by a block in the protein synthesis. Since it is observed that the frequency of lysogenization increases with increasing time of CHL treatment, one is led to conclude that during the blockage of protein synthesis the repressor accumulates inside the cell. The increase of the frequency of lysogenization with the duration of CHL treatment should then reflect the time-course of repressor synthesis. If the rate of synthesis is independent of time we should obtain, if applying equation 2, a 'one-hit' increase of the lysogenizations with the duration of CHL treatment. Any other kind of kinetics of increase would mean that either equation 2 is incorrect or the rate of repressor synthesis is not independent of time, or both proposals are incorrect. Fig. 1*a* shows that the experimental data fit well enough the expectation for a rate constant in time. In each of the four experiments of Fig. 1*a* the absolute number of infections (lysis + lysogenization) remained constant during all periods of treatment with CHL; therefore the observed change in frequency of lysogenization is not caused by selective killing or curing from lytic or

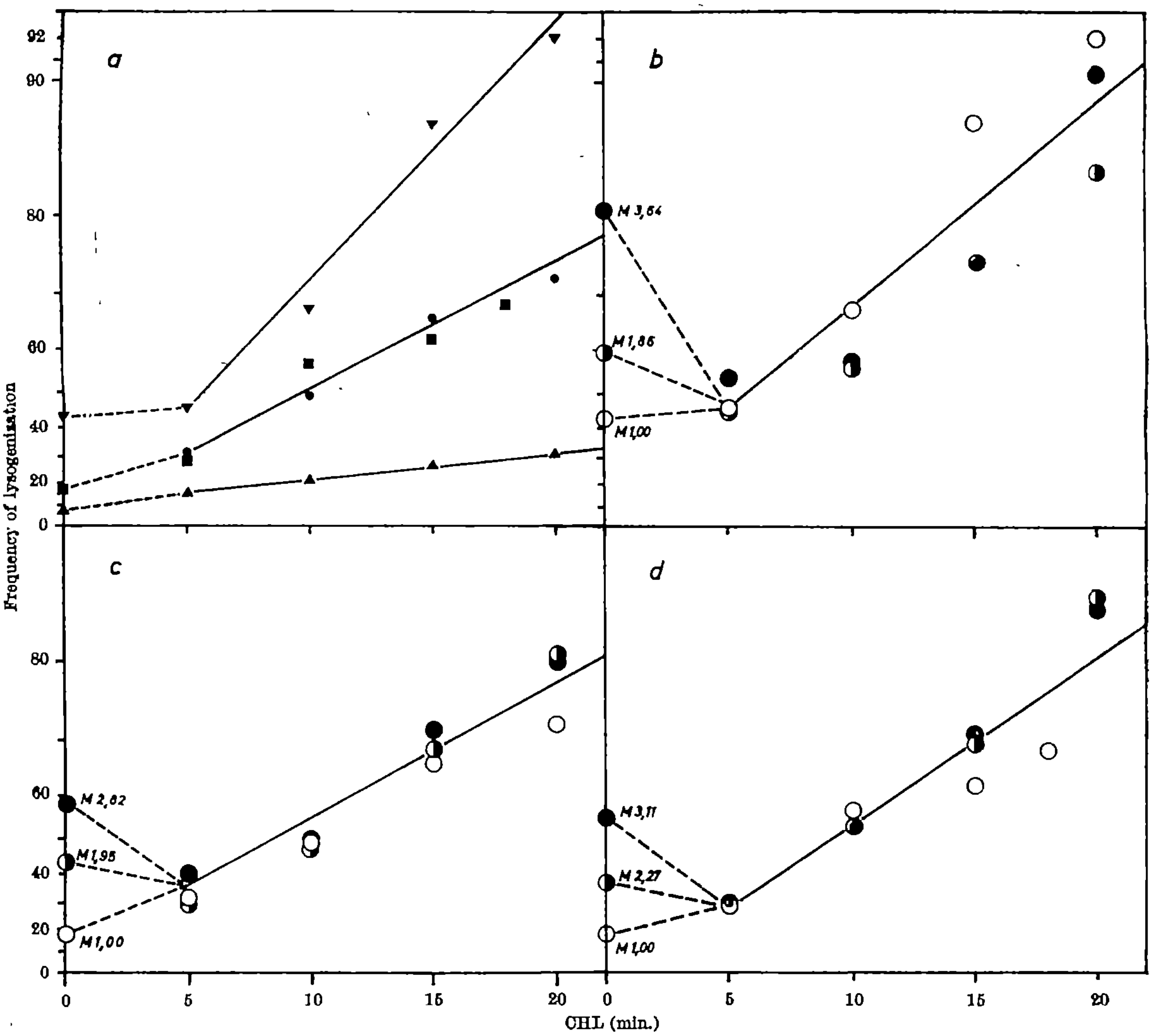

Fig. 1. *a*, Stationary cells of *Salmonella typhimurium try*8⁻ have been infected in phosphate buffer at 37° C. with $P22\ H_1$ in multiplicities ranging from 1/20 to 1/100; after 10 min. adsorption period (99 per cent adsorption) they were diluted 1/10 into anti-*P22*-serum in nutrient broth ($t = 0$). At time $t = 5$ min. the complexes have been transferred in nutrient broth + 12·5 μgm. CHL. The treatment lasting 5, 10, 15 or 20 min. has been terminated by dilution 1/50 in nutrient broth. At time $t = 25$ all samples with the different duration of treatment were plated after appropriate dilution with 'Tgal⁻' indicator in soft agar on nutrient agar plates with galactose (ref. 2). All treatments have been carried out at 37° C. Four different experiments

b–d, Same principle and same procedure as described for Fig. 1*a* with the exception that three parallel samples with different multiplicities of infection (*m*) were tested. In the figures the multiplicity of the complexes (*M*) is indicated at the corresponding points on the ordinate ($M = m/1 - e^{-m}$). Fig. 1*b*. $m = 0\ 046$, $M = 1{\cdot}00$ (○); $m = 1{\cdot}40$, $M = 1\ 86$ (◑); $m = 3\ 51$, $M = 3{\cdot}64$ (●). Fig. 1*c*. $m = 0\ 047$, $M = 1\ 00$ (○); $m = 1{\cdot}52$, $M = 1{\cdot}95$ (◑); $m = 2\ 64$, $M = 2{\cdot}82$ (●). Fig. 1*d*. $m = 0\ 017$, $M = 1\ 00$ (○); $m = 1{\cdot}94$, $M = 2{\cdot}27$ (◑); $m = 2{\cdot}95$, $M = 3{\cdot}11$ (●). The frequencies of lysogenization at $M = 1\ 00$ are the same experimental points as ▼, • and ■ in Fig. 1*a*

lysogenic infections. Hence we may formulate as follows:

$$1 - G = \exp(-t_{\mathrm{CHL}} \times k_{\mathrm{CHL}}) \text{ resp.}$$
$$\ln(1 - G) = -t_{\mathrm{CHL}} \times k_{\mathrm{CHL}} \qquad (3)$$

where k_{CHL} denotes the probability of lysogenization for an infecting phage under the physiological conditions of CHL treatment extrapolated back to time zero, and t_{CHL} the duration of treatment.

This result suggests that: (1) the repressor concentration inside the cell determines the probability of lysogenization according to equation 2; (2) that repressor formation indeed proceeds in a linear manner during the presence of CHL. It is likely that both proposals apply to conditions without treatment with CHL in the same way.

Since the synthesis of primary gene products proceeds in the same linear manner[11] we reach the conclusion that the repressor of lytic phage development behaves like a primary gene product. This idea is in full accordance with the concept developed by Jacob and Monod[3].

(*B*) *Influence of chloramphenicol on the multiplicity effect*. The repressor formation seems to consist of several steps each dependent on special biochemical reactions as the following experiments will show. Since the increase of lysogenic responses with multiplicity (see equation (1)) and the increase of frequency of lysogenization during treatment with CHL (see equation (2)) of the complex follow the same kinetics, and since both can be explained on the basis of different repressor concentrations, one is led to speculate that both the number of phages per infected cell (that is, multiplicity of the complexes M; $M = m/1 - e^{-m}$) plus the treatment of the complex with CHL should yield a higher frequency of lysogenization than without treatment with CHL. Surprisingly, this is not the case; rather the frequency of lysogenization in multiple-infected complexes decreases to the level of single infected ones after 5-min. treatment with CHL (Fig. 1*b*–*d*). (An influence of selective killing or curing of lytic or lysogenic infections at different multiplicities is excluded experimentally by the same reason as in the experiment illustrated by Fig. 1*a*.) If the treatment is increased further up to 20 min., the frequency of lysogenization increases in single and multiple-infected cells approximately with the same kinetics. This result can be taken as indicating that the blockage of protein synthesis prevents an important step in the establishment of the multiplicity effect by permitting formally only one of the infecting phages to synthesize repressor. The protein synthesis necessary for the expression of the multiplicity effect occurs within the 5th–10th min. after the start of the intracellular phage development, as can be shown experimentally. Accordingly, treatment with CHL after that time-interval (for example, starting at $t = 10$ or $t = 15$ min. after start of intracellular phage development) permits again the expression of the multiplicity effect, although slightly reduced.

Within the chain of reactions leading to repressor formation we now can distinguish two steps. The first one depends on the synthesis of some kind of protein which must be initiated by each infecting phage in order to contribute to the multiplicity effect of lysogenization. This contribution itself is a part of the second step, the synthesis of the repressor, which seems to be more or less independent of protein synthesis. A noteworthy feature of the first step is, that even in the presence of CHL formally one phage can initiate it, but all other infecting phage particles seem to be excluded from it. In other words, CHL treatment limits the 'capacity' of the cell for performance of the first step to one phage only.

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UREA EXCRETION IN RUMINANTS

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VARIOUS authors[1,3–7] have published conflicting reports on the level of urea excretion in the urine of ruminants. Read[1], investigating the urine nitrogen excretion of a pregnant female camel (*Camelus bactrianus*) fed on sorghum leaves, sweet potato and salt, reported that there was only a trace of urea in the urine. As it has been generally accepted that urea is the most important of the nitrogenous waste products in mammalian urine[2], this finding excited the interest of other investigators. Petri[3] and Smith and Silvette[4] reported that they could not duplicate Read's results using either pregnant or non-pregnant camels (*Camelus bactrianus* and *Camelus dromedarius*). They state that the urine of the camels they investigated contained a normal amount of urea nitrogen, amounting to approximately 60 per cent of the total. Smith and Silvette[4] also reported that the urine of the llama (*Auchenia huanacos*) and the alpaca (*Auchenia vicunna*) contained normal amounts of urea. Meyer, Weir and Smith[5], studying sheep, observed low total urine nitrogen values when the animals were deprived of water and feed for limited periods.

The controversy was re-opened by Schmidt-Nielsen *et al.*[6], who examined the water metabolism of the camel (*Camelus dromedarius*) in Algeria. They reported that the urea excretion of the growing camel became extremely low under conditions of low intake of nitrogen and deprivation of water, and they maintained that their results suggested that there was no reason to doubt those of Read[1].

Schmidt-Nielsen and Osaki[7] have also shown that the excretion of urea nitrogen by sheep maintained on a very low crude protein content diet and *ad lib.* water is also low.

Current work in this laboratory, planned to investigate the nitrogen metabolism of *Bos taurus* and *Bos*

Table 1. ACTUAL AMOUNT UREA NITROGEN IN THE URINE AND PERCENTAGE UREA NITROGEN IN THE TOTAL URINE NITROGEN OF *Bos taurus* AND *Bos indicus* IDENTICAL TWIN SETS DURING FIVE 10-DAY EXPERIMENTAL PERIODS

Twin set	Species	Animal	Experimental periods	Approx. C.P. of ration	Urea-N per day gm.	Urea-N as per cent total urine-N
1	*Bos taurus*	A_1	I	12	32·0±2·19	67·7±1·60
			II	12	28 6±1·55	69·0±1·44
			III	12	30·3±2·52	62·6±1 23
			IV	12	36·8±1·46	67·7±1 20
			V	12	36·7±3·31	60·8±2·37
		A_2	I	12	36·1±2 28	70·2±1·71
			II	8	29 0±2·41	63·1±1 76
			III	4	13·0±5·23	55·4±2·60
			IV	4	5·3±1·03	32 5±3·82
			V	4	2·3±0·06	15·0±2 95
2	*Bos indicus*	Z_1	I	12	29·8±3·45	74·1±1·70
			II	8	20·4±2·07	64·9±1·96
			III	4	16·6±3 50	71·6±2 54
			IV	4	9·0±4·29	52·7±6·61
			V	4	3 4±0·06	35·9±6·71
		Z_2	I	12	32·6±3·53	72·0±1·20
			II	12	28·4±1·83	70·3±1·35
			III	12	20·2±3·76	68·5±1 51
			IV	12	33·6±0·10	71·0±2·71
			V	12	39·6±1·84	68·7±2·23

In periods IV and V, twins A_2 and Z_1 were deprived of water for 96-hr. intervals.

indicus cattle under conditions of low intake of nitrogen and deprivation of water (stimulating nutrient stress conditions in the semi-arid areas of East Africa), has produced results which might help to explain these conflicting reports.

Two sets of identical steer twins, one *Bos taurus* (Ayrshire) and the other *Bos indicus* (Boran), were fed a ration containing approximately 12 per cent crude protein for a long standardization period, the final ten days being period I of this experiment. The crude protein content of the ration fed to one twin in each set was then progressively lowered, first to approximately 8 per cent (period II), then to approximately 4 per cent (period III). During these periods all the animals received water *ad lib.* The twins receiving the 4 per cent crude protein ration were then deprived of water but not of feed, only being allowed to drink for a very restricted period (20 min.) at 96-hr. intervals (periods IV and V). Period IV was essentially a change-over period when water deprivation was first introduced, while by period V it was assumed that the animals were accustomed to deprivation. During the five 10-day experimental periods 24-hr. collections of urine were made and this was analysed for total nitrogen, ammonia nitrogen and urine nitrogen. Determinations of intake of total nitrogen and total fæcal nitrogen were made at the same time. The percentages of urea nitrogen in the total urine nitrogen of the four experimental animals for five 10-day experimental periods are given in Table 1, together with data on actual urea nitrogen excretion.

It will be seen that a progressive reduction in the crude protein content of the ration immediately effects the actual amount of urea nitrogen excreted, and that deprivation of water leads to a further reduction. The percentage of urea nitrogen to total urine nitrogen does not, however, radically alter until the animals are deprived of water (periods IV and V). Within twin-set differences are statistically far more significant in periods IV and V than in periods I, II and III.

A comparison of the results published by Read[1], Petri[3], Smith and Silvette[4] and Schmidt-Nielsen *et al.*[6] with those obtained to date in this laboratory suggests that the experimental results of all these authors fit into a consistent pattern. Read[1] and Schmidt-Nielsen *et al.*[6], who claim that the urea content of camel urine falls below the normal standards were experimenting with ruminants receiving a low crude protein content diet and deprived of water to a greater or less extent; while Petri[3] and Smith and Silvette[4], who claim that the urea content of camel and other ruminant's urine is normal, were utilizing animals receiving a ration with an adequate crude protein content and water *ad lib.* The results published by Meyer, Weir and Smith[5] and Schmidt-Nielsen and Osaki[7] are also in agreement with the observations made in this laboratory.

It would appear from our preliminary results that variation in the excretion of urea nitrogen of cattle could be a function of both the crude protein content of the diet and deprivation of water, whereas variation in the percentage of urea nitrogen in the total urine nitrogen may be more a function of deprivation of water than the crude protein content of the diet.

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A POSSIBLE MODE OF ACTION OF SOME ANTI-FUNGAL AND ANTI-BACTERIAL CHELATING AGENTS

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RECENTLY it has been reported[1] that the rate of incorporation of metal ions into protoporphyrin dimethyl ester solubilized in aqueous detergent solutions can be influenced both by the charged nature of the detergent and by the presence of chelating agent, 8-hydroxyquinoline (oxine). The maximum rate was attained in anionic detergent solutions in the presence of equimolar concentrations of the metal ion and oxine. Further work has now indicated that a number of other chelating agents also catalyse this reaction, the most active ones belonging to a group of biologically active chelating agents which are believed to have a common mode of action.

The reaction between the 1 : 1 metal chelate complex (MKe^+) and the porphyrin (PH_2) in an aqueous detergent solution can be described by the bimolecular reaction:

$$MKe^+ + PH_2 \rightleftharpoons MP + H_2Ke^+$$

Table 1 summarizes the second-order rate constants (k) for the incorporation of copper into mesoporphyrin dimethyl ester in 2·5 per cent aqueous sodium dodecyl sulphate solution (SDS) at pH 7·0 and 20° C. in the presence of equimolar concentrations of cupric ions and various chelating agents. The experimental conditions were chosen so that the initial cupric ion concentration was approximately ten-fold the porphyrin concentration. The results were corrected for the formation of the inactive 1 : 2 copper chelate species during the course of the reaction.

Table 1. SECOND-ORDER RATE CONSTANTS (k) AT 20° C. FOR FORMATION OF PORPHYRIN-COPPER COMPLEX AT pH 7·0 IN 2 5 PER CENT SDS

Initial concentrations present: copper sulphate 5×10^{-4} M, chelating agent 5×10^{-4} M and mesoporphyrin dimethyl ester 6×10^{-5} M

Chelating agent	$k \times 10^{-3}$ (l./mole-min.)
Sodium diethyldithiocarbamate	8,300
Potassium ethylxanthate	1,100
2-Hydroxypyridine-*N*-oxide	1,000
8-Hydroxyquinoline	750
Kojic acid	550
Salicylaldehyde	110
Control (no chelating agent)	20
Salicylic acid	10
Glycine	10
Terramycin	10
Nitrilotriacetic acid	10
o-Phenanthroline	5
2-2′Dipyridyl	5
Histidine	5
Ethylenediamine tetraacetic acid	2

It will be noted that only certain of the chelating agents shown in Table 1 are catalytically active (some indeed are inhibitory). Moreover, the most active compounds, including sodium diethyldithiocarbamate, 2-hydroxypyridine-*N*-oxide, 8-hydroxyquinoline and kojic acid, although structurally unrelated to each other, belong to a class of anti-fungal and anti-bacterial substances which are believed to have a common mode of action[2,6].

A high level of activity has been shown to occur only in the presence of certain metal ions. Copper is the most universally active in this respect being co-toxic with the chelators against a variety of fungi and bacteria.

Dosage response studies indicate that the 1 : 1 metal chelate species is the primary active form.

On the basis of the results reported here and the earlier biological observations[2,6] it seems reasonable to suggest that the mode of action of these compounds might be associated with the incorporation of copper into a porphyrin precursor of some essential hæm-type pigment. In support of this hypothesis we may note that:

(1) The correlation between biological activity and catalytic activity occurs in a group of substances which are structurally unrelated and have little in common apart from their ability to chelate metals, a property shared by a vast number of other substances biologically and catalytically inactive in the sense described here.

(2) The 1 : 1 metal chelate complex is the active species both in the biological and catalytic systems.

(3) The cupric ion is the ion most readily incorporated into a porphyrin nucleus from an aqueous solution[3,4].

(4) The wide spectrum and high level of biological activity observed would be consistent with an interference in some essential hæm synthesis.

This work is being followed up both at the biological and chemical level.

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OXYGENATION OF LEGHÆMOGLOBIN IN SOYBEAN ROOT-NODULES IN RELATION TO THE EXTERNAL OXYGEN TENSION

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LEGHÆMOGLOBIN, the characteristic red pigment of legume root nodules, seems to be involved in some component reaction of the fixation of atmospheric nitrogen which these organs accomplish[1]. The role of this hæmoprotein has variously been postulated to be an oxidation-reduction catalyst, an oxygen carrier, a binder of nitrogen and an electron carrier[2-5]. The state of this pigment in functioning nodules has not been easy to determine, and it has been thought to be oxygenated or partially oxygenated. Appleby[6] has prepared purified leghæmoglobin as separate components and has determined their oxygen equilibria. He found that the components were half oxygenated at partial pressures of oxygen from 0·04 to 0·06 mm. mercury. He also reported that young nodules frozen in liquid nitrogen contained a mixture of ferro-leghæmoglobin and oxyleghæmoglobin, but refers to the difficulty of spectroscopic examination of intact nodules.

In the work to be reported here, soybean root nodules of known age were obtained as previously described[7] and used immediately after being detached from the roots. Spectroscopic methods were based on those described by Hartree[8] using a Beck microspectroscope mounted on a Galileo model *VFC* microscope equipped with a built-in light source. A preliminary investigation was made of the optical properties of intact and sliced nodules. It was found that only a small proportion of light entering an intact nodule from any angle passed through the central tissue: most of it 'tracked' around the cortex. The spectrum of this emerging light was therefore characterized by very faint absorption bands of leghæmoglobin masked with what may be regarded as stray light. In contrast, when light entered a cut surface of the central tissue, the emerging light showed intense absorption bands of oxyleghæmoglobin which were rapidly replaced by the less-intense band of ferroleghæmoglobin when the cut surface was pressed against a microscope slide[6,9,10]. No amount of masking or focusing of the incident light improved the absorption spectra of light emerging from intact nodules.

A glass chamber was therefore constructed from pieces of microscope slide cemented together (Fig. 1*a*) and equipped with large hypodermic needles for entry and exit of gas. The chamber was closed at the top by another piece of microscope slide which was pressed down by the microscope objective. Nodule pieces were cut which were 0·25 mm. thicker than the depth of the chamber by means of the guide illustrated in Fig. 1*c*. The cut surface was quickly pressed down on to a drop of 0·5 per cent agar in *p*H 7·0 phosphate buffer on the lower surface of the gassing chamber; the top was placed in position and pressed gently closed by lowering the microscope objective. The cut surface of the nodule was thus sealed against the lower surface of the chamber and held in position with gentle compression, while the rest of the intact surface was exposed to the atmosphere in the chamber.

The microscope optics were adjusted as in Fig. 1*a* so that the incident light was focused on the cut surface of the central tissue and the emerging light was focused on the entrance slit of the spectroscope by adjustment of the extension tube of the microscope without the eyepiece. In this way stray light was eliminated.

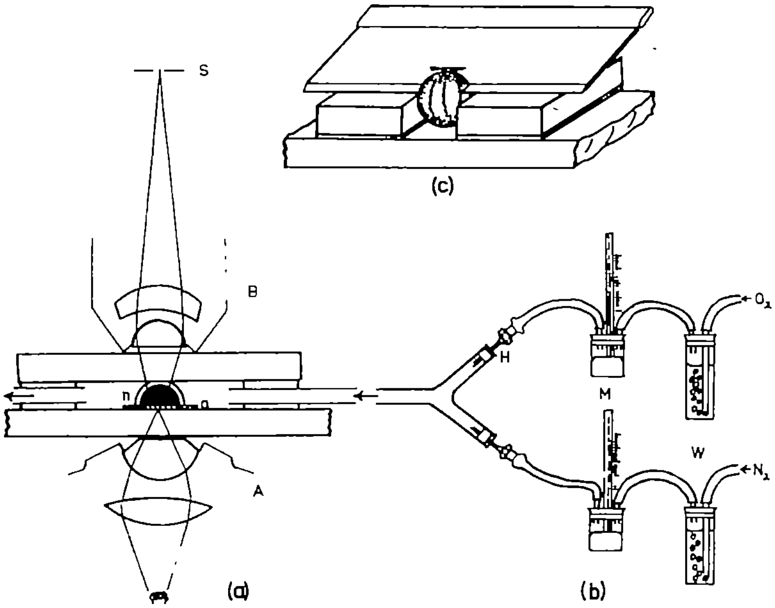

Fig. 1. (*a*), The optical system and gassing chamber. *A*, microscope condenser, *N.A.* 1·4; *B*, ×25 microscope objective; *S*, spectroscope entrance slit; *n*, nodule piece; *a*, 0·5 per cent agar drop. (*b*), The gassing line. *H*, hypodermic needles internal diam. 0·25 mm.; *M*, mercury manometers; *W*, warm water humidifiers. (*c*), The slicing guide constructed of microscope slide glass of the same thickness as that of the gassing chamber. Extra depth was obtained by the addition of pieces of 0 25 mm. thick microscope coverslip

Gas mixtures of various oxygen tensions were passed through the gassing chamber by means of the apparatus illustrated in Fig. 1*b*. The construction was such that a manometer reading of 300 mm. mercury gave a flow-rate of 500 c.c./min. By varying the relative pressures of oxygen and nitrogen with a combined pressure of 300 mm. mercury various partial pressures of oxygen were maintained in the chamber, measurement and calculation having shown that for both gases flow-rate was proportional to pressure. All experiments were done at 23° C. using good-quality commercial gases of about 98 per cent purity.

Initial experiments showed four readily recognizable types of absorption spectra. These are illustrated in Fig. 2 and were corroborated by independent observers. With actively nitrogen-fixing nodules aged 20–35 days in air the spectra showed a sharp band at 550 mμ, the bacteroid cytochrome *c* (ref. 11) and the broad rather diffuse band of ferro-leghæmoglobin extending from 550 to 570 mμ with the maximum absorption between 550 and 560 mμ. Except in cases where the seal between the cut surface and the glass was afterwards found to be faulty due to drying out of the tissue, no changes in this type of spectrum were observed until the partial pressure of oxygen in the chamber exceeded 50 per cent. Higher oxygen tensions than this caused progressively increased oxygenation of the pigment as shown in Fig. 2, reaching a maximum at 80–100 per cent when the prominent bands of oxyleghæmoglobin at 540–547 and 570–574 mμ (ref 12) were unmistakable. Older nodules showed oxygenation at lower oxygen tensions; for example, 39-day-old nodules began to show changes in the absorption spectra at 20–30 per cent oxygen. In no case was the 625 mμ band of ferrileghæmoglobin observed at any oxygen tension.

The progressive oxygenation of the leghæmoglobin of soybean nodules was next estimated at various oxygen tensions with the aid of a wedge trough in the light path of the comparison beam of the spectroscope.

In the first experiments fresh nodule extract was used as the source of hæmoglobin, but because of its instability, human hæmoglobin was substituted in later work. However, the same results were obtained irrespective of the source of the comparison pigment The procedure was as follows: 15 ml. phosphate buffer (*p*H 7·0, *M*/15) were placed in each compartment of the wedge trough. A nodule was prepared and placed in the gassing chamber and 100 per cent oxygen passed through until the hæmoglobin in the nodule was fully oxygenated. Oxyhæmoglobin was then added to one compartment of the wedge trough with a graduated pipette until the comparison spectrum matched the nodule spectrum with the trough in the position in which the comparison light passed through the maximum width of the oxyhæmoglobin compartment. The oxygen was turned off and nitrogen passed through the chamber and an equal volume of hæmoglobin solution was added to the buffer in the other side of the wedge trough together with enough sodium dithionite to maintain the pigment in the reduced state for the duration of the measurements. The matching of the oxy-forms of the pigment in nodule and wedge trough was more reliable than matching of the reduced forms. However, when spectra were matched as oxy-forms they also matched well when reduced. Gas mixtures of various oxygen tensions were then passed through the gassing chamber for 1·5 min. to allow equilibration, after which the nodule and comparison spectra were matched by sliding the wedge trough across the path of the comparison beam.

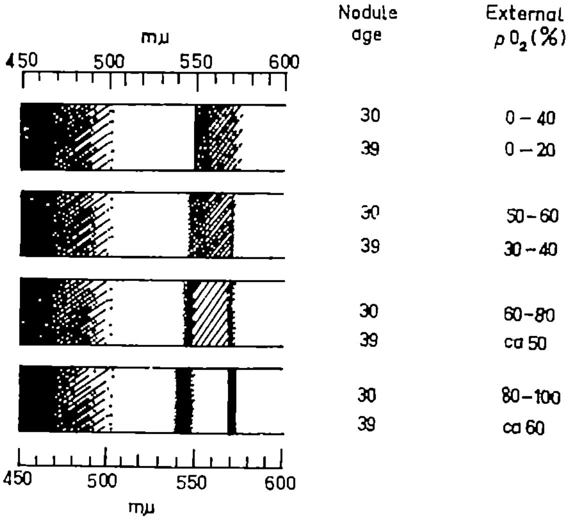

Fig. 2. Diagrammatic representation of the four types of absorption spectra identified by independent observers at various oxygen tensions (pO_2)

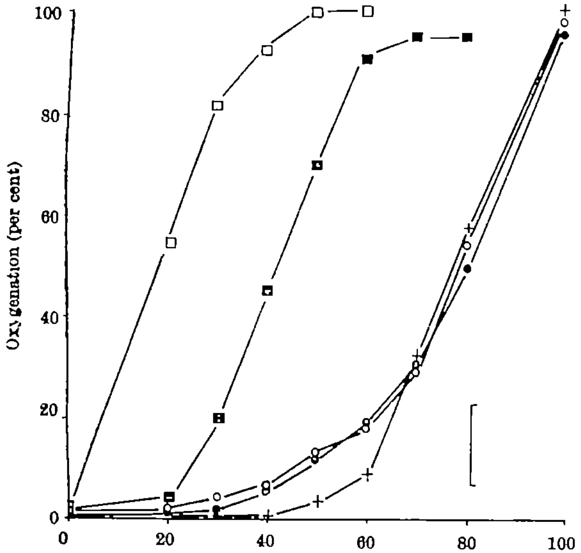

Fig. 3. The progressive oxygenation of nodule leghæmoglobin with increasing oxygen tension (pO_2) for nodules of various ages. The maximum standard deviation obtained for any of the points is shown at lower right. Nodule age (days): +, 28; ●, 34; ○, 35; □, 35 (green centre); ■, 39

That this brief equilibration time was sufficient is shown by the following results for a typical nodule: After slicing, the fresh nodule piece in the chamber in nitrogen was fully reduced in 15·5 sec.; on changing the gas to oxygen the nodule was fully oxygenated in 18·0 sec.; after measurements lasting 15 min. the fully oxygenated leghæmoglobin was reduced after 43 sec. in nitrogen and on changing to oxygen the oxyleghæmoglobin bands appeared after 10 sec. and were fully developed in 44 sec. The wedge trough positions were converted to percentage oxygenation of the pigment from the ratios of the lengths of the comparison light path through oxy- and ferro-hæmoglobin solutions.

The results of experiments with nodules of various ages are shown in Fig. 3, in which each point is the mean of determinations with 3 or 4 nodules: an exception is the curve for the 35-day-old green centred nodule, only one of which was found. The magnitude of the maximum standard deviation of the data for any point is also shown. The reliability of the methods used is shown by the close agreement of the data for 34- and 35-day-old nodules obtained on successive days. Nodules which were actively fixing nitrogen showed little if any oxygenation of hæmoglobin until the oxygen tension in the chamber exceeded 40–50 per cent. At higher oxygen tensions oxygenation increased rapidly to the maximum values. The low percentage oxyleghæmoglobin values obtained at low oxygen tensions are not very reliable because of the difficulty in detecting by eye the small changes in absorption spectra which occur. It is not possible to say that the pigment was entirely reduced at 20 per cent oxygen, but it was certainly less than 5 per cent oxygenated and because of the shape of the curves was probably less than 1 per cent oxygenated. The results also show that as the nodules aged or degenerated (the green centred nodule) so did the oxygenation of the pigment commence at lower oxygen tensions.

The nitrogen-fixing central tissue of soybean root nodules consists of large host cells, each of which contains large numbers of bacteroids (the nodular form of the nodule bacteria) which are enclosed in groups of 4–6, together with the hæmoglobin, in double-layered membrane envelopes[5]. The results presented support the conclusions drawn from respiratory and nitrogen fixation studies[13] that there is an oxygen barrier in the nodules which does not permit the passage of oxygen into the envelopes until a certain oxygen tension has been exceeded. The curves of Fig. 3 also bear a striking resemblance to those obtained when the Michaelis constant for nitrogen fixation is plotted against external oxygen tension[13]. The oxygenation curves for leghæmoglobin in pure solution obtained by Appleby[6] indicate that the oxygen tension within intact nodules in air is much lower than that derived from other work[14] and probably lies below 0·005 mm. mercury or less than 0·0007 per cent. It should also be emphasized that these results could only be obtained with nodules with homogeneously aged central tissue cells such as occur in soybean nodules; nodules with growing points such as those of clover, peas, etc., present a confused spectrum due to the gradation in age of the central tissue cells.

I thank Dr. C. A. Appleby for help in observation and discussion and other colleagues of the Division of Plant Industry for independent observation of spectra.

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INFLUENCE OF MACROCLIMATIC CONDITIONS ON THE DEVELOPMENT OF BLAST AND HELMINTHOSPORIOSE OF RICE

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IN spite of limitations of meteorological records to explain the intimate association of host and parasite[1], such observations have been found to be valuable in forecasting disease and in epidemiological research[2]. While microclimate (environment close to the plant) controls the process of initial establishment of a pathogen[3], macroclimate helps in disease correlation and spread.

Forecasting of disease in rice, particularly with reference to blast (*Piricularia oryzae* Cav.), has been

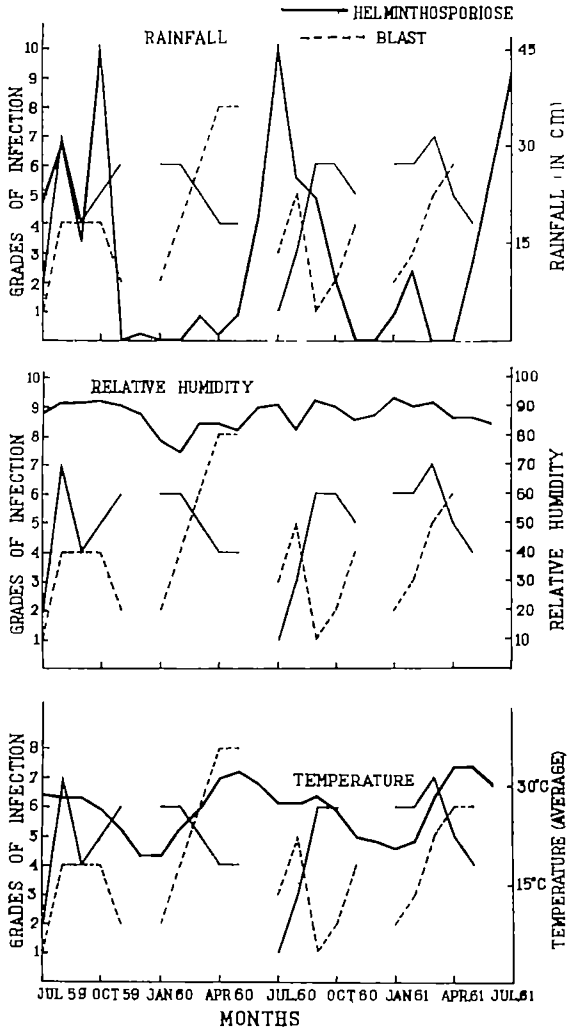

Fig. 1. Graphs showing relationships of the incidence of blast and helminthosporiose to meteorological conditions of rainfall, relative humidity and temperature

investigated to a certain extent in Argentina[4] and Japan[5,6]. Hashioka[7] stated that the prevalence to blast in temperate areas is dependent on the temperature suitable for growth of the pathogen, in sub-temperate areas on the age of the plant (which affects their resistance) and in the tropics on the relation of temperature to the resistance of the host. Published records show that little work has been done in India in this connexion[8].

During a survey of incidence of blast (*P. oryzae* Cav.) and helminthosporiose (*Helminthosporium oryzae* Breda de Haan) of rice due to natural infection in Cuttack and Puri Districts of Orissa State, I made an attempt to correlate them with the meteorological conditions of rainfall, relative humidity and temperature. Incidence of diseases was studied statistically by the method of multi-stage stratified random sampling technique (formulated by the Indian Council for Agricultural Research). Ten grades of infections of both blast and helminthosporiose were made, depending on the number of undeveloped and developed spots of infections (including neck and node blast), which were followed as standard infection score-charts. In an area of more than 8,249 square miles altogether about 20 improved and more than 100 unimproved local varieties of rice plants were cultivated. Soil conditions varied from alluvial to laterite, sandy to loamy and clayey; *p*H ranging between 5·2 and 6·8.

Helminthosporiose. The development of helminthosporiose on rice plants showed a direct relationship with the rainfall during the four rice-growing seasons examined. The relationship seemed to be more with the amount of moisture present in the soil than that on the plant surface or atmospheric humidity, since the incidence of disease showed an increase only in the months following rainfall and not during the months of actual rainfall. It might be mentioned here that the two peaks in the rainfall curve (Fig. 1) caused widespread floods over an extensive area, submerging the standing crop for a short time. But even under this submerged condition (October 1959 and July 1960) incidence of disease did not rise rapidly. It showed a slow and gradual increase, reaching its severest form during the months of November 1959 and September 1960, when there was practically little or no rainfall. Thus, it appeared that the expression of helminthosporiose on rice plants seemed to bear a correlation to soil moisture and plant uptake. The same was true for the cultivation season of January–June, when soil dried up and moisture percentage decreased. There was relatively a steady fall in the incidence of helminthosporiose; but a sudden increase in rainfall in February 1961 caused an immediate increase in disease incidence in March 1961.

Since the average temperature of minimum and maximum never fell below 20° C. or went above 34° C., limiting conditions of temperature in the development of helminthosporiose could not be determined. Between 20° and 34° C. atmospheric temperature did not seem to have any relationship with the incidence of the disease.

Blast. Of the three principal meteorological conditions studied, temperature appeared to show the closest parallelism to the development of blast on paddy in all the seasons. Between the average temperatures of 20°–34° C. the relationship was more or less directly proportional. The optimum temperature for highest incidence of blast was recorded within 28°–30° C. Above 30° C. the development of the disease was checked, and below 24° C. it showed a marked fall. Thus, during August–October 1959 incidence of blast remained constant, when the average temperature ranged between 26°–28° C.; thereafter a fall in the temperature was followed immediately by a fall in the prevalence of the infection. Again, with the rise of temperature from January 1960 onwards, there was direct increase of blast incidence. The season January–June 1961 was remarkably consistent with the above observation.

But the preceding season (July–December 1960) posed a problem in this respect in that, when temperature was most suitable (24°–28° C.) for development of blast, there was a sharp fall followed again by an increase in the incidence of the disease. It may be noticed that, during the period, the relative humidity was as high as 80–90 per cent, which was optimum for the development of blast[9]. The explanation for this retardation might lie in the excessive rainfall during the period. Earlier literature[10] supported that susceptibility to blast varied in inverse ratio to the amount of water contained in the soil. Similar observations were made by me during the survey of rice fields subjected to floods. In one particular field, 45-day-old seedlings carried blast infection symptoms up to Gr. 5 before the area was flooded for 3–4 days. After the flood water receded, there was practically no sign of the disease in that area during that season. This observation might offer a possible explanation of the above problem arising out of a sudden retardation in blast incidence during August–September 1960 and may in itself carry a latent solution in the control of blast.

In general, for the development of blast, temperature seemed to be more important than humidity[11]. During the present survey study humidity was always very high (above 80 per cent); so a condition of relative humidity becoming limiting never arose.

I thank Dr. R. H. Richharia, director of this Institute, for assistance.

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EFFECT OF GIBBERELLIN ON A NON-FLOWERING GENOTYPE OF RED CLOVER

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DURING the multiplication of pedigree stocks of Aberystwyth *S*.123 extra late-flowering red clover (*Trifolium pratense* L.) it has been noted that a low frequency of non-flowering segregates appears. These plants tiller vigorously, but do not form stem internodes or initiate inflorescences even after prolonged periods under continuous light.

Previous work on the responses of red clover to gibberellic acid[1,2] suggested that failure to produce stems might be due to inadequate synthesis of endogenous gibberellin, and to test this hypothesis a series of estimations of 'gibberellin-like' activity were made on extracts of normal and non-flowering genotypes.

Extraction of 500 gm. fresh weight samples with 95 per cent ethanol was carried out at 0° C. over a period of 12 hr., and, after decanting, the alcohol was removed in a rotary evaporator, leaving an amber-coloured aqueous residue. After further purification procedures the extracts were line-loaded on to strips of Whatman 3*MM* paper and chromatographed in 80 per cent isopropanol. The developed strips were each cut into segments and assayed for 'gibberellin-like' activity using a red clover seedling test similar in general method and sensitivity to the lettuce hypocotyl assay of Frankland and Wareing[3].

In Fig. 1 typical activity histograms derived from early- and late-flowering red clover varieties at the third extended internode stage are compared with results from non-flowering material grown for two months under continuous light. While the general pattern of activity is similar in all three cases, large quantitative differences clearly exist. The pronounced 'gibberellin-like' activity present in the extracts of the early-flowering variety at R_F 0·7–0·8 can be correlated with the fact that floral initiation occurs when 2–3 extended internodes are externally visible, this process being delayed until about the sixth internode stage in extra late-flowering types[4]. Similarly, a parallel can be drawn between the R_F 0·4 activity and the rate of stem extension, which is more rapid in early- than in late-flowering varieties, and is non-existent in non-flowering types. The corre-

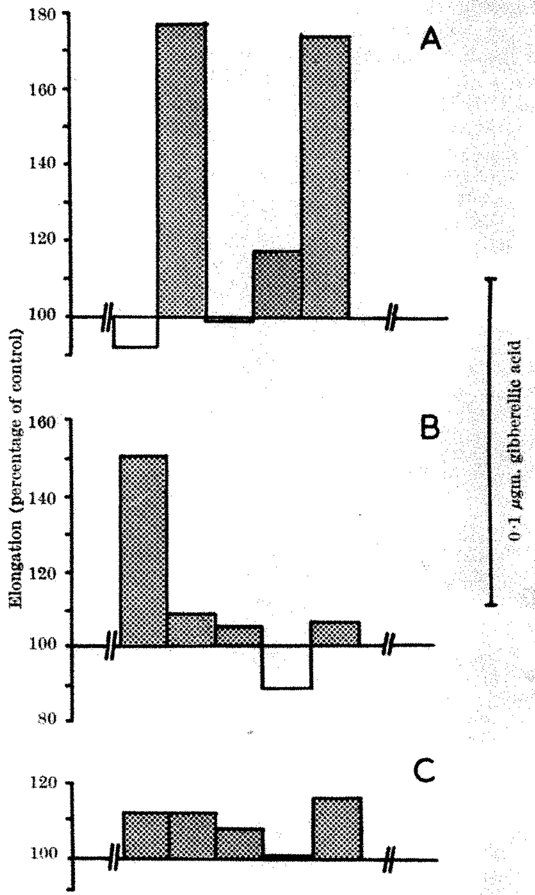

Fig. 1. Activity histograms of red clover extracts after chromatography in 80 per cent isopropanol. Red clover seedling bioassay. *A*, Dorset Marl, early-flowering; *B*, Aberystwyth *S*.123, extra late-flowering; *C*, non-flowering genotype

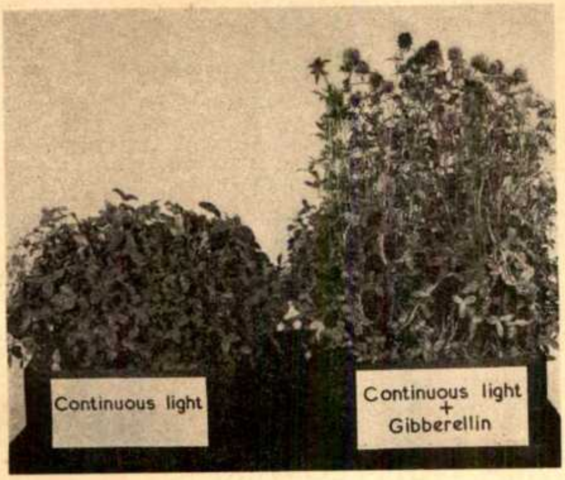

Fig. 2. Non-flowering genotype with and without gibberellin treatment after two months growth under continuous light

spondence in chromatographic mobility between the R_F 0·4 activity and authentic gibberellin A_3 suggests they may be identical or interchangeable, and, therefore, that treatment of non-flowering types with gibberellic acid might be expected to bring about internode extension.

Clonal material of several non-flowering genotypes was placed under inductive day-length conditions and half the propagants were given a foliar spraying of 1 mgm. of gibberellic acid in 0·1 per cent ethanol weekly for five weeks, making a total dosage of 5 mgm./plant. The remainder were sprayed with 0·1 per cent ethanol only on each treatment date. As may be seen in Fig. 2, the gibberellin promoted considerable internode extension and also profuse flowering, a response which is typical of all the genotypes tested. The induction period under continuous light for the treated material was of the order of 28 days, compared with the 12 days required by normal, related plants of *S*.123 red clover. In terms of yield and quality of seed the gibberellin-induced plants compared favourably with the parent variety.

Repeated treatment with gibberellic acid will not bring about floral initiation in normal plants of *S*.123 under non-inductive day-length conditions, but considerable internode extension is promoted, a response which can also result from exposure to low-intensity directional light under high-temperature (70° F.) conditions. This suggests that a two-factor system is operative, the first part, regulating stem, petiole and leaf expansion, consisting principally of a temperature-controlled process producing endogenous gibberellin similar to, or identical with, gibberellin A_3. Under inductive day-length conditions the secondary process is initiated whereby this gibberellin, or subsequent products, are modified in form substances capable of altering the course of differentiation at the shoot apex towards the reproductive state. It may be postulated that the R_F 0·7–0·8 'gibberellin' already described is directly concerned with apical differentiation and that the day-length controlled reaction consists of a chemical transformation from one form of gibberellin to another with a different activity pattern.

On this basis it is possible to interpret the non-flowering condition as a reduced ability to synthesize endogenous gibberellin A_3, a deficiency which would limit response to inductive treatments. Failure of normal plants to flower under unfavourable day-length conditions in the presence of gibberellic acid would then be due to the absence of the secondary day-length mediated system.

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LIFE-FORMS IN CARBONACEOUS CHONDRITES

IN the figures published by Claus and Nagy[1] there are several organized elements. I do not recognize that called type 5 by those authors, but would like to direct attention to certain similarities between two different photographs entitled type 2 organisms and certain anemophilous pollen grains.

In Fig. 2 of their original article Claus and Nagy portray an object which has, clearly visible, pores. Although the plane in which these pores lie is not accurately discernible, they resemble the pore arrangement in pollen grains of, for example, *Corylus*. Pollen of this genus is approximately 22–31μ in diameter, psilate, aspidate, and usually having three pores on the equator. In polar view the grains are more or less triangular, in equatorial view sub-oblate[2]. It should be noted that several other genera occur in temperate latitudes alone which have similar pollen grains, for example, *Betula* and *Myrica*.

With regard to the other photograph of a type 2 organism, Claus and Nagy do not say whether this is the same structure as that shown in Fig. 2. However, similar considerations to those outlined suggest that this is not the case. The rather ill-defined object in the photograph resembles the oblate-spheroidal to sub-oblate form of the pollen of *Alnus*, a suggestion which is supported by the presence of what might be interpreted as pores at the three visible corners of the object. In fact, the size indicated on the photograph suggests that the specimen, although illustrated obliquely, is rather too small for the European species of *Alnus*[2].

Claus *et al.* further mention other structures which, although similar, had external appendages. I am not clear from their texts[1,3] how many they had of these, but note that they state that these varied in size from 8μ to 30μ in diameter. Although the small objects represented in the photographs of their recent publication[3] do not fit any angiosperm pollen grain or fungal spore met by me in my limited experience, I would like to point out that there are many pollen grains of approximately 30μ in diameter which have spines or appendages of some sort, for example, the Campanulaceae, which are echinate, and *Crepis paludosa*, which is ridged.

Pollen grains occur in vast numbers in the atmosphere[2,4,5] and accumulate in quantities in organic deposits. Claus and Nagy suggest that since these organic structures and others were present the meteorite could not have undergone a temperature in excess of 200° C. May I suggest that before accepting this conclusion we should consider:

(1) Were the subsequent investigations which are reported in ref. 3 carried out at the same time of the year as the earlier ones? Pollen of *Corylus* and *Alnus* is only produced for a limited period and that from a nearby tree can attain high values as a biological contaminant of laboratories during this period.

(2) At what temperature would the meteorite have solidified had it, in fact, exceeded the suggested maximal temperature of 200° C. and been molten during entry of the Earth's atmosphere? Pollen grains are very resistant to oxidation and indeed the normal palynological procedures involve oxidizing off the other plant material to leave only pollen grains, fungal spores and such structures as the thecæ of the Desmothoracan *Clathrulina*.

It would be preferable to disprove the simplest explanation that these structures are of terrestrial origin before moving to more elaborate hypotheses. It is, incidentally, of considerable interest that what might be explained as a pollen tube can be seen leading away from one organism (Fig. 2 of ref. 1).

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Identity of Organized Elements from Meteorites

THE publication[1] of further illustrations of organized elements from the Orgueil and Ivuna meteorites raises the question of whether the possibility of their terrestrial origin has been adequately explored. The illustrations, although prepared under extremely difficult conditions, prompt the following tentative suggestions towards identification. The elements illustrated by Nagy, Claus and Hennessy in their Fig. 1*a* and *b* could well be the spore of a cryptogam, possibly a basidiospore. The object depicted in Fig. 5 (of Nagy *et al.*) in size, shape, rough wall and equatorial crest strongly resembles an ascospore of one of the larger members of the *Aspergillus glaucus* group. The object in Fig. 4*b* (Nagy *et al.*) resembles in size, shape, possible septation and surface characters a spore that occurs frequently in the air spora of southern England, though we have not yet been able to identify it or trace it to its source. It is presumably a spore of one of the Fungi Imperfecti, and a specimen from air has been illustrated recently[2]. The element illustrated in Fig. 3 (Nagy *et al.*) has some resemblance to a pollen grain of nettle (*Urtica*) except that it is only about two-thirds the usual diameter.

To refer to illustrations in the earlier article[3], it seems that Figs. 2 and 3 could well be a fungus spore. The remarkable object depicted in Fig. 4, except for the halo, which may be an extraneous deposit, has some resemblance in size and three thickened areas to a germinating pollen grain.

Any one person can be familiar with only a fraction of the morphological diversity of plant spores, and a large series of drawings and photographs of these organized objects from meteorites should be published so that as wide a range of specialists as possible can attempt identifications.

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Further Life-forms in the Orgueil Meteorite

I OBTAINED some test material derived from the Orgueil meteorite—at my request—from G. Claus and B. Nagy. I crushed the meteorite sample in a sterile porcelain mortar to powder and, putting it under sterile circumstances partly into glycerol, partly into 'Caedax', I examined it under a microscope. During my examinations I found in the meteorite powder six different filamentous formations which, relying on their qualities known so far, remind one of the algae.

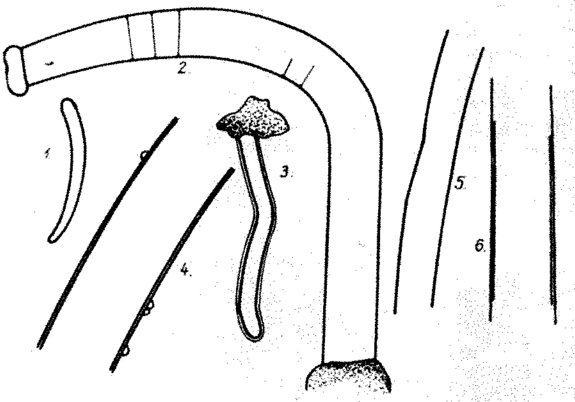

Fig. 1

Characteristics of the filaments are as follow: breadth, 3μ; length, 40μ; somewhat curved. One end of the filament is rounded off, the other slightly acuminate (Fig. 1, 1). Part of the filament is 4μ wide and 55μ long. It is covered with a follicle, the apex is rounded off (Fig. 1, 2). Part of the filament is 12μ wide and 170μ long. The filament is slightly tapering towards the apex. The apical cell is like a head (Fig. 1, 3). Part of a 23-μ wide filament is shown in Fig. 1, 4. On the double wall here and there small warts can be seen. Part of a 10-μ wide filament is illustrated in Fig. 1, 5. In part of a 16-μ wide filament, here and there the double cell wall can be easily seen (Fig. 1, 6).

Part of the filaments described here may possibly be indigenous to the meteorite.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Polarization of the Light from the Magellanic Clouds

The importance of studies of the polarization of starlight in galactic research has been recognized for some time, and interpretation of the results in terms of paramagnetic interstellar grains preferentially orientated in the galactic plane by a general galactic magnetic field appears well supported. The possibility of observing polarization of light from other galaxies has been investigated[1–3]. Two extragalactic systems which seem to have escaped attention in this respect are the Magellanic Clouds.

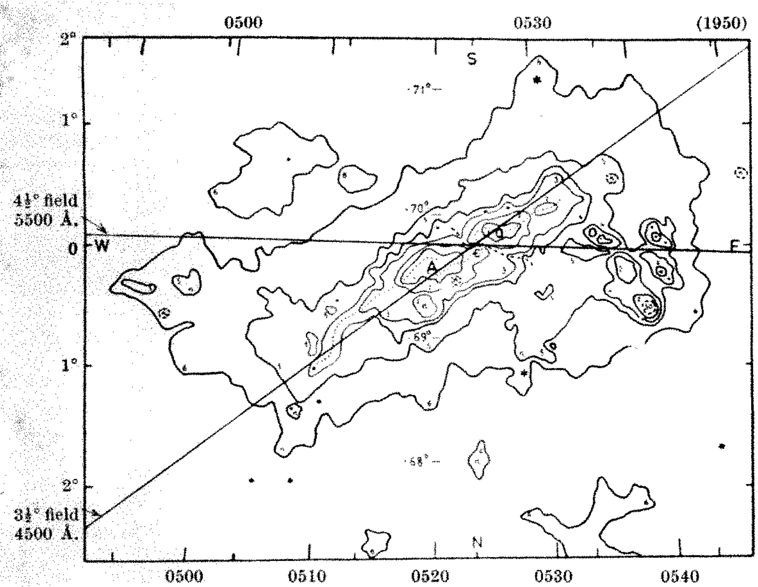

Fig. 1. Plane of polarization of the Large Magellanic Cloud for two field sizes drawn on the isophote map in blue light as determined by de Vaucouleurs (*Astron. J.*, **62**, 71; 1957)

In June–July 1961, during an expedition to a high-altitude observing site at Chacaltaya in the Bolivian Andes (16° 19′ S., 68° 10′ W.; 17,100 ft.) with the principal aim of making a detailed photometric study of the Zodiacal Light, the polarizations of the Large and Small Magellanic Clouds (LMC and SMC) were measured. Because of the very transparent skies experienced at Chacaltaya the extinction corrections in the photometry of extended objects such as the Zodiacal Light or the Magellanic Clouds could be reliably made. The instrument used was a photoelectric polarimeter with an electronically operated variable retardation plate as analyser and a piece of polaroid as detector. The instrumental and observational details will be described fully in a later publication. The observational results are summarized in Table 1.

Table 1

	Field (deg.)	Position angle (deg.)	Degree of polarization (per cent)	Effective wave-length (Å.)	No. of observations
SMC	4½	177	24 ± 5	5500	16
LMC	3½	126	25 ± 5	4500	46
LMC	4½	88	33 ± 7	5500	13

The position angle gives the orientation of the plane of polarization, here defined as the plane of vibration of the electric vector, measured east of north. The errors in the degree of polarization are due mostly to the variation in the intensity of the background light of the sky from night to night; this was because the night sky photometer records, from which the background correction was determined, were obtained on different nights from those on which the polarization measurements were made. This night-to-night variation amounted to ± 15 per cent. The difference of 38° between the position angle as determined in blue light for the LMC with a 3½° field and the position angle in yellow light with a 4½° field is not entirely understood but may possibly be due to the following cause. The telescope was set using the circles and then adjusted by the slow motion to the Cloud centre using the rather sharp maximum on the meter recording intensity. This means that the field centre may have been different in the two cases because the yellow and blue isophotes will not coincide for the same field size; also the 4½° field may introduce additional structural features which may further separate the centres of the two fields and also directly influence the orientation of the polarization plane. Fig. 1 shows an isophote map of the LMC for blue light with the planes of polarization for the two field sizes drawn in. Each observation consists of one determination of the degree of polarization at a given position angle. While there seems to be no obvious correspondence between any optical feature and the plane of polarization in the case of the Small Cloud, for the Large Cloud the polarization plane for the 3½° field lies very close to the bright bar, shown as a dotted line in Fig. 1. The values found for polarization of the light from other galaxies[1–3] lie between 4 and 12 per cent for 11 galaxies of either spiral or elliptical type; two other galaxies, *NGC* 3077 and *NGC* 4449, both of irregular type, were found by Vashakidze[3] to have polarizations equal to 15 and 21 per cent, respectively. The latter is a Magellanic type irregular galaxy. Any interpretation of these results in terms of structural features, differing ratios of gas to dust and of magnetic fields is clearly premature; but it is likely that a detailed polarimetric study of bright nearby systems, particularly the Magellanic Clouds, will enable such an interpretation to be made.

I thank Prof. D. E. Blackwell, who planned the expedition to Bolivia and so made this work possible, the Royal Society, who financed the expedition, Mr. D. W. Beggs, who designed the electronic equipment, and the Department of Scientific and Industrial Research for a maintenance grant.

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PHYSICS

Burst-Type Signal from Small-Field Aurora

Photoelectric observations of luminosity fluctuations in small-field aurora have already been reported[1]. This communication illustrates some cases of burst-type signal recorded in two channels at Saskatoon (52·1° N., 106·6° W.). As briefly outlined in the earlier report, signals from two fields each 0·55° in size and separated from each other by 3° were recorded using a two-channel Sanborn recorder.

Fig. 1 *a*, *b* and *c* shows the signals from different auroral forms. The signal-burst has a pulse-like structure. These pulses may be sharp as well as broad. There could be one or several pulses in a burst. The pulse-length has a range of 0·5–5 sec. and the pulse recurrence time varies from 0·5 to 10 sec. The duration of the signal is 60–80 sec. The auroral form as visually observed in each of the three cases was of intensity II–III according to the international brightness coefficient scale or corresponding to 10–100 kilo rayleighs.

Fig. 2 *a* and *b* shows the signal from an unusual pulsating form. The intensity of this form as observed visually was only I–II or 1–10 kilo rayleighs. The signal looks like a combination of several bursts having pulse-like structure with relatively longer smooth oscillations. These low-amplitude short pulses last 0·1–0·3 sec. with recurrence time in the range 0·1–0·5 sec. The relatively longer oscillation periods vary between 4 and 5 sec. and less sometimes. This type is a less frequently recorded signal. When recorded it is seen to last for several minutes.

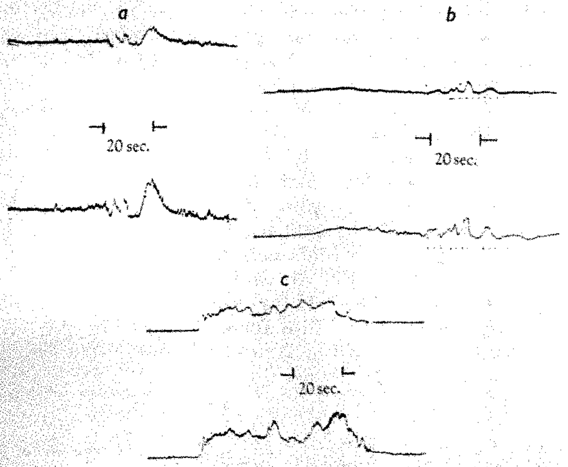

Fig. 1. *a*, Signal from a flaming aurora; *b*, signal from a corona; *c*, signal from a rayed band

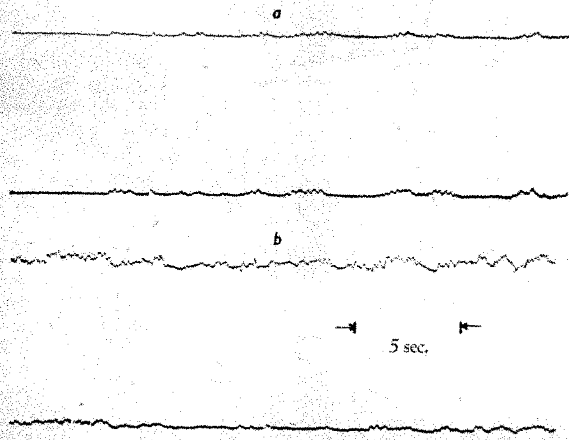

Fig. 2. *a* and *b*, Signal from a pulsating aurora

The similarity in the signal-pattern in the two channels is striking. Quiescent forms of aurora are not seen to give this type of signal in the two channels. In the cases of active forms illustrated in Fig. 1, possibly but not certainly, the effect of simultaneous intensity coruscations and motion might be involved. Perhaps, the excitation processes have also something to do particularly in the type of signal as in Fig. 2.

I thank Dean B. W. Currie and Dr. G. G. Shepherd for their help in this work.

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Heating in Capillary Flow

The temperature profile in an essentially Newtonian liquid in laminar flow in a capillary has been measured experimentally. Previously the phenomenon of capillary heating (that is, heat produced by the internal friction of liquids in flow in a capillary) had been the subject of extensive theoretical treatment[1,2]; but with little experimental verification. These experiments show that the system is essentially adiabatic, in contradiction to widespread belief that the 'isothermal wall' condition existed.

Measurements were made on a mineral oil thickened with 3 per cent polyisobutylene, with a viscosity of 1 poise. The degree of non-Newtonian behaviour at the shear-rates used is insignificant. Stainless steel, conically tipped capillaries were used and the thermocouple was made of very fine (0·003 in.) iron and constantan wire. The thermocouple was mounted on a microscope feed and could be moved across the diameter of the stream in increments of 0·0002 in. The temperature measured was, of course, some integrated temperature over an increment of the

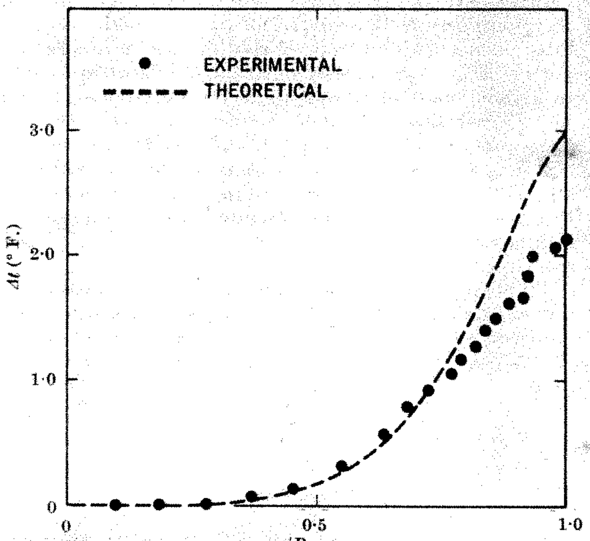

Fig. 1

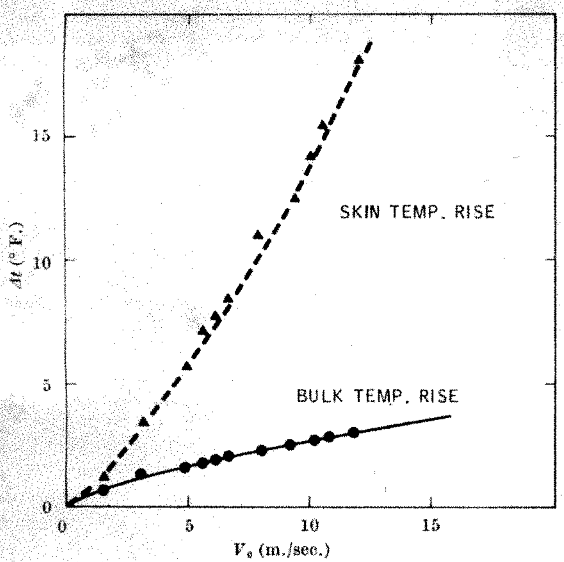

Fig. 2

stream equal in width to the thermocouple's diameter. The diameter of the stream was approximately 1 mm., and the measurements in the stream were made within 0·004 in. of the outlet of the capillary. Temperature measurements were also made of the conical tip of the capillary itself in order to verify the measured 'skin' temperature. The two temperatures were approximately equal over the entire range of experiments.

A typical profile is shown as measured in Fig. 1. Contrary to popular belief, the point of maximum temperature rise is seen to be at the wall rather than in the centre of the stream, proving the existence of the adiabatic condition. The dotted line is the prediction, under the same experimental conditions, from Brinkman's[1] equations for the adiabatic case. As would be expected, since the system is truly in steady state and not quite adiabatic, the heat transferred manifests itself at the wall in a measured temperature lower than that which would exist for the completely adiabatic case. Notwithstanding the simplifying assumptions of Brinkman's calculation and the experimental deviation from true adiabatic conditions, the comparison is seen to be quite good. Fig. 2 illustrates the variation of rise in skin temperature with velocity. A velocity of 12 m./sec. (corresponding to a shear rate of about 10^5 sec.$^{-1}$) gives rise to an 18° F. increase in temperature at the wall. On the same plot can be seen the stream's bulk temperature rise. At 10 m./sec. the increase in skin temperature is about 7 times the increase in bulk temperature. Preliminary results also show the temperature profile to depend on capillary length as predicted from theory.

These observations are not only of significance in high shear capillary viscometry, but also are, on the basis of initial tests, critical in the evaluation of viscous and elastic components in free-flying jet work and in polymer extrusion investigations.

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Electrical Resistance of Oxide Films formed on the Roll of a Corona Discharge Roll-Type Separator

During the course of an investigation into the underlying principles of a corona-discharge roll-type separator (Fig. 1), very inconsistent results were obtained, which were eventually traced to the erratic formation of oxide film on the brass roll.

In order to examine these oxide films more conveniently, a polished brass plate, about 6 in. × 12 in., was set in an upright position in front of, and touching, the roll so that the corona discharge was beamed on to the plate from a distance equivalent to that of the nearest point of the drum (3¾ cm.). The corona discharge was then passed for 3 hr. under normal conditions (30 kV. at 200 μamp). A clearly visible oxide film was formed, which had striations at right angles to the corona wire due to non-uniform emission from the wire (Fig. 2). The experiment was repeated using plates made of stainless steel, chromium-plated brass, and duralumin.

Some trouble was experienced in making contact with the oxide film so as to measure the electrical resistance through the thickness of the film by the conventional d.c. Wheatstone bridge method. 'Free' electrodes using mercury droplets or small gold-plated spheres on a loose copper 'spring' were tried; results from the former were very erratic, while it proved impossible to put sufficient pressure on the latter without breaking through the delicate film of oxide. Various types of conducting paint were then tried; it was eventually found that slightly diluted colloidal graphite applied with a hypodermic syringe (without a needle) gave a 2–3-mm. diameter electrode which was stable and strong, had no appreciable intrinsic contact resistance and did not tear the oxide film on drying. Contact was made by allowing a soft pencil lead to rest on the graphite dot.

Apart from its physical hardness, three properties of the oxide film which can be inferred from the resistivity curves (Fig. 3) and which determine the suitability of a metal for roll construction are: (*a*) The sensitivity of film formation to ion current. The curves shown in Fig. 3 are smoothed plots drawn

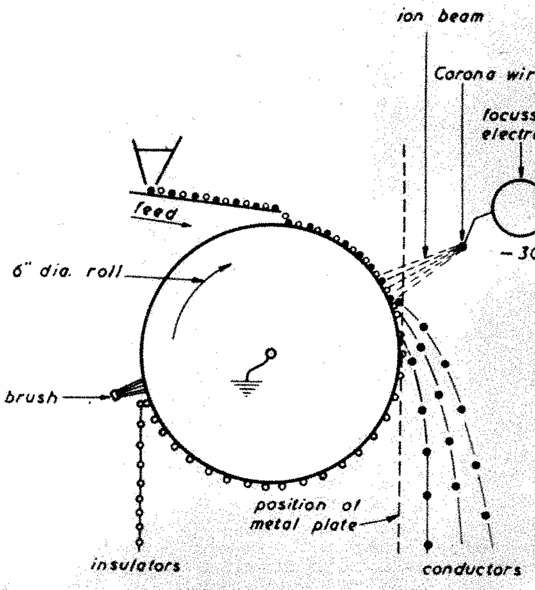

Fig. 1. Corona discharge roll-type separator

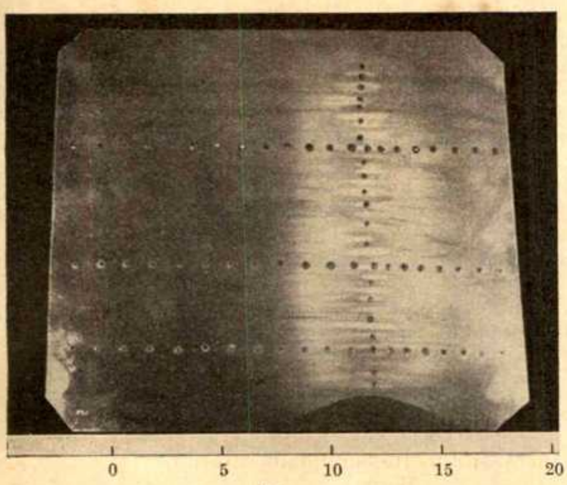

Fig. 2. Oxide film on brass plate

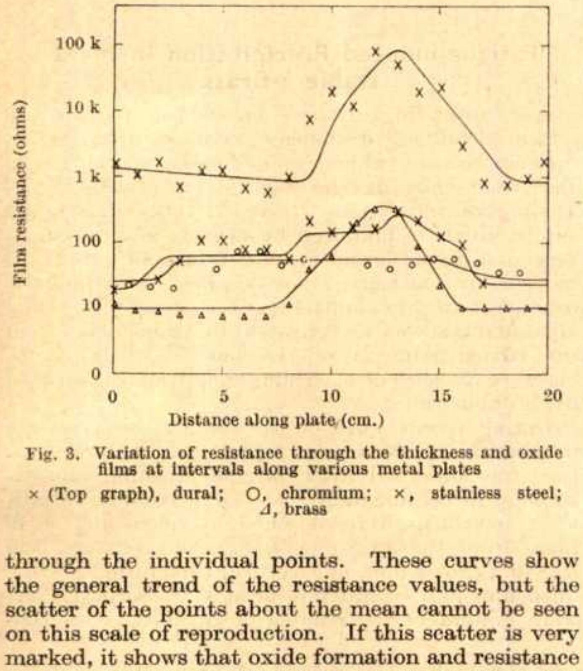

Fig. 3. Variation of resistance through the thickness and oxide films at intervals along various metal plates

× (Top graph), dural; O, chromium; ×, stainless steel; Δ, brass

through the individual points. These curves show the general trend of the resistance values, but the scatter of the points about the mean cannot be seen on this scale of reproduction. If this scatter is very marked, it shows that oxide formation and resistance are very sensitive to the local intensity of the corona discharge and this leads to erratic results in mineral separation. Similarly, the sharper the main peak of the curve, the more sensitive is the oxide film to ion current. (*b*) The absolute value of the resistivity of the oxide film exclusive of the main peak. This should preferably be low. (*c*) The overall range of resistivity values. The lower this range, the more suitable is the metal for roll construction.

Table 1. OXIDE FILM PROPERTIES

Metal	Mechanical* hardness	Sensitivity to corona	Range of resistance (π)
Brass	Soft (unsuitable)	Very sensitive (unsuitable)	10–300 (fair)
Chromium	Hard (suitable)	Insensitive (suitable)	20–60 (suitable)
Duralumin	Hard (suitable)	Not very sensitive (suitable)	500–80 k (unsuitable)
Stainless steel	Hard (suitable)	Not very sensitive (suitable)	20–300 (fair)

* This is apparent abrasive resistance.

Table 1 shows the results of the experiment for the four metals used, and the degree of suitability of each metal for use as roll material.

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METALLURGY

Fatigue Failure in Copper Single Crystals at Low and High Amplitudes of Torsion

IN the discussions of fatigue failure under cyclic stresses a distinction should be made, as has been pointed out by Wood[1], between cycling at low-strain amplitudes and cycling at high-strain amplitudes. By low-strain amplitudes are meant amplitudes in the flat part of the *S–N* curve where the specimens are relatively long lived. High-strain amplitudes are those on the steep part of the *S–N* curve corresponding to relatively short lives. In Fig. 1 is sketched an *S–N* curve wth the *H* (hardening) and *F* (fatigue) regions indicated according to Wood. In the course of work being carried out on the modes of fatigue failure of oriented single crystal copper rods cycled in alternating torsion some rather striking examples of the distinction between high- and low-amplitude fatigue were observed.

The single crystals were grown in two orientations by Bridgman's technique from 99·999 per cent pure copper in graphite moulds. As grown, the crystals were about 4 in. long and 3/8 in. in diameter. Prior to cycling in the torsional fatigue machine a gauge-length of 1¼ in. long and 1/4 in. in diameter was electrolytically machined into the central portion of the as-grown crystals. The crystals shown in Fig. 2*a* and 2*b* have a cube edge or $<100>$ direction along the longitudinal axis, while the crystals shown in Fig. 3*a* and 3*b* have a cube diagonal or $<111>$ direction along the longitudinal axis.

The crystal shown in Fig. 2*a* was cycled in torsion at an amplitude of ± 0·0019 surface shear strain for 5×10^6 cycles. It appears that in this case the crystal failed by growth of fissures on the octahedral planes until the remaining portion of the cross-section of the crystal was no longer able to support the

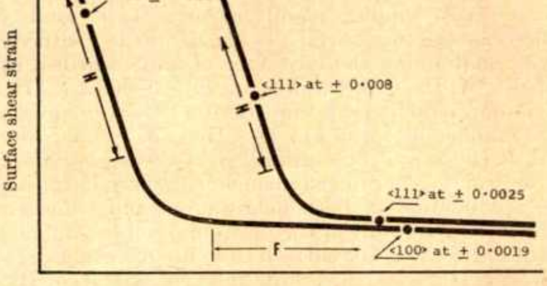
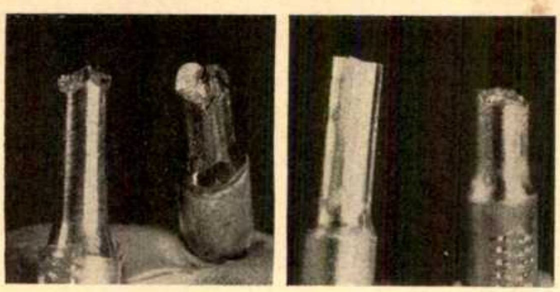
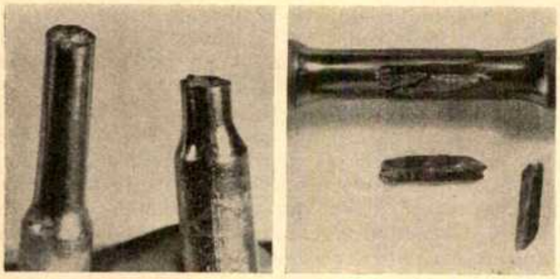


Fig. 1. *S–N* curve showing the *H* and *F* regions. The relative locations of the crystals described in the text are as indicated. Preliminary experiments lead to the establishment of distinct *S–N* curves for crystals of different orientation as shown in the figure



(*a*) (*b*)

Fig. 2. *a*, < 100 > axis crystal cycled at low amplitude (*F* region) to failure. Fracture surface follows {111} planes. *b*, < 100 > axis crystal cycled at a higher amplitude (*H* region) to failure. Fracture surface is irregular



(*a*) (*b*)

Fig. 3. *a*, < 111 > axis crystal cycled at low amplitude (*F* region) to failure. Fracture surface composed of segments of {111} planes. *b*, < 111 > axis crystal cycled at a higher amplitude (*H* region) to failure. Chips with sides approximating {211} planes are loosened during the cycling

applied stresses. In this case there is no evidence of the formation and propagation of irregular microcracks in the usual sense. The crystal in Fig. 2*b* was cycled at an amplitude of ± 0·012 for 6×10^3 cycles. In this case no fissures were formed of the kind described by Wood; but, rather, a series of ridges and grooves formed in 90° sectors around the circumference of the crystal. Failure occurred following the formation and propagation of cracks in the grooves.

The mode of failure of the < 111 > axially oriented crystal is shown in Figs. 3*a* and 3*b*. The crystal shown in Fig. 3*a* has been cycled at a surface shear strain amplitude of ± 0·0025 for 4×10^6 cycles. Fissures have penetrated to some distance along the octahedral plane perpendicular to the crystal axis. Due to the fact that at certain positions around the circumference of the crystal the resolved shear stress on the cross slip plane is equal to that on the primary plane, cross slipping readily occurs. This results in the final fracture surface being segmented although still maintaining the character of an octahedral slip plane. At the higher amplitude of ± 0·008 a < 111 > axially oriented crystal failed after 3×10^5 cycles in the manner shown in Fig. 3*b*. Here, as in the case of the < 100 > axis crystal cycled at the higher amplitude, there is no development of fissures but rather the production of both longitudinal and transverse cracks which result in the ejection of chips similar to that in Fig. 3*b*. The sides of this chip have an interior angle of 120° which, taking into consideration their orientation on the crystal, means that they may be bounded by planes of the form {211}. Here, as in the case of the < 100 > axis crystal, the appearance of the crystal on failure is entirely different for low and high amplitudes; but, nevertheless, the mode of failure is still governed by the crystallographical symmetry of the crystal. It would appear, from these observations, that any theory of fatigue damage based on dislocation interactions will have to be for a specified amplitude; that is, the cyclic movement of dislocations at small amplitudes leads to an entirely different mode of crystalline damage than the dislocation movement at higher amplitudes. This difference may possibly be correlated with the observed sequence in work hardening, that is, the easy glide, linear hardening, and parabolic regions of the work hardening curve.

A more detailed account of the crystallography of the torsional fatigue failure of the oriented single crystals is being submitted for publication elsewhere.

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Fatigue-induced Precipitation in Metastable β-Brass

A NUMBER of instances have been recorded in which metallurgical changes have occurred due to fatigue stressing which would not have occurred in the same time at the same temperature in the absence of the fatigue stress[1–4]. The changes are similar to those produced by heating and appear to be caused by either an increased diffusion-rate or an increase in nucleation sites. Two most obvious examples are the apparent over-ageing of certain aluminium alloys investigated by Hanstock[1,2] and the virtual removal of the long incubation time observed in normal aged aluminium alloys reported by Stubbington[4].

During recent work on the fatigue behaviour of metastable β-brass similar fatigue-induced precipitation was observed after fatigue stressing and on subsequent heating at 200° C. All metastable β-cantilever specimens were obtained by quenching 60/40 brass from the all β-region. Specimens were held at 840° C. for 2 min. in a vertical tube furnace before quenching into saturated brine at 4° C. Surface layers were ground off to remove de-zincified layers con-

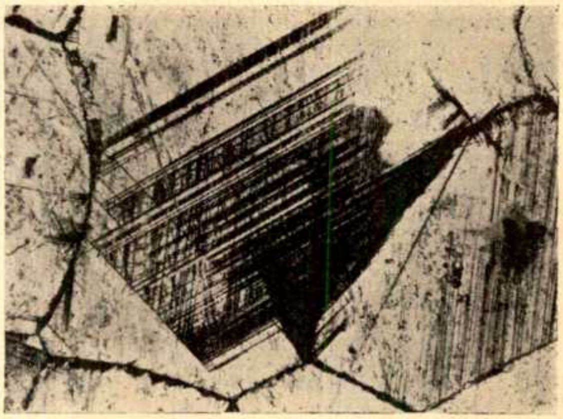

Fig. 1. Surface of fatigued metastable β-brass specimen. Fatigue-induced precipitation in slip markings. Etched in 10 per cent aqueous ammonium persulphate. (× *c*. 63)

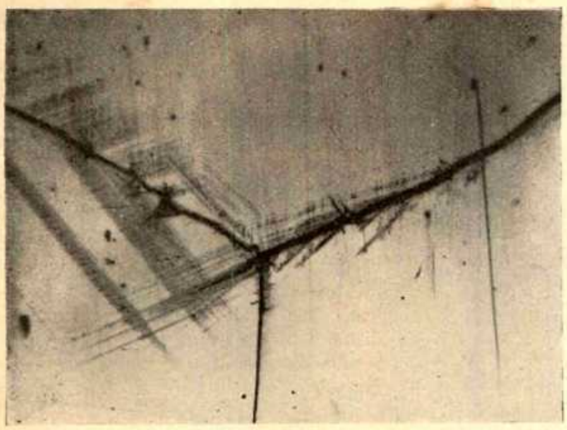

Fig. 2. Surface of fatigued metastable β-brass specimen. Fatigue-induced precipitation adjacent to grain boundaries. (Unetched. × c. 420)

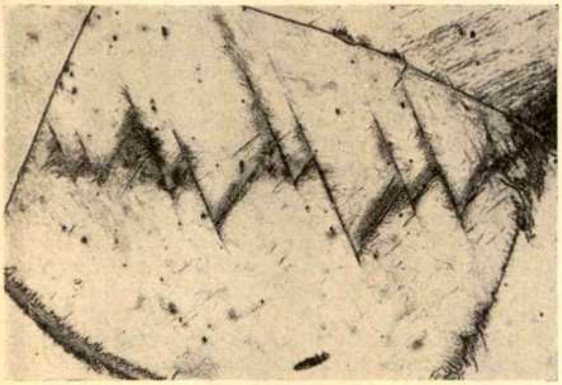

Fig. 3. Surface of fatigued metastable β-brass specimen after tempering for $1\frac{1}{2}$ hr. at 200° C. Preferential precipitation at a fatigue crack. (Etched in alc. $FeCl_3$. × c. 248)

taining phases other than metastable β. The resulting specimens were carefully mechanically polished and finally electro-polished before fatiguing in reverse plane bending at a frequency of 8,400 c./min. Certain specimens were tempered after fatiguing for $1\frac{1}{2}$ hr., at 200° C.

Fatigue-induced precipitates were observed to form either in slip markings or adjacent to grain boundaries (Figs. 1 and 2). A glancing-angle X-ray photograph indicated that these precipitates were face-centred cubic in type and that their cell dimensions were approximately 3·65 Å. (at 21° C.). Fatigued material showing no precipitation, after tempering showed preferential precipitation of α-solid solution in areas where damage had been greatest and in general an increased amount of precipitation (Fig. 3).

The fatigue-induced precipitation observed is considered to be α-solid solution. The precipitation is attributed to an acceleration of diffusion due to vacancies and interstitials produced in concentrations above their equilibrium values by the motion of dislocations under fatigue stresses. The precipitation observed after tempering fatigued material is thought to be due to the large number of nuclei present in fatigued material. Thomas and Whelan[5] have shown that dislocation loops formed by the collapse of vacancy disks can act as precipitation nuclei and recent observations have shown many such dislocation loops in fatigued material.

One of us (B. J. S. W.) acknowledges the British Iron and Steel Association for the award of a bursary.

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CRYSTALLOGRAPHY

Non-Crystalline Excrescences from Heated Silica

THREE different forms of filamentary silica have been discussed recently. Jaccodine and Kline[1] reported the growth of whiskers on the devitrified surface of vitreous silica which had been heated in a nitrogen stream for 24 hr. at 1,425° C. Abrahams and Stockbridge[2] showed that these growths were proper whiskers (Nabarro and Jackson[3]) of high-cristobalite with [111] as whisker axis. They also examined whiskers found on hydrothermally grown crystals of α-quartz[4], and showed that these were α-quartz crystals elongated along [0001]. Haller[5] grew fibres of silica, non-crystalline and optically isotropic, on heated platinum surfaces (temperature about 1,100° C.) exposed to a mixture of silicon tetrafluoride and water vapour in a nitrogen stream.

We wish to report the formation of excrescences of silica in circumstances different from the foregoing. These have been found in blisters formed on the outer surfaces of 'Vitreosil' furnace-tubes held, in air, at temperatures of 1,100–1,200° C. for periods of up to 8 weeks. The material of the tubes was originally vitreous silica; but this partially devitrified after some weeks at temperature. Blisters formed during this period and the tubes eventually fractured. On breaking open the blisters it was found that these contained excrescences of silica. Although there were opaque crystalline regions within the blisters, the excrescences all originated from regions that were still vitreous. They were optically isotropic, and X-ray diffraction patterns (copper $K\alpha$ radiation) showed only the diffuse rings characteristic of non-crystalline

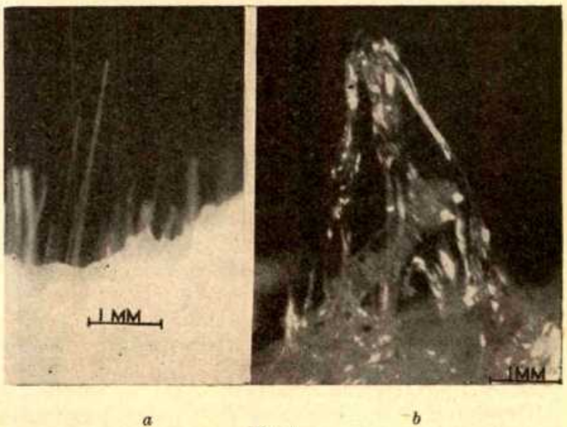
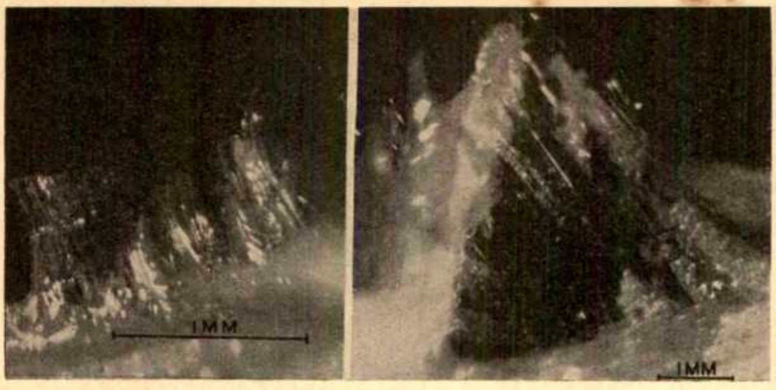


Fig. 1



a *b*

Fig. 2

material, with no sign of preferred orientation. Some excrescences were filamentary in form (Fig. 1*a*), while others showed more complex arrangements of threads (Fig. 1*b*). Another characteristic shape was that of long, narrow ridges (Fig. 2*a*).

In many cases the excrescences stretched across the narrower regions of a blister (Fig. 2*b*). This observation suggests that the excrescences described here were formed by vitreous silica, which after softening to a plastic condition was stretched between roof and floor of the slowly extending blister. Filaments formed in this way would break if the extension became too large, and a number of examples were in fact seen of 'stalactite–stalagmite' pairs in complementary positions on top and bottom inner surfaces of blisters. No growths were seen on the outer surfaces of the blisters.

The devitrified regions of the tube were shown by X-ray diffraction to be low-cristobalite. Spotty powder patterns (crystallite size about 0·05 mm.) were obtained. No excrescences were seen to emerge from these crystalline regions and it is probable that the temperature was too low for the growth of proper whiskers.

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Thermal Formation of Etch Pits after Polymorphic Transformation

LINES of etch pits form at phase, twin, and subgrain boundaries on the surface of crystals of thallous nitrate maintained at temperatures greater than 70° C.

The room temperature orthorhombic form, in which the cations are in a primitive pseudo-tetragonal array[1], is in equilibrium at 76° C. with a related pseudo-cubic trigonal structure[2]. In one mechanism of the change orthorhombic to trigonal the orientation relationship $(010)_o//(011)_{pc}$ and $<101>_o//<1\bar{1}1>_{pc}$ is maintained[3], the interface being parallel to $(010)_o$. As there is also a shape deformation, the change shares the main features of martensitic transformations[4]. The pseudocubic form is in equilibrium at 143° C. with a closely related cubic phase. Good crystals about 0·3 mm. long with an impurity content of approximately 500 p.p.m. were grown from aqueous solution. To investigate etching in untransformed crystals slip and twinning were introduced by applying pressure. Undeformed specimens were used for experiments involving transformation.

When the deformed crystals were held at 70° C. pits appeared along twin boundaries but not along the slip boundaries. In the orthorhombic to trigonal transformation, pits formed at stationary interfaces, becoming clearly visible under the microscope after 5 hr. at 85°–90° C. After this period the number and position remained constant. The spacing of pits was uniform within each row, but differed at separate interfaces within the same crystal by a factor of up to 2. The spacings in different crystals ranged from 0·6μ to 2·4μ. No pits were observed along striations left on the trigonal form parallel to the interface.

Crystals annealed in the trigonal phase for 10 hr. at 110° C., then kept in the cubic form at 150° C. for one to two days, showed either almost randomly placed pits, or an irregular network. In the former case there were always close to 10^7 pits per cm.^{2}. In the latter case a typical value for the spacing of pits was 1μ, and the rows clearly outlined subgrains of mean area 50μ^2. The presence of subgrains is consistent with our X-ray observations that this transformation creates some strain and annealing produces polygonization[5].

Although proof is as yet lacking, the appearance and arrangement of the pits make it highly probable that these correspond to dislocations, though not necessarily to all the dislocations present. Thermal etching of dislocations has been observed on a few metals[6] and on aluminium oxide crystals[7]. Dislocations at the phase interface could be associated with the arrays by means of which the moving interface in a martensitic transformation has been described[4,8].

This work suggests that: (1) preferential thermal etching of dislocations occurs on this ionic material; (2) dislocations expected to be present in the coherent interface between two solid phases have been revealed by etch pits; (3) subgrains are present after some polymorphic transformations, and in this example their mean area was 50μ^2.

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GEOCHEMISTRY

Separation Techniques in Sedimentary Geochemistry illustrated by Studies of Boron

THE geochemical distribution of elements in sedimentary rocks is genetically complex because they may be concentrated in the detrital fraction or resistates; the clay fraction; the chemically precipitated fraction; and the organically precipitated fraction. It follows that advances in sedimentary geochemistry depend on increased knowledge of the distribution of elements in these four constituents. Hence, analysis of the bulk rock, which is generally the standard practice in igneous geochemistry, may not be a successful method when applied to problems in sedimentary geochemistry.

An investigation of boron in British sedimentary rocks illustrates some of the problems and techniques involved in the separation of different constituents of a sedimentary rock. Boron is concentrated in clays and in the resistates mainly in tourmaline. A method to differentiate between boron in the clay and in the resistate fractions is described here and may prove applicable to other problems of sedimentary geochemistry. Detailed results and conclusions concerning the geochemistry of boron will be reported fully elsewhere.

Harder[1] followed by Frederickson and Reynolds[2] showed that much of the insoluble boron in sediments is concentrated in the clay mineral illite. In these early studies clays were isolated from the resistates by size fractionation and were assumed to be tourmaline free. During the present investigation it was found that size fractions less than 0·5μ could be isolated by sedimentation in beakers of one litre capacity. However, consistent success with this simple method was only achieved when the experimental conditions were rigidly controlled.

Carbonate rich rocks were disaggregated by solution in 2 normal hydrochloric acid. Low carbonate rocks were ground to B.S.S. 100 mesh size and treated with hydrochloric acid. After reaction with acid ceased, the samples were washed at least ten times by repeated decantation with distilled water and dispersed in 1/25 normal sodium carbonate solution. Different size fractions were isolated by sedimentation in beakers of one litre capacity, followed by decantation at the time-intervals shown in Table 1.

Table 1. EMPIRICAL RELATIONSHIP BETWEEN GRAIN SIZE AND SETTLING TIME FOR A 10-CM. COLUMN AT 70° F.

Grain size (μ)	Settling time Days	Hr.	Min.	Sec.
60·0	–	–	–	30
30·0	–	–	1	40
10·0	–	–	18	0
5·0	–	1	13	0
2·0	–	7	47	0
1·0	1	7	7	0
0·5	5	4	30	0

The coarse fractions were recovered by filtration and the finest fractions were concentrated in a centrifuge. If a suitable centrifuge is not available, a few drops of concentrated hydrochloric acid are added to the fine fractions. The acid causes flocculation and aids recovery of the finest fractions.

The amount of illite in any size fraction could be computed from potassium analyses according to the following formula, because X-ray diffraction studies showed that other potassium-bearing minerals were absent.

$$\text{Per cent pure illite} = \frac{\%K_2O}{8{\cdot}5} \times 100$$

The amount of illite calculated by the above method is much less than the amount observed from X-ray diffraction studies. However, illite in sediments is degraded compared with muscovite mica and the potassium content provides a measure of the number of muscovite-type layers in the illite. Since Harder[3] has shown that boron is incorporated in the muscovite structure it is better to compare boron concentration with potassium content rather than with illite content estimated from X-ray studies.

Potassium was estimated in solution by a standard flame photometric procedure. The solution was prepared by digesting 100 mgm. of sample with 15 ml. of hydrofluoric acid and 3 ml. of sulphuric acid in a small polythene beaker. The acid was fumed off over a steam bath for 24 hr. and the remaining salts were washed into a small glass beaker. The contents of the glass beaker were reduced by evaporation to about 3 ml. and strongly heated. Nitric acid was cautiously added dropwise to oxidize carbonaceous matter. After this treatment, warming with distilled water brought all salts into solution.

The above technique involves slightly more handling than the standard procedure in which platinum crucibles are used. However, the number of samples that can be prepared at any one time is not restricted by availability of expensive apparatus. If samples are analysed in batches of 25 the speed of the method compares favourably with X-ray fluorescence used by Frederickson and Reynolds[2].

The boron content of the different size fractions was determined spectrographically. Boron 2497·7 was used as the analysis line and a beryllium triplet at 2494·6 was used as an internal standard line. The true boron content of pure illite (adjusted boron content) was calculated according to the following formula:

$$\text{Adjusted boron} = \frac{8{\cdot}5}{\%K_2O} \times \text{observed boron}$$

The observed and adjusted boron contents of illite in size fractions of the insoluble residue from the Main Limestone, Yoredale Series, Hawes, Yorkshire (Nat. Grid Ref. 34/835944), is shown in Fig. 1. Although observed boron decreases in the coarser fractions due

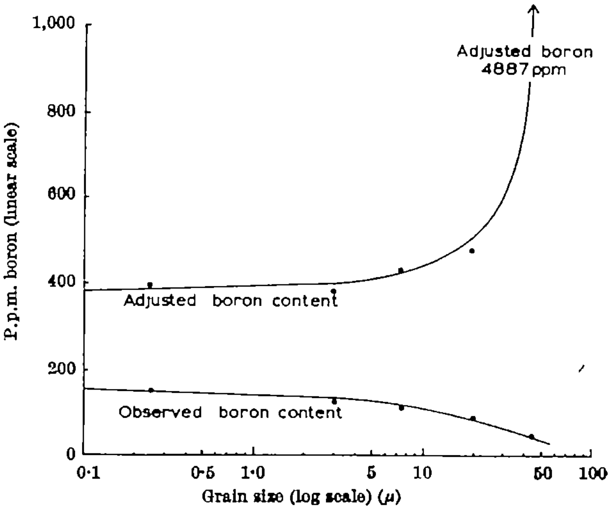

Fig. 1

to decrease in the amount of illite, adjusted boron increases. This is believed to indicate that some of the boron in the coarser size fractions is contributed by tourmaline. In particular, the boron content of pure illite decreases to a constant value in size fractions less than 5µ, indicating that the effect of tourmaline in these very fine fractions is negligible. Six other samples which were investigated showed a similar relationship between grain size and boron.

Frederickson and Reynolds[2] proposed that the boron content of pure illite was closely related to the salinity of the water in which the illite was originally deposited. The boron content of pure illite has been determined for seventeen rocks the inferred depositional environment of which varies from fresh or brackish water to evaporitic. The adjusted boron content of pure illite from normal marine rocks in the Yoredale series ranged between 400 and 650 p.p.m. In dolomites from the top of the Lower Magnesian Limestone of Yorkshire the adjusted boron content of pure illite ranged between 691 and 768 p.p.m., and in halite of unknown age from a borehole in Tanganyika, Africa, the adjusted boron content was 961 p.p.m. These results agree with the published results of Frederickson and Reynolds[3].

The adjusted boron contents of illite from two supposedly freshwater limestones from the Upper Jurassic Purbeck Beds of the south of England were 509 p.p.m. and 554 p.p.m. Marine illite from the same formation was found to have an adjusted boron content of 520 p.p.m. These values do not agree with the very low concentration of boron recorded by Frederickson and Reynolds[2] in illite from freshwater environments.

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RADIATION CHEMISTRY

Phase Effects in the Radiation Chemistry of Organic Compounds which may arise from Long-Range Energy Transfer

WE have recently investigated the γ-ray-induced destruction of the solute (S = either I_2, $FeCl_3$ or DPPH) in dilute liquid and solid solutions in the organic solvents listed here. For all liquid solutions at 30° C. $G(-S)$ is independent of solute concentration and dose and shows the expected dependence on solvent structure (Table 1). In contrast to this simple situation the corresponding solid solutions show the following two distinct types of radiation response:

(1) Crystalline solutions in diethyl ether or dioxan and glassy solutions in phenetole or 2-methyl tetrahydrofuran have $G(-FeCl_3)$ values which are independent of dose, temperature and solute concentration and are smaller than the values for the corresponding liquid solutions.

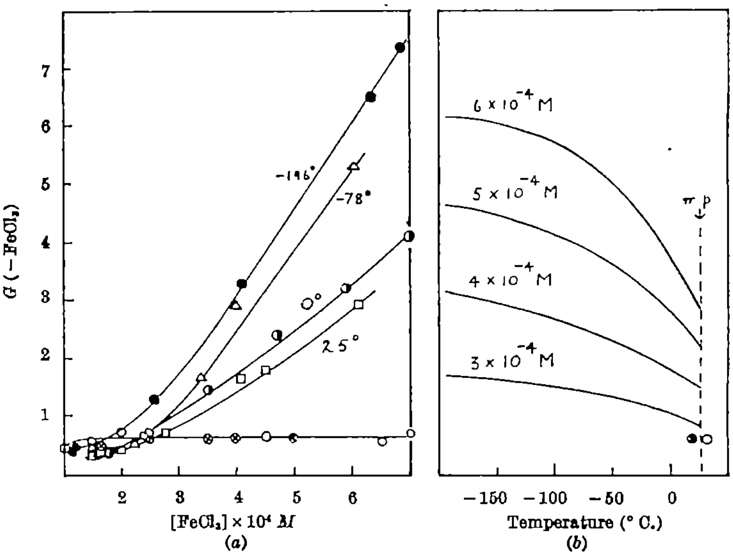

Fig. 1. $G(-FeCl_3)$ for solutions of $FeCl_3$ in diphenyl ether. (*a*) Effect of changing solute concentration. Liquid at 30° (○), supercooled liquid at 25° (⊗), crystalline solid at 25° (□), 0° (◐), −78° (△) and −196° (●). (*b*) Effect of temperature at different solute concentrations

(2) $G(-S)$ for crystalline solutions of $FeCl_3$ in the first four solvents in Table 1 and of iodine in diphenyl ether or phenetole[2] is markedly dependent on concentration, increases with diminishing temperature of irradiation and may be very much larger than the value in the liquid phase. This behaviour is illustrated in Fig. 1.

Low-temperature spectrophotometric studies of 2-methyltetrahydrofuran glass shows that a reaction actually occurs in the solid phase[3]. Although the same technique is inapplicable to the crystalline systems, a number of other experiments indicated that there is no segregation of the solute and that type (2) behaviour is determined by reaction occurring in the solid solution during irradiation. That type (2) behaviour is controlled by the state of aggregation rather than by any effect of temperature *per se* is shown by the observations that supercooled solutions in diphenyl ether show behaviour characteristic of the liquid at a higher temperature rather than the solid at the same temperature (Fig. 1), and that glassy phenetole solutions conform to type (1), whereas crystalline phenetole solutions at the same temperature conform to type (2). Since the solute is itself immobile, the action of the radiation must be to produce some entity in the solvent which is able to migrate to, and cause chemical change in, the solute. The marked dependence of $G(-S)$ on $[S]$ in type (2) systems indicates that the mean migration distance

Table 1

Solute / Solvent	$FeCl_3$	I_2	DPPH
Benzene	0·86 ± 0·12[1]	0 41 ± 0 04[1]	0 69 ± 0·03[1]
Diphenyl ether	0·66 ± 0·05	0·38$_5$ ± 0 04[2]	0·40 ± 0·01
Diphenyl methane	0·90 ± 0·05		
Phenetole	0·85 ± 0 05		
p-Dioxan	3·5 ± 0 25		
2-Methyl-tetrahydrofuran	4 0 ± 0·2		
Diethyl ether	12·5 ± 1		

must be limited and since $G(-S)$ is still increasing with $[S]$ at the highest value of $[S]$ which can be used (10^{-3} M) this distance cannot exceed 75 Å. The migrating entity could be either energy, ionization or an electron. We consider ionization transfer to be unlikely because $\mathscr{I}_{FeCl_3}$ is greater than the ionization potential of the aromatic molecules comprising the solvent in type (2) systems. Electron transfer can be excluded because the large G_{e^-} values which it would be necessary to postulate to account for the highest $G(-S)$ values (>12 for the benzene–$FeCl_3$ system) are irreconcilable with probable W values and ionization potentials of the solvent, and are much larger than those recently measured by Hamill *et al.*[3]. Moreover, if naphthalene were a more efficient electron trap than $FeCl_3$, its presence should cause $G(-FeCl_3)$ to be smaller. In fact, although it does cause $G(-FeCl_3)$ in 2-methyl tetrahydrofuran to become zero, it has no effect on $G(-FeCl_3)$ in phenetole. We are therefore left with energy transfer as the only possible mechanism, and this hypothesis has many merits. If the process is one of transfer by inductive resonance, as described by Forster[4], a necessary condition is overlap of the absorption spectrum of the solute with the emission spectrum of an excited state of the solvent. This condition is satisfied by all those systems which give type (2) behaviour and by none which give type (1) when $FeCl_3$ or I_2 is the solute. Furthermore, the absorption spectrum of naphthalene overlaps more completely than that of $FeCl_3$ with the emission spectrum of phenetole, and the inhibiting effect of naphthalene in this medium and its lack of effect in 2-methyl tetrahydrofuran are thus readily explicable. It is also known that irradiation of solutions of $FeCl_3$ or I_2 by light of wave-length within the range 2500–3500 Å. causes photo-dissociation of these solutes so that chemical change in the solute is a likely consequence of energy transfer. The resonance transfer mechanism also predicts a dependence of $G(-S)$ on $[S]$ which is broadly in agreement with that observed. The efficiency of this transfer increases with the coupling energy, which is probably large in the type (2) systems studied and is therefore likely to increase with increasing density, that is, decreasing temperature, and it might also be expected that a high degree of structural regularity such as exists in benzene crystals would favour energy transport.

It is interesting to speculate whether persistence of structural regularities in liquids could be sufficiently large to enable a degree of donor-acceptor coupling which would permit energy migration. The radiation chemistry of dilute solutions of $FeCl_3$ in benzene[1] does provide evidence of the participation of excited benzene molecules, and unusual quenching effects in the radiation-induced fluorescence of terphenyl in benzene[5] support the notion that aggregates of 10–20 benzene molecules do exist.

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CHEMISTRY

Intrinsic Viscosity of Gelatin

THE intrinsic viscosity ($[\eta]$) is commonly used to characterize high polymers; but its determination is often complicated by adsorption of the high-polymer solute to the apparatus[1]. It has been widely assumed and has recently been categorically stated[2] that adsorption effects can be neglected in the determination of the intrinsic viscosity of gelatin. The examination of more than a score of gelatin samples in this laboratory indicates that under normal working conditions this is not so. Considerable errors attend the use of sintered glass filters in the preparation of samples through removal of significant amounts of gelatin by adsorption. The extent of this error depends on the size and porosity of the filter disk. For example, when 50 ml. of a 0·1 per cent solution of alkali-bone gelatin ($[\eta] = 65$ ml./gm.) in molar potassium chloride at pH 7 was filtered through a 2-cm. diameter disk, the adsorptions determined by chemical analysis of the filtrate[3] were 4, 7 and 13 per cent, for disks of porosity 1, 2 and 3 respectively. It is possible that the influence of this adsorption on the viscosity is greater than indicated by these figures since high-molecular-weight species of polymers are usually more readily adsorbed. When membrane filters (Membranfilter Gesellschaft No. 1) were used for filtering the solution, the adsorption was not detected analytically and may be presumed to be less than 1 per cent.

Adsorption also occurs to the capillary of the viscometer. A similar 0·1 per cent solution of gelatin was placed in an Ostwald viscometer (British Standard, size A) at 40° C. and the time of flow was determined. The solution was then removed and the viscometer was rinsed with saline solvent and filled with solvent. The time of flow of solvent was observed to be about 0·5 per cent higher than with a cleaned (50 per cent v/v nitric acid) viscometer. This small change in flow-time (1·0s) will, however, account for an 8 per cent error in viscosity number. The thickness of the adsorbed layer has been calculated from the increase in flow-time to be of the order of several hundred Ångstrom units. The thicknesses of 25–50 Å. determined by Pouradier and Roman[4] for the layers of gelatin adsorbed to silver bromide are not necessarily inconsistent with the above, since these authors derived the thickness from the weight of gelatin per unit area of surface and do not refer to the thickness of the layer in contact with solution. Both adsorption errors become increasingly important as the concentration is reduced, particularly below about 0·3 gm. gelatin/100 ml. Since the two errors are in opposition the shape of the viscosity number/concentration curves will vary with the exact experimental conditions. We have on two or three occasions obtained, we believe, quite fortuitously, linear relationships over the gelatin-concentration range 0·1–0·6 gm./100 ml. If membrane filters are used the viscosity number always increases as the concentration falls below about 0·25 gm./ml. This effect is most marked for high molecular weight fractions.

These two errors can be reduced but not eliminated by making intrinsic viscosity determinations in molar potassium thiocyanate at pH 7 (Fig. 1). The amount of gelatin adsorbed is somewhat less and the viscosity is about 10 per cent higher than in molar potassium chloride. Both facts tend to reduce the percentage error. With this solvent and membrane filters,

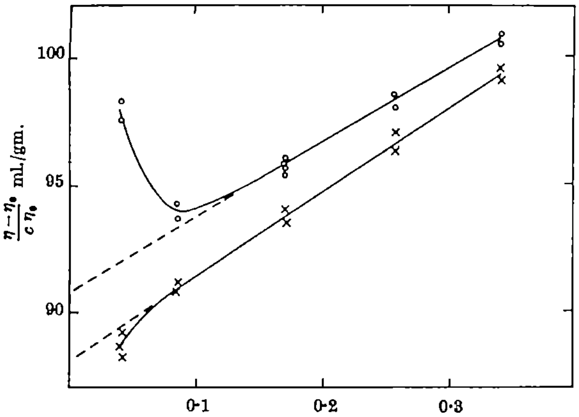

Fig. 1. Reduced viscosity ($\eta-\eta_0/c\eta_0$) as a function of the nominal concentration for solutions of an alkali-process gelatin fraction in molar potassium thiocyanate. ○, Filtered through a membrane filter; ×, filtered through a No. 2 sintered glass filter. The filters used were 2 cm. diameter in each case and 20 ml. of solution was allowed to pass before taking samples for test. The fall in gelatin concentration indicated by analysis amounted to 1 per cent for the membrane filtration at all concentrations, within experimental error, but varied for sintered glass filtration from 8 per cent at 0·1 gm. gelatin/dl. to 4 per cent at 0·3 gm./dl.

viscosity numbers determined at concentrations above 0·2 gm./100 ml. bear a linear relationship to concentration which can be extrapolated to zero concentration to obtain the intrinsic viscosity. This applies even to fractions of alkali-processed gelatins having an intrinsic viscosity of 170 ml./gm.

In the foregoing experiments all glassware (viscometers and filters) after use with gelatin were cleaned by soaking in 50 per cent v/v nitric acid and washing with water before using again.

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Activation Energy and Fractional Order

CONSIDER the case of a unimolecular gas reaction catalysed by a surface obeying the Langmuir adsorption isotherm, with true velocity constant k, adsorption coefficient b, heat of adsorption λ, surface coverage θ, pressure p. It is possible to derive a very simple relation between apparent activation energy E_a, true activation energy E_t, and θ, which should prove of considerable utility. The rate r is:

$$r = k\theta = kbp/(1 + bp)$$

where

$$b = b_0 e^{\lambda/RT}$$

hence:

$$E_a = RT^2 \frac{d \ln r}{dT} = E_t - \lambda + (1 + bp)^{-1} \lambda bp$$
$$= E_t - \lambda\,(1 + bp)^{-1}$$

substituting $\theta/1 - \theta = bp$

$$E_a = E - \lambda\,(1 - \theta) \qquad (1)$$

For the extreme cases of $\theta \rightarrow 0$ and $\theta \rightarrow 1$ this reduces to Hinshelwood's well-known result[1].

The result may be related to Sandler's relation[2]. The Freundlich and Langmuir isotherms often coincide over a limited pressure range, and where this is so we may equate $d\ln\theta/dp$ derived for the two isotherms. This leads to the relation[3]:

$$n = 1 - \theta$$

where n is the exponent of the pressure in the Freundlich isotherm. Substituting in equation (1), we obtain Sandler's relation:

$$E_a = E_t - \lambda . n. \qquad (2)$$

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Reactions of Sodium with Active Oxygen

IN a recent investigation of the chemiluminescent flame formed when sodium diffuses into hydrogen coming from a discharge, Polanyi and Sadowski[1] found that the sodium was being consumed at a rate which was 10^4–10^5 times faster than could be accounted for on the basis of ternary reactions. Since electronically excited hydrogen could be excluded as a reagent, they accounted for the sodium consumption in terms of the binary reaction:

$$\mathrm{Na} + \mathrm{H_2}\dagger\,(v \geqslant 6) \rightarrow \mathrm{NaH} + \mathrm{H} \qquad (1)$$

where $\mathrm{H_2}\dagger(v \geqslant 6)$ refers to molecular hydrogen in at least the sixth vibrational level of the ground electronic state. In the present work, the depletion of sodium in an atmosphere of active oxygen produced in a Wood's tube discharge was measured by means of the Polanyi sodium diffusion-flame technique[2]. The apparatus has been previously described[1]. Argon was used as a carrier gas for the sodium vapour.

The product, kp, was calculated from the Polanyi diffusion-flame equation[2]:

$$kp = \frac{\delta[\ln(x_0 r_0/xr)]^2}{(r_0 - r)^2} \qquad (2)$$

where k is the rate constant associated with the sodium-consuming reaction, p is the concentration of the atmospheric reagent, δ is the diffusion coefficient of sodium in the reaction mixture, r_0 is the nozzle radius, x_0 is the partial pressure of sodium at a distance r_0 from the nozzle, x is the partial pressure of sodium at the visible periphery of the flame, and r is the distance from the nozzle to the flame periphery.

In a typical experiment, the Wood's tube current was adjusted until a flame of radius 1·7 cm. was obtained. In this experiment, the total reaction vessel pressure was 0·4 mm. consisting of 0·3 mm. oxygen and 0·1 mm. argon; the reaction vessel temperature was 300° C., x_0 was approximately $1{\cdot}7 \times 10^{-3}$ mm., r_0 was 0·057 cm., x was 7×10^{-6} mm., r was 1·7 cm. and δ was $2{\cdot}01 \times 10^3$ cm.^{2} sec.$^{-1}$. Although δ for sodium in oxygen is not known, a good approximation is δ in nitrogen. Therefore, the value listed was obtained by correcting Heller's[3] determination of δ in nitrogen to the present experimental conditions.

The product, kp, was found to be $3{\cdot}0 \times 10^3$ sec.$^{-1}$. If we consider the reaction:

$$\mathrm{Na} + \text{active oxygen} + M \xrightarrow{k_3} \text{products} + M \quad (3)$$

and suppose that the partial pressure of active oxygen is 0·4 mm., that is, is equal to the total pressure of the reaction vessel, then k_3 would have to be at least 3×10^{19} c.c.^{2} moles^{-2} sec.$^{-1}$ to account for the consumption of sodium. This is between 10^2 and 10^3 times larger than any termolecular rate constant reported. Termolecular association reactions between sodium and oxygen atoms would require rate constants at least an order of magnitude higher, since it is doubtful that the level of oxygen atoms exceeded 10^{-2} mm. It seems therefore that a termolecular reaction cannot account for the observed sodium consumption.

In support of this conclusion is the fact that whereas contained flames could easily be obtained when the oxygen was activated in an electrical discharge, a contained flame could not be obtained in normal oxygen. Bawn and Evans[4] were able to obtain contained flames only at total reaction vessel pressures which were from ten to sixty times as great as those used in the present investigation. The reaction prevailing in their system was:

$$\mathrm{Na} + \mathrm{O_2} + M \xrightarrow{k_4} \mathrm{NaO_2} + M \quad (4)$$

The most likely sodium-consuming reaction in the present system appears to be the bimolecular process:

$$\mathrm{Na} + \mathrm{O_2}^* \xrightarrow{k_5} \mathrm{NaO} + \mathrm{O} \quad (5)$$

where, in order to render reaction (5) thermoneutral, $\mathrm{O_2}^*$ would have to be molecular oxygen in at least the 9th vibrational level of the ground electronic state, at least the 5th vibrational level of the $^1\Delta_g$ state, or at least the 2nd vibrational level of the $^1\Sigma_g^+$ state. Both these latter states are metastable with respect to the transition to the ground state ($^3\Sigma_g^-$), since a magnetic dipole transition is required. The radiative lifetime for the $^1\Sigma_g^+ - {}^3\Sigma_g^-$ transition has been estimated to be 7 sec.[5]. Foner and Hudson[6] and Herron and Schiff[7] have detected good yields of $\mathrm{O_2}^*(^1\Delta_g)$ in oxygen which had been passed through an electrodeless discharge.

For k_5 between 10^{12} and 10^{14} c.c. mole^{-1} sec.$^{-1}$, the pressure of $\mathrm{O_2}^*$ would have to lie between 1×10^{-1} and 1×10^{-3} mm., a not unreasonable range in view of the findings of Foner and Hudson[6], and of Herron and Schiff[7]. The relative importance of the postulated oxygen species in reaction (5) could not be determined in the present investigation.

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Fundamental NH$_2$ Stretching Frequencies in Amines and Amides

In molecules with functional groups of the type $-AB_2$, two stretching vibrations corresponding to the symmetric (ν_s) and asymmetric (ν_{as}) modes of vibration of the B atoms are observed. Bellamy and Williams[1] have demonstrated that so long as the two A–B bonds are equivalent, and mass and coupling effects are negligible, polar effects acting on the group induce proportional changes in ν_s and ν_{as}. For sixty-two primary amines covering a wide range of molecular type in solution and in the pure liquid state, they found:

$$\nu_s = 345{\cdot}5 + 0{\cdot}875\,\nu_{as} \quad (1)$$

with a standard deviation of 4·8 cm.$^{-1}$. Where neighbouring groups destroyed the bond equivalence, as in hydrogen bond interactions with only one hydrogen atom of the amino-group, this relationship no longer held. Stewart[2] has shown that equation (1) can be predicted from the force field treatment of Linnett[3], assuming identical NH bond stretching force constants.

For a more restricted range of molecular type, *meta*- and *para*-substituted anilines in very dilute carbon tetrachloride solution, Krueger and Thompson[4] found:

$$\nu_s = 1{,}023 + 0{\cdot}682\,\nu_{as} \quad (2)$$

with an average deviation of less than 1 cm.$^{-1}$. The greater precision of equation (2) with respect to equation (1) can undoubtedly be ascribed to the fact that the correlation of Bellamy and Williams was based on experimental measurements under slightly different conditions from a number of laboratories. The possibility that the nitrogen atom is in different hybridization states in this very broad correlation may also be a contributing factor. A solvent effect on the ν_s/ν_{as} correlation in substituted anilines has also been noted[5].

For the corresponding first overtone bands in substituted anilines, Whetsel *et al.*[6] have reported:

$$\nu_s = 1{,}304{\cdot}6 + 0{\cdot}790\,\nu_{as} \quad (3)$$

with a standard deviation of 3·2 cm.$^{-1}$. For both the fundamental and overtone aniline frequencies, *ortho*-substitution (except *ortho*-alkyl) lowers ν_s with respect to ν_{as} by destroying the exact equivalence of the NH bonds. Recently, Puranik and Ramiah[7] have shown that the NH$_2$ stretching frequencies in primary amide monomers follow:

$$\nu_s = 1{\cdot}1214\,\nu_{as} - 542{\cdot}5 \quad (4)$$

with an average deviation of about 3 cm.$^{-1}$, except in hydrogen bonded systems such as those using dioxane as a solvent. Cannon[8] has previously presented evidence for extensive conjugation between the carbonyl group and the amino-group in amides, leading to planarity.

Fig. 1 shows the correlations corresponding to equations (1) and (4) respectively in relation to the data for the substituted anilines, where both the symmetric and asymmetric NH$_2$ frequencies rise with increasing electron-withdrawing power of the substituent groups[4]. The significantly lower slope of the aniline correlation (A) as opposed to the general amine correlation (B) and the amide correlation (C), and the displacement of line C to the right with respect to B, is interpreted in terms of a systematic decrease of the sp^3/sp^2 character of the amino-group in the aniline series as the electron-withdrawing

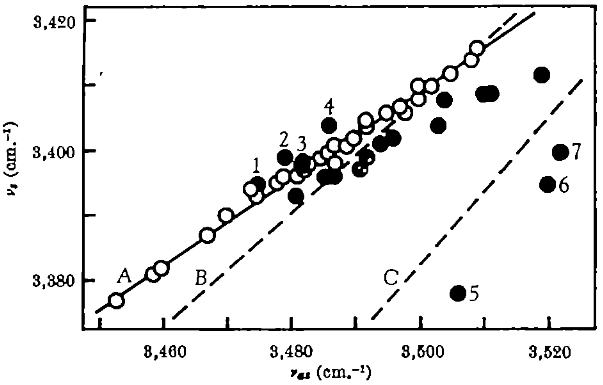

Fig. 1. Symmetric and asymmetric NH_2 frequency relationships. *A*, for substituted anilines; *B*, for primary amines generally, *C*, for primary amides. *Ortho*-substituted compounds of special interest: (1) 1-naphthylamine, (2) 2,4,6-trimethylaniline; (3) *o*-toluidine; (4) 2,6-dimethylaniline, (5) ethyl anthranilate; (6) 2,4-dinitroaniline; (7) *o*-nitroaniline

nature of the substituents increases. This concept is in harmony with more detailed calculations which show an HNH angle of 109·4° in *p*-phenylenediamine, and a smooth rise to 111·1° in aniline and 113·6° in *p*-nitroaniline. No recent NH_2 frequency measurements on simple primary alkyl amines (where the nitrogen atom bonding orbitals are certainly sp^3) in dilute carbon tetrachloride solution appear to be available.

Ortho-substitution in general leads to angle opening, or more extensive sp^2 contribution to the nitrogen-bonding orbitals. Where the substituents are 2-NO_2, 2,4-diNO_2, and 2-$COOCH_2CH_3$, HNH angles of 121·7°, 122·6° and 123·6° respectively were calculated from infra-red frequency measurements. Fig. 1 shows these three compounds displaced farther from the general amine correlation than are the amide values, in agreement with the above angles.

Little definite evidence concerning the geometry of the amino-group in aromatic amines has been presented so far, as Brand and Speakman[9] have recently pointed out. From Kerr constant measurements they suggest a non-planar structure. Wepster[10] has also reported supporting evidence for a pyramidal amino group in these compounds. My infra-red measurements confirm these deductions, and also show that strongly electron-donating substituents drive the amino-group fairly completely into a pyramidal ammonia-like structure, whereas strongly electron-withdrawing substituents tend to pull the amino hydrogen atoms towards the plane of the aromatic ring, thereby increasing the HNH angle. *Ortho*-substituents involved in strong intramolecular hydrogen bonding appear to lead to planar molecules in which the HNH angle may exceed 120°.

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BIOCHEMISTRY

Infra-Red Spectra of Some Melanins

MELANINS are pigments of high molecular weight formed by enzymatic oxidation of phenols and usually occur as granules in which the melanin may be combined with protein[1]. The classical work of Raper[2] established the basic role of indolyl-5,6-quinone in the formation of melanin from 3,4-dihydroxyphenylalanine (dopa), and it is now accepted that melanin structures result from polymerization of enzymatically produced *o*-quinone substrates. The detailed structure of melanin is still unknown, and it has been the practice to identify melanins in living organisms by a number of non-specific tests[3], for example, insolubility in common solvents other than strong aqueous alkali (from solution in which the pigment is precipitated on acidification), solubility in ethylene chlorhydrin, reduction of ammoniacal silver nitrate, bleaching with hydrogen peroxide and reduction by sodium thiosulphate and re-oxidation with potassium ferricyanide.

During the course of an investigation of the melanin pigment in *Arenicola marina*, it was decided to compare the properties of this pigment with those from other invertebrate animals (*Holothuria niger*, *Sepia* ink-sac and a beetle) and with ox-eye choroid. As the tests outlined were found to be of little value, an investigation of the infra-red absorption spectra of carbon tetrachloride mulls of the pigments was carried out with the Perkin Elmer Infracord. The main difficulty is the isolation of the pigment in a pure form, particularly in removing any attached protein. Protein material was hydrolysed by digestion with trypsin at 37° for one week followed by hydrolysis with 6 *N* hydrochloric acid for some hours, and the black insoluble material remaining was filtered. Treatment of trypsin (and other proteins) with hydrochloric acid alone gives rise to a black insoluble substance, 'humin', but it was found that this product from trypsin was completely soluble in tetrahydrofuran; all samples of melanin after the treatment with acid were extracted with this solvent by boiling under reflux before the infra-red analyses were carried out. Since tetrahydrofuran is miscible with water, this treatment probably provides a water-free sample of pigment. The pigment was ground to a fine powder which was mixed with carbon tetrachloride and re-ground; the sample was then transferred to a sodium chloride plate, a few drops of carbon tetrachloride added and the second plate pressed on the mixture and the two plates rotated to achieve a uniform distribution of material. The infra-red spectrum was then recorded as soon as possible, since slow evaporation of the solvent occurs.

Typical spectra are shown in Fig. 1. Only beetle melanin had a marked solubility in *N* sodium hydroxide, and the spectrum recorded is that of the sample precipitated from the alkali solution by acidification. Ox-eye melanin was partially soluble in alkali and the spectra of the soluble and insoluble fractions are shown. Melanins from *Arenicola* and *Holothuria* had only a very slight solubility in alkali while *Sepia* melanin was completely insoluble. The most significant feature of these results is the implication of the similarity of the pairs of pigments, *Arenicola* and *Holothuria*, and alkali-soluble ox-eye and beetle and their dissimilarity from *Sepia*. Synthetic melanins, obtained from catechol and dopa by keeping their aqueous solutions (containing a little tyrosinase)

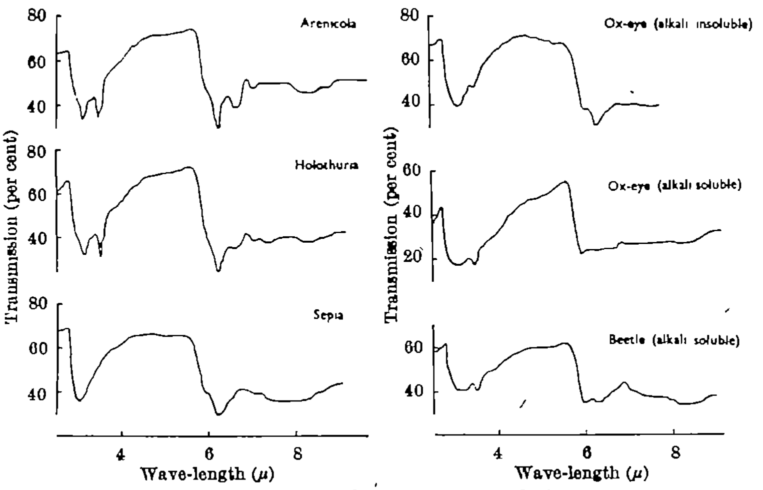

Fig. 1. The infra-red spectra of some melanins

exposed to air at 37° at pH 6·8 (phosphate buffer) and then boiling with 6 N hydrochloric acid and extracting the black residues with tetrahydrofuran, gave infra-red spectra closely similar to that of *Sepia*. In all spectra there are characteristic absorptions which can be attributed to NH (or OH) bonds (at c. 3μ) and conjugated carbonyl groups (at c. 6μ) in accord with the known basic units of melanin structure.

Confirmation of these differences in infra-red spectra was obtained through the generous co-operation of workers at the Max Planck Institute of Biochemistry in Munich during a recent visit. The samples were then prepared in potassium bromide disks and the spectra were found to be identical with those obtained with carbon tetrachloride mulls. The potassium bromide disk method is clearly capable of providing at least a semi-quantitative comparison of the characteristic absorptions recorded.

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Ascorbic Acid, Oxygen, and the Disappearance of Adrenochrome and Noradrenochrome

Adrenochrome undergoes change in the presence of ascorbic acid[1,2]. Despite the fact that derivatives of this process have been demonstrated by chromatography[2], the reaction mechanism remains obscure. For example, the necessity for a great excess of ascorbic acid is not characteristic of its reducing action elsewhere[3]. The role of ascorbic acid in the destruction of adrenochrome may well have physiological significance, especially as an explanation for the presence of 400–500 mgm. of ascorbic acid in 100 gm. of fresh adrenal tissue[4]. The present investigation probes the mechanism of the interrelationship of ascorbic acid and adrenochrome or noradrenochrome *in vitro*.

Aqueous solutions containing 0·057 M reagent-grade ascorbic acid were prepared immediately before use. Solutions containing $3 \cdot 0 \times 10^{-3}$ moles of epinephrine or norepinephrine per litre of 0·01 M hydrochloric acid were kept in the refrigerator, and aliquots were removed for daily use. Serum was separated from specimens of freshly clotted human blood and then pooled. The buffer was 0·1 M sodium phosphate of pH 7·4.

Adrenochrome or noradrenochrome was formed immediately before each experiment by bubbling oxygen for 1 min. through 15 ml. of a solution containing the indicated amount of catecholamine, 0·3 gm. of manganese dioxide[5], and 0·02 M sodium phosphate. The manganese dioxide settled within 1 min. To 4·5 ml. of the supernatant solution was added the indicated amount of ascorbic acid. The residual absorbancy of the adrenochrome or noradrenochrome was measured after various periods of time at 480 mμ in a Beckman *DU* spectrophotometer with tungsten lamp. The absorbancy of ascorbic acid at this frequency was negligible. Subtraction of the reading of the appropriate blank yielded the net absorbancy of the experimental solution. Some of the adrenochrome or noradrenochrome was added to serum immediately prior to ascorbic acid.

In view of the large excess of ascorbic acid, possible mechanisms for the disappearance of the chrome compounds might include a first-order reaction or a rearrangement in an environment modified by the ascorbic acid. The results of a series of experiments with various concentrations of ascorbic acid and noradrenochrome are presented in Table 1. Concentrations of ascorbic acid below $1 \cdot 20 \times 10^{-3}$ M had no effect on adrenochrome or noradrenochrome. With ascorbic acid constant, the noradrenochrome disappeared relatively more rapidly as its concentration increased. Increase in ascorbic acid accelerated the disappearance also. Thus, the process was not a first-order reaction, since the relative rate of disappearance of noradrenochrome was not independent of its initial concentration, nor was variation in the excess of ascorbic acid without significant effect[6].

Table 1. DISAPPEARANCE OF VARIOUS AMOUNTS OF NORADRENOCHROME IN THE PRESENCE OF ASCORBIC ACID

Concentration of noradrenochrome ($\times 10^{-3}$ M)	Concentration of ascorbic acid ($\times 10^{-3}$ M)	Time for disappearance of 20 per cent of noradrenochrome (min.)	Time for disappearance of 40 per cent of noradrenochrome (min.)
0·24	2·40	12·1	25·0
0·59	2·40	7·4	18·0
1·18	2·40	5·0	13·5
0·24	7·20	5·2	10·0
0 59	7·20	2·2	3·8
1·18	7·20	1 3	2·3

In a further series of experiments, one of three identical solutions containing noradrenochrome was vigorously oxygenated, the second nitrogenated, while the third remained in free contact with air (Fig. 1). Immediately thereafter, ascorbic acid was added to each tube. The bubbling of oxygen essentially prevented the disappearance of the noradrenochrome until air had an opportunity to diffuse into the solution, while nitrogen accelerated the disappearance. Bubbling of nitrogen for prolonged periods of time was without effect in the absence of ascorbic acid. Thus, the disappearance of the noradrenochrome was strongly dependent on the oxygen in the

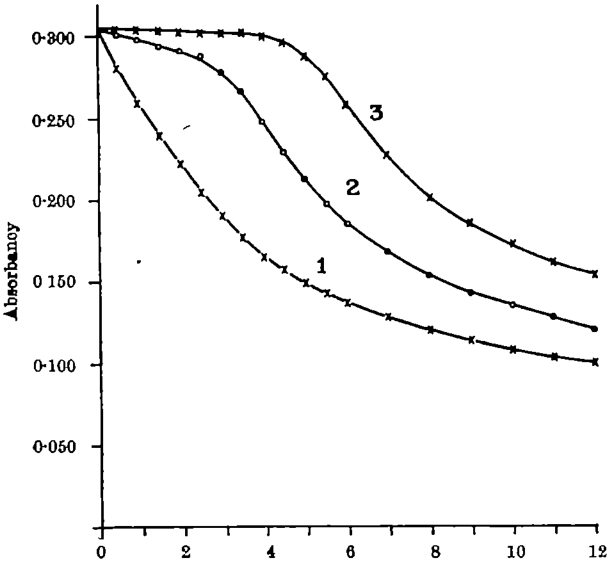

Fig. 1. Nitrogen was bubbled for 1 min. through 4·5 ml. of a noradrenochrome-containing aqueous solution (curve 1). Oxygen was simultaneously bubbled through a second similar volume (curve 3), while a third solution remained open to the air (curve 2). Immediately thereafter, at zero time, 0·4 ml. of 0·057 *M* ascorbic acid was added to each tube. The final solutions contained $0{\cdot}59 \times 10^{-4}$ *M* noradrenochrome and $4{\cdot}80 \times 10^{-3}$ *M* ascorbic acid. The absorbancies were read at 480 mμ after various periods of time

solution. Ascorbic acid ,appeared to modify the environment through reduction in the free oxygen.

Increase in the concentration of ascorbic acid by a factor of 3 caused adrenochrome and dopachrome to disappear in the same way as noradrenochrome. Replacement of 60 per cent of the water by serum did not change the results.

The formation of many stable compounds from adrenochrome indicates the existence of more than one reaction pathway. At least the first step—the disappearance of adrenochrome, noradrenochrome, or dopachrome—proceeds only in an environment modified by a large excess of ascorbic acid. The importance of the interrelationship of ascorbic acid and oxygen is that it provides a way for the disappearance of adrenochrome and noradrenochrome, which requires relative anoxia, to proceed in oxygen-containing physiological fluids such as serum. This investigation is compatible with the possibility that the maintenance of an optimum oxygen environment for physiological reactions in general may be an important role of ascorbic acid and similar substances.

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Preparation of Profibrinolysin-free Fibrinogen and Thrombin from Human Plasma

AMBRUS[1] has described an *in vitro* assay for fibrinolytic enzymes and has correlated his results with the therapeutic effectiveness of the preparations tested. Both the fibrinogen and thrombin used in this assay must be free or nearly free of profibrinolysin in order to distinguish between fibrinolysin and profibrinolysin activator activity.

Several investigators[2-4] have reported procedures for the preparation of profibrinolysin-free fibrinogen, and others[5-8] have published methods for preparing profibrinolysin-free bovine thrombin. We wish to report our methods for the preparation of profibrinolysin-free clotting reagents from human plasma which result in greater yield of product.

Profibrinolysin-free human fibrinogen was prepared by blending the Fraction I paste of Cohn[9] derived from 1 litre of normal human plasma into 45 ml. of *p*H 6·4, 0·055 *M* sodium citrate: 0·2 *M* sodium chloride buffer at 5°. The resulting suspension was warmed and stirred at 25° for at least 30 min., and then recooled to 5°. An equal volume of 2 per cent aqueous ethanol was added to precipitate the cold-insoluble globulins. After centrifugation the opalescent supernatant was adjusted to *p*H 3·8 with 2 *M* hydrochloric acid to precipitate fibrinogen with a purity of more than 80 per cent. The precipitate, recovered by centrifugation, was dissolved in 135 ml. of *p*H 3·7, 0·05 *M* acetate buffer at 5°. While stirring without aeration, 10·8 ml. of 2·9 *M* sodium chloride was added to precipitate fibrinogen of better than 90 per cent purity. This fibrinogen was recovered by centrifugation and dissolved and precipitated again in the same manner to further reduce profibrinolysin contamination. After the reprecipitation, the fibrinogen was again dissolved in 135 ml. of the *p*H 3·7, 0·05 *M* acetate buffer at 5°. To this solution was added 6·1 ml. of 2·9 *M* sodium chloride, and precipitate No. 1 was recovered by centrifugation at 5°. To the cold supernatant was added 3·1 ml. of 2·9 *M* sodium chloride and precipitate No. 2 was recovered by centrifugation at 5°. Precipitates Nos. 1 and 2 were each suspended at 5° in 15 ml. of *p*H 7·2, 0·055 *M* sodium citrate buffer containing 4 per cent dextrose. The *p*H value of each suspension was carefully adjusted to 6·5 with *M* sodium hydroxide, after which time the mixtures were warmed to 20° before further adjusting to *p*H 7·2. This *p*H was maintained while the fibrinogen was dissolved. Generally all the precipitates dissolved after stirring for 60 min. at 20°. Occasionally a small amount of insoluble material remained and was removed by centrifugation at 20°. The resulting fibrinogen solutions were adjusted to a protein concentration of 4·5 per cent by the addition of further citrate–dextrose buffer. These solutions of precipitate Nos. 1 and 2 were dried from the frozen state, and designated 'profibrinolysin-free' fibrinogen No. 1 and No. 2, respectively. We have routinely recovered 1·0 gm. of No. 1 and 1·6 of profibrinolysin-free fibrinogen No. 2 from each litre of plasma processed. These dried powders contained about 60 per cent protein of which at least 94 per cent was coagulable by thrombin.

Profibrinolysin-free human thrombin was prepared from fraction III-2 fractionated from human plasma by the method of Oncley *et al.*[10]. The prothrombin of this fraction was converted to thrombin by the use of human placental thromboplastin[11]. Two gm. of crude thrombin powder (which was obtained from about 1 litre of human plasma) was suspended in 135 ml. of 5°, *p*H 7·3, 0·5 *M* phosphate buffer. Four gm. of powdered calcium orthophosphate (J. T. Baker Chemical Co.) was added, and the mixture stirred at 5° for 30 min. prior to centrifugation. To the supernatant was added 90 ml. of cold 50 per cent (w/v) ammonium sulphate to precipitate inactive protein and trace amounts of residual profibrinolysin. After centrifugation at 5° the clear supernatant was dialysed against *p*H 7·3, 1·5 × 10^{-4} *M* phosphate buffer until free of ammonium sulphate. The imperviate, containing profibrinolysin-free thrombin, was dried from the frozen state. A yield of about 0·33 gm. of powder containing at least 30 per cent of the starting thrombin activity was obtained.

For use in the Ambrus[1] assay the dry profibrinolysin-free fibrinogen was dissolved in *p*H 7·2, 0·05 *M* imidazole buffer containing 0·05 *M* sodium chloride to give a protein concentration of 0·6 per cent. The profibrinolysin-free thrombin was dissolved in *p*H 7·2, 0·05 *M* imidazole buffer to give 10 NIH thrombin units per ml. When tested for profibrinolysin contamination, by titration with streptokinase according to the procedure described by Ambrus, lysis times of over 5 hr. (less than 0·005 profibrinolysin units) were obtained with profibrinolysin-free fibrinogen No. 1—profibrinolysin-free thrombin clots, while lysis times of well over 10 hr. (less than 0·001 profibrinolysin units) were obtained with profibrinolysin-free thrombin clots. Since the addition of 1 RPMI unit of fibrinolysin to similarly prepared clots will result in a lysis time of 2 min., the profibrinolysin contamination in these reagents may be considered as of no consequence.

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Structure of a Monophosphoinositide from Brain

SEVERAL workers[1-4] have reported the presence of monophosphoinositide (MPI) in phospholipid fractions originating in brain tissue. Only Dittmer and Dawson[3], however, claim to have isolated a pure sample of a phospholipid analysing as a simple MPI, and they did not elucidate its structure. Brockerhoff and Ballou[4] have suggested that the D-glycerol 1-(L-myoinositol 1-phosphate) residue is contained in brain MPI, but they did not isolate the parent lipid or its deacylation product in a pure state.

A chromatographically pure sample of MPI has been isolated from brain and its structure established using degradative methods similar to those already applied successfully to horse liver MPI[5].

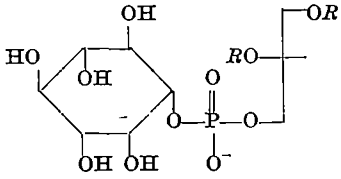
Following a modified Folch procedure[5,6], an ethanol-insoluble fraction of phospholipids was isolated in a yield of 1·35 per cent of wet bovine brain tissue. Chromatographic analysis of this brain fraction on formaldehyde-impregnated paper[7] showed that it consisted largely of phosphatidyl ethanolamine and phosphatidyl serine and that it also contained small amounts of MPI, phosphatidyl choline, and complex phosphoinositides.

We were unable to separate chromatographically pure MPI from nitrogen-containing phospholipids by chromatography on aluminium oxide or silicic acid columns. However, dinitrophenylation by means of fluorodinitrobenzene (FDNB) of the ethanol-insoluble phospholipids with a modification of a procedure due to Wheeldon and Collins[8] led to complete reaction of the nitrogen-containing phospholipids and a consequent increase in R_F values. The chromatographic properties of the MPI were unchanged and a separation of the latter from the yellow dinitrophenylated lipids was readily achieved. A pure sample of horse liver MPI, when treated under the same conditions, but omitting the FDNB, was essentially unaffected; a small proportion (< 10 per cent) was converted to a phosphatidic acid-like compound.

A sample (6·5 gm.) of the ethanol-insoluble fraction in dry benzene (100 ml.) was treated with FDNB (1·45 gm.) in the presence of triethylamine (2·5 ml.) at 30–40° C. for 2 hr. Evaporation of the reaction mixture to dryness gave a yellow residue which was dissolved in chloroform for application to a silicic acid–Hyflo Supercel column (2 : 1 w/w, 405 gm.; 4·2 cm. diameter). A stepwise eluting technique was used. Chloroform/methanol (6 : 1 v/v) first removed non-lipid material; further elution with chloroform/methanol (6 : 1 v/v) and then (4 : 1 v/v) brought dinitrophenylated phospholipids and small amounts of a substance chromatographically similar to phosphatidic acid off the column. Chromatographically pure MPI was then eluted by chloroform/methanol (3 : 2 v/v), giving the proportion of MPI in brain tissue as 0·03 per cent. This sample contained an unestimated amount of plasmalogen groups.

The chromatographically pure brain MPI (100 mgm.) was treated with hydroxylamine for 10 min., which led to complete deacylation. Working up as before[5] gave an aqueous phase containing glycerol myoinositol phosphate, with traces of glycerol 1- and 2-phosphate. After the separation of the glycerol myoinositol phosphate by preparative paper chromatography, the *cyclo*hexylammonium salt was formed in aqueous solution. It had an $[\alpha]^{20}_{589}$ in water of − 11 ± 2°. Its specific rotation and chromatographic properties showed that it was identical with the corresponding compound from horse liver MPI; the latter has been proved to be *cyclo*hexylammonium D-glycerol 1-(L-myoinositol 1-phosphate)[5].

The phosphate-myoinositol linkage position was confirmed by a degradation to myoinositol 1-phosphate. The *cyclo*hexylammonium salt (6 mgm.) of the glycerol myoinositol phosphate was treated with an excess of sodium metaperiodate for 15 min. at room temperature. Glycerol was then added to destroy the remaining periodate. After a further 15 min., phenylhydrazine formate at *p*H 6 was added to the solution. The reaction mixture was incubated at 40° for 5 hr. and then centrifuged. The clear supernatant was acidified with 'Dowex-50 (H+)' resin, neutralized with dilute ammonia, and investigated by paper chromatography. The product was myoinositol 1-phosphate; traces of inorganic phosphate were also observed. Acidic equilibration of the myoinositol 1-phosphate gave a chromatographically resolvable mixture of myoinositol 1-phosphate and myoinositol 2-phosphate. This establishes the structure of the deacylated product as I (*R* = H).



I

The locations of the fatty acid groups were determined from the raction of the MPI with acetic acid[8]. MPI (3 mgm.) was heated in 88 per cent acetic acid (0·2 ml.) at 100° for 20 min. The solvent was then evaporated off leaving a residue which was partitioned between chloroform and water. Myoinositol 1-phosphate and myoinositol 2-phosphate, but no glycerol phosphate, were detected in the aqueous phase. The chloroform solution contained diglyceride and monoglyceride and in addition small amounts of unreacted MPI and lysophosphatidyl inositol showing that the acyl groups are on the glycerol residue. The nature of the fatty acid residues has not been investigated.

From the above evidence it is concluded that the brain monophosphoinositide is 2,3-di-*O*-acyl-D-glycerol 1-(L-myoinositol 1-phosphate) (I; *R* = acyl residue), accompanied by a smaller amount of a corresponding plasmalogen.

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Mechanism of Antitumour Action of 1-Amino-*Cycl*opentane Carboxylic Acid

The unnatural amino-acid 1-amino-*cyclo*pentane carboxylic acid (ACPC) has been reported to inhibit the growth of Novikoff rat hepatoma[1], Walker rat carcinoma 256 [2], sarcoma 180 and carcinoma 755 [3]. Berlinguet *et al.*[4] recently reported that this unnatural amino-acid does not undergo any metabolic change in normal rat, and so far nothing is known concerning the mechanism of its action.

Steinberg *et al.*[5] and many other workers[6,7] previously demonstrated that synthetically prepared amino-acid analogues like the amino-acids themselves could be incorporated into proteins and they can thus compete with the natural amino-acids for the latter's incorporation into proteins. That ACPC might act as an amino-acid antagonist was first mentioned by Ross[3]. Mickelson[8], using bacterial systems, failed to demonstrate any such effect of ACPC. In order to elucidate the mechanism by which this unnatural amino-acid exerts its antitumour effect, the following experiments were performed.

A 20 per cent rat liver homogenate was prepared in a Dounce homogenizer at 0° C. in a medium containing 0·35 *M* sucrose, 0·25 *M* potassium chloride, 0·035 *M* potassium bicarbonate and 0·004 *M* magnesium chloride, and centrifuged at 2,300*g* in the cold. 0·05 ml. of the supernatant fraction containing the microsomes and cell sap was incubated at 37° C. for 30 min. with ^{14}C-ACPC (1·5 × 10^5 counts/min.) or with ^{14}C-valine (0·5 × 10^5 counts/min.) in the presence of 3 μmoles of ACPC, 5 μmoles of Mg^{++}, 2 μmoles of ATP, 5 mgm. of creatine phosphate and 50 μgm. (protein) of purified creatine kinase. The reaction was terminated by adding 5 ml. of 5 per cent TCA. The TCA precipitable material was washed, plated and counted according to the procedures previously described by Devi *et al.*[9].

Table 1. Incorporation of ^{14}C-Valine into Proteins in the Absence and Presence of 1-Amino-*cyclo*pentane Carboxylic Acid

	c.p m /mgm. TCA-precipitable material			
Source of microsome-cell sap preparation	No. of Expts.	Range of values	Range of values in presence of ACPC	Percentage of inhibition
Liver of normal rat	5	142–160	84–96	40
Liver of tumour bearing rat (Walker)	4	130–146	86–98	32
Walker tumour	4	150–166	96–108	36

For *in vivo* experiments, five rats weighing 120–140 gm. each received an injection of 20 mgm. of ACPC 48 hr. prior to the administration of ^{14}C-valine (1·2 × 10^6 counts/min.); in another set of experiments ^{14}C-valine was injected after 3 hr. of the administration of ACPC. A similar number of rats received only ^{14}C-valine, which served as controls. The animals were killed 3 hr. after injection of carbon-14. 1 gm. of liver from each rat was homogenized in 5 ml. of 0·25 *M* sucrose and to 1 ml. of such homogenates 5 ml. of 5 per cent TCA added. The washings, plating and counting of the TCA-precipitable material were similar to those described before. Similar experiments were also performed with tumour (Walker)-bearing rats, where 1 gm. of the tumour tissue was homogenized, precipitated and the precipitate was washed, plated and assayed for its radioactivity.

The results can be summarized as follows. When the microsome-cell sap fraction was incubated with ^{14}C-valine and ACPC, the incorporation of carbon-14 into proteins was reduced to varying degrees depending upon the amount of ACPC present. The maximum inhibition noted was 41–47 per cent in the presence of 10 μmoles of ACPC. This effect of ACPC to inhibit the incorporation of ^{14}C-valine was also noted when microsome-cell sap preparation obtained from Walker tumour was used. When ^{14}C-ACPC was used instead of ^{14}C-valine no incorporation of carbon-14 into proteins could be demonstrated.

In *in vivo* experiments where ACPC (1·56 × 10^7 counts) was injected 48 hr. prior to the injection of ^{14}C-valine, the extent of the latter's incorporation into proteins was reduced by 32–40 per cent. Somewhat less (25–28 per cent) inhibition was noted when ACPC was administered only 3 hr. prior to the injection of ^{14}C-valine. When ^{14}C-ACPC alone was injected, a very small but definitely measurable amount of it was found to be incorporated into proteins.

Table 2. Inhibition of Incorporation of ^{14}C-Valine, *in vivo*, into Proteins by 1-Amino-*cyclo*pentane Carboxylic Acid

Source of homogenate	c.p.m./mgm. TCA-precipitable material Controls	ACPC administered 8 hr. prior to ^{14}-C injection	Percentage of inhibition	ACPC administered 48 hr. prior to ^{14}-C injection	Percentage of inhibition
Liver of normal rat	90	66	27	48	47
Liver of tumour bearing rat	82	58	29	44	46
Tumour	102	75	27	60	41

Each value is an average of four such determinations.

The incorporation of ^{14}C-uridine into RNA was not inhibited by ACPC whether the incorporation of ^{14}C-uridine was carried out *in vitro* or *in vivo*. The procedure for these experiments is similar to those of Devi and Sarkar[10]. ACPC did, however, prevent the incorporation of ^{14}C-valine into transfer RNA by 40–50 per cent in the experiment where ^{14}C-valine was incubated with high-speed supernatant fraction in the presence of ACPC, Mg^{++} and ATP.

It follows from the foregoing results that ACPC does inhibit the incorporation of ^{14}C-valine into proteins to an extent of 40–50 per cent.

The initial stages of protein synthesis, as we know them to-day, are the activation of amino-acids by specific amino-acid-activating enzymes, followed by their incorporation (attachment) into specific soluble RNA's. In the second stage, the amino-acids from *S*RNA-aa's complexes are transferred to template RNA's whereby some hitherto unknown mechanism, protein with proper amino-acid sequences, is formed and released[11]. The fact that ACPC does inhibit the incorporation of ^{14}C-valine by 40–50 per cent suggests that this unnatural amino-acid perhaps exerts its antitumour effect by suppressing protein synthesis although no significant amount of it has been found to be incorporated into proteins. This is not unexpected as several analogues have been recently shown to inhibit protein synthesis without being incorporated into proteins[12,13]. Our results also indicate that the most likely site of its action is at the initial stage of protein synthesis because in the presence of ACPC the incorporation (attachment) of ^{14}C-valine into *S*–RNA is reduced by 40–50 per cent.

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Hormone Influence on the Thiol Content of *Hevea brasiliensis* Latex

Field experiments carried out in Malaya[1] have proved that the application of certain growth regulators, such as 2 : 4-dichlorophenoxyacetic acid (2 : 4-D), to mature *Hevea brasiliensis* trees will effect substantial increases in rubber yield. This communication shows that a further and possibly correlated effect of these growth regulators is to alter the ratio of reduced to oxidized low-molecular-weight thiol content of the latex.

It has previously been shown[2,3] that the redox potential of fresh latex is probably controlled by such sulphydryl components the nature and concentration of which have been determined[4]. The application of growth regulators influences markedly the general metabolism of pea seedling tissues and of bean[5] seedlings, with concomitant changes in sulphydryl redox[6].

Using the colorimetric method for thiol previously adopted, the —SH and —S : S— content of the acid-soluble portion of small (50 mgm.) samples of latex containing upwards of 1·6 μgm. —SH were determined, before and after reduction with zinc and hydrochloric acid, with a reproducibility within 3 per cent. It was found that when young seedlings of *Hevea brasiliensis* are maintained in tropical conditions of temperature and humidity, but under subdued and constant illumination, then the endogenous acid-soluble thiol content of the latex, the 'equilibrium' content, is reached at a value of 1·5–2·0 μequiv./gm. latex.

The percentage of thiol in the reduced condition, a reflexion of the redox potential, is rather variable. Application of hormone to the leaves or stem of such plants generally increases the reduced thiol content and lowers the oxidized content towards zero in the space of 48 hr. Table 1 shows the effect of gibberellic acid, applied in alcohol–water (50 : 50) solution to freshly tapped 1 year-old seedlings.

Table 1

	μ equiv. —SH/gm. latex (1-yr.-old seedlings)						
Reduced	1 9	1·7	1·7	1·6	2·5	2·5	3·7
Oxidized	2·5	2·7	2·9	2 7	0	0·6	0
Hours after application	− 20	− 10	− 5	+ 5	+ 45	+ 55	+ 60

The reduced thiol content, prior to treatment, in this case averaged 40 per cent, rising to 100 per cent thereafter. There was a tendency in a few other instances for this percentage to rise slightly during the pre-treatment period, possibly due to increased metabolic activity resulting from loss of latex on

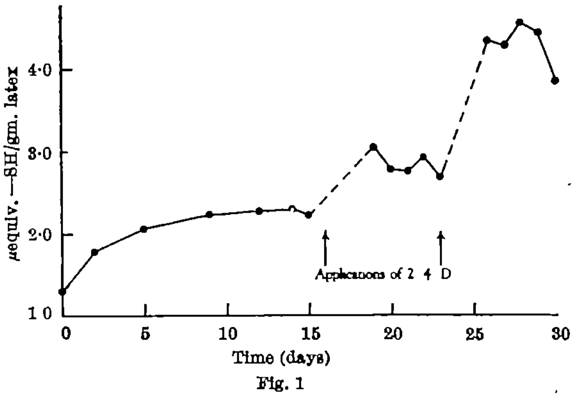

Fig. 1

tapping. This effect was noticeable on more prolonged tapping.

Fig. 1 illustrates the influence of 2 : 4-dichlorophenoxyacetic acid on 2-year-old potted seedlings. Here the pretreatment percentage of reduced material varied in the 60–80 range, at a final 'equilibrium' thiol level of about 2·3 μequiv./gm. Several applications of the hormone increased the percentage to 100 at a final thiol content of approximately 4·5 μequiv./gm. This was followed by a fall towards the equilibrium values.

Since the conversion of 'active isoprene' (isopentenyl pyrophosphate) to rubber may be dependent on the activity of an isomerase which is inhibited by sulphydryl inactivators[7], the possibility exists that the incorporation of this precursor into rubber is influenced by the redox potential and that the latices from growth regulator-treated plants will exhibit greater efficiency in this respect. Experiments are to be carried out to test this possibility.

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Effect of Ascorbic Acid in Aniline Poisoning

ASCORBIC acid has been utilized extensively in large doses in the therapy of industrial methæmoglobinæmia, although Halsted[1] commented that at developments greater than 60 per cent methæmoglobinæmia, it is ineffective because of its lack of ability to bring on rapidly a reversion mechanism. But the rapidity of reversion is only the first problem. The second problem to be settled is whether, in chemical cyanosis, the effect of ascorbic acid may not be restricted only to bring about the reduction. It is known that ascorbic acid promotes the rate of hydroxylation of aniline to *p*-aminophenol[2], which is a potent agent in the production of Heinz bodies not only *in vivo* but also *in vitro*[3], and ascorbic acid is able to produce Heinz bodies *in vitro* without any toxic agents[4]. We have found that in rats poisoned with aniline the formation of Heinz bodies is promoted by treatment with ascorbic acid.

Twenty male white rats each weighing 270–320 gm. were used. All animals received intraperitoneal injections of 1 per cent aniline (0·08 ml. aniline/kgm.), and 250 gm. ascorbic acid (in 10 per cent buffered solution) was administered to 10 of them by the tail vein immediately before injecting aniline. The methæmoglobin concentration was determined as a percentage of total hæmoglobin by means of Evelyn and Malloy's method[5] modified by Magos[6] at 1, 3, 5, 7, 24 and 48 hr. after the treatment. Proportions of Heinz body red cells were determined at 48 hr. in wet preparations stained with methyl violet. Not only was the percentage of Heinz body red cells determined, but also the intensity of Heinz body formation by means of an index. 250 red blood cells were carefully examined and the intensity of Heinz body formation in a single cell was signed with index numbers as follows: no Heinz body, 0; one small Heinz body, 1; two small Heinz bodies or of one moderate size, 2; two moderate Heinz bodies or one conglomerate, 4. In the following equation n is the number of cells in the group.

$$\text{Heinz body index} = \frac{(1 \times n_1) + (2 \times n_2) + (4 \times n_4)}{n_0 + n_1 + n_2 + n_4}$$

On the basis of the results given in Table 1, it seems that, although ascorbic acid decreases the degree of maximal methæmoglobinæmia (the period of the methæmoglobinæmic phase remained unchanged), its effect on intensifying Heinz body formation is much more significant.

Table 1. MAXIMAL METHÆMOGLOBINÆMIA, HEINZ BODY RED CELLS (H.B.R.C.) AND HEINZ BODY INDEX (H.B.I.) IN ANILINE POISONING WITH OR WITHOUT ASCORBIC ACID TREATMENT

	Treatment				
	Aniline	Aniline ascorbic acid	S_D	t	P
Maximal methæmoglobinæmia	26·5 ± 4·27	22·7 ± 3·06	1·68	2·262	$0·05 > P > 0·02$
H.B.R.C.	46·5 ± 10·4	65·0 ± 11·4	4·88	3·818	$0·01 > P > 0·001$
H.B.I.	1·34 ± 0·54	2·23 ± 0·37	0·210	4·833	$0·001 > P$

These findings indicate that, apart from the lack of ability to bring about rapidly the methæmoglobin reduction, it is advisable to avoid the therapeutic use of ascorbic acid in chemical cyanosis because of its irreversible effect.

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PHYSIOLOGY

Regular Impulse Activity of Single Units in the Cat Striate Cortex

IN the course of an investigation into the spontaneous and evoked activity of single cells in the visual cortex of unanæsthetized, unrestrained cats, a number of cells have been discovered, remarkable for their regularity and rate of discharge. Each of these cells, of which five have so far been observed, discharged a single spike whenever it fired, and each spike was followed by a long silent period lasting many seconds. As may be seen in Fig. 1, the inter-spike intervals for a given cell are, with few exceptions, distributed over a very narrow range. In calculating the mean value of the inter-spike intervals of cells 2, 3 and 4, certain intervals were omitted. In cell 2, for example, the interval which was omitted lasted 20·29 sec., that is, approximately twice the modal inter-spike interval (9·5–9·99 sec.) for this unit. Similar prolonged periods of silence occurred occasionally between action potentials from cells 3 and 4, but always these gaps were, to a close approximation, a multiple of the modal interval. It is possible that the missing spikes were lost in the base-line of the

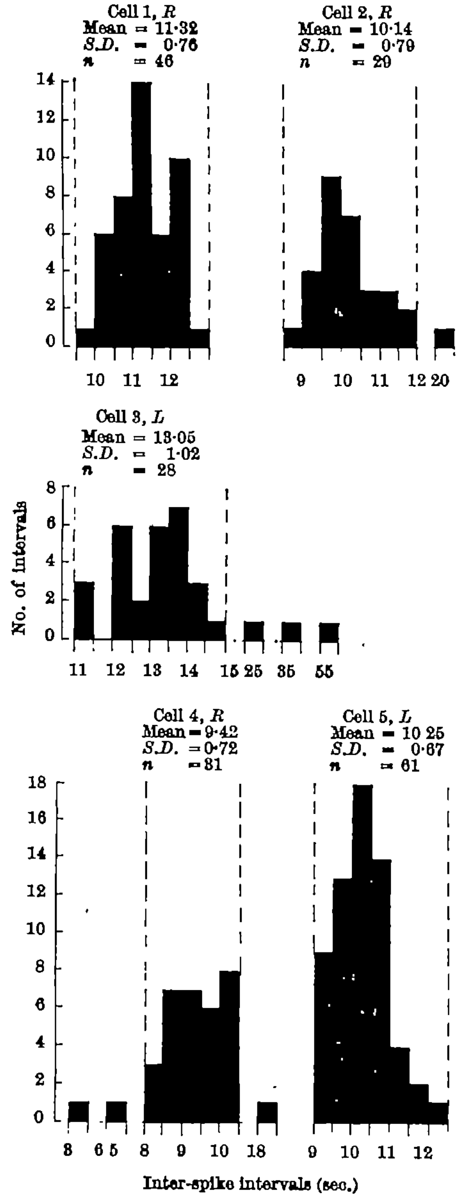

Fig. 1. Histograms of inter-spike intervals. The hemisphere in which each cell was found is indicated by *L* (left) and *R* (right). The mean (in sec.) and standard deviation (*S.D.*) for each cell refer to the intervals (*n* in number) enclosed by the broken lines

record; but there were instances where the failure to fire appeared genuine, for careful examination of those records where the signal/noise ratio was particularly good failed to reveal the missing spike. That this property of maintaining the overall rhythm is real is suggested from the behaviour of cell 4. The mean value of the regular inter-spike intervals of this cell was 9·42 sec.; but of the three intervals excluded in calculating this value, one was 18·39 sec. (approximately twice the expected interval), one was 6·60 sec. and one was 3·21 sec. These last two intervals occurred in succession so that the overall regularity of discharge was maintained.

The five cells were recorded at various depths below the pial surface of the striate cortex of both right and left hemispheres. They were all found in one cat from which it had been necessary, for reasons relevant to the original experiment, to take prolonged and continuous film records of cortical impulse activity. The existence of these cells was not suspected until the film records were analysed, and they would certainly not have been discovered without such extensive records, for there would then have been too few spikes to give a statistically useful number of intervals. Each unit was discovered while investigating the activity of another cell which discharged irregularly and with a much higher frequency, and the action potentials of which were larger than those of the regularly firing units. The microelectrode thus recorded simultaneously from two cells. The regularly firing cells were not seen on the oscilloscope at the time of filming, in part because they fired so rarely, but also because they were relatively small, with an amplitude in the region of 0·5 mV. Action potentials of this size could be generated by a small cell lying close to the tip of the microelectrode. However, since these small spikes appeared with other, larger, spikes from another cell, one cannot exclude the possibility that the smaller spikes were generated by a cell situated some distance from the microelectrode tip. In this position the cell would be less exposed to the risk of mechanical stimulation by the electrode than might otherwise be the case. Finally, the rate of discharge of the regularly firing cells was quite unaffected by diffuse retinal illumination or by weak electric shocks applied to the skin of the animal, although the other cells the spikes of which appeared on the same trace were all affected by one or both of these stimuli.

In considering the mechanism of activation of the regularly firing cells, two simple hypotheses present themselves. On one hand, the cells might be part of a very long chain of cells showing reverberating activity. However, with the exception of cells 2 and 5 (which came from right and left hemispheres respectively) the mean value of the intervals of each cell differed significantly ($P < 0·001$) from that of all the other cells. Since all these units came from the same animal, it is necessary to suppose that there are at least four such chains showing reverberating activity, each firing independently of the other. On the other hand, the pattern of discharge could be an expression of some chemical excitatory process occurring independently within each of the five cells. On the basis of the small range of variation of the inter-spike intervals of each unit, and the relatively wide range of variation of the means between units, the latter hypothesis seems the more economic and attractive.

I thank Miss Angela Reed for help and the Medical Research Council for financial support.

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Osmotic Changes caused by the Accumulation of Dulcitol in the Lenses of Rats fed with Galactose

CATARACTS produced experimentally in rats maintained on a diet high in galactose or xylose or by alloxan injection have similar clinical manifestations[1]. Since the development and appearance of these cataracts are similar, there is the suggestion that a common mechanism or factor may be involved. We wondered whether the accumulation of polyols first

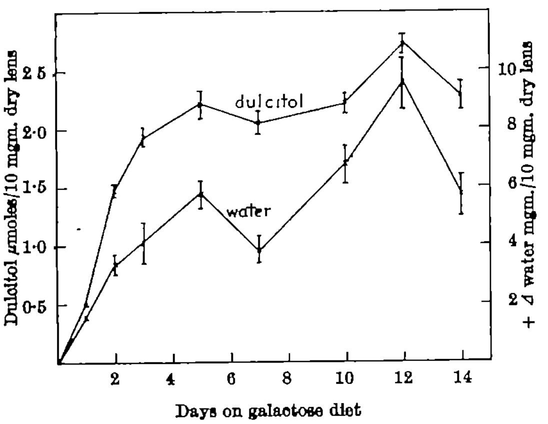

Fig. 1. Accumulation of water and dulcitol in the lenses of galactose-fed rats

observed by van Heyningen[2,3] might possibly be the initiating factor in these cataracts. It seemed possible that the accumulation of polyols, along with the keto sugars, in the lens fibres might be sufficient to create an osmotic disturbance. Because of the increase in the osmotically active substances, water would be drawn into the lens fibres. Since sugars, especially the sugar alcohols, do not readily diffuse through biological membranes, their concentrations might reach a level which would increase the volume of water sufficiently to cause the lens fibres to swell and perhaps to rupture their membranes.

To test this possibility we measured the accumulation of hexitol in the lenses of the galactose-fed rats. Sprague–Dawley strain of rats weighing about 100 gm. were put on a diet of 50 per cent galactose as described by Yudkin and Arnold[4]. Control rats were fed the same diet but with 50 per cent glucose. The analyses of various sugars in the lens were made on a protein-free filtrate after precipitation by barium hydroxide and zinc sulphate. Hexitol was determined by the procedure of West and Rapoport[5] as modified by Faulkner[6]. The other sugars were determined by conventional methods.

The galactose cataract appears to be a simpler disorder to examine than the alloxan diabetic cataract. First of all, the cataract develops earlier than in diabetes. Secondly, the level of blood sugar does not need to be as high as that in diabetes to produce a cataract. The most significant difference is that dulcitol, the sugar alcohol form of galactose, is the only derivative which accumulates in the lens. Enzymatic studies using lens extracts indicated that two enzymes are involved in the metabolism of polyols. In the presence of reduced triphosphopyridine nucleotide, galactose and glucose are converted to their alcohol form by aldose reductase. A second enzyme, polyol dehydrogenase, in the lens of the rat, man, rabbit, cat and calf catalyses the oxidation of sorbitol to fructose, but apparently does not attack dulcitol. Since dulcitol does not appear to penetrate the lens membranes readily, once formed it accumulates.

In Fig. 1 is shown the accumulation of dulcitol and water in the lenses of rats fed galactose for varying periods of time. In these experiments approximately 100 rats were used, half of them placed on the galactose diet and the other half on the control glucose diet. One lens from each animal was used to determine the dulcitol content while the other was used to obtain wet and dry weights. The lenses of the rats fed glucose for a period up to 15 days showed no appreciable increase in the hexitol content from that observed in the normal rat lens (0·04 µmole per lens). On the other hand, as shown in Fig. 1, there was a rapid increase in both dulcitol and water in the lenses of the galactose-fed rats. In most cases the increase in water and dulcitol appears to be proportional. The results are based on 10 mgm. of dry weight of lens, the approximate weight of the rat lenses used.

The accumulation of dulcitol in these lenses was considerable. The lenses of rats on the galactose diet for 3 or more days contained dulcitol, which accounted for 3–5 per cent of the dry weight of the lens. Since galactose-1-phosphate has been suspected of playing a part in the development of cataracts in galactosæmic patients and rats fed galactose[7,8], it was of interest to relate the amount of dulcitol found in these experiments to that amount of galactose-1-phosphate accumulating under similar circumstances. Korc[9] has reported that in rats fed galactose for 11 days there was less than 0·005 µmole of galactose-1-phosphate accumulated per lens. The results as shown in Fig. 1 revealed that 2·2 µmoles of dulcitol per lens were found after 10 days on the diet. Paper electrophoretic and chromatographic examinations by the procedures of van Heyningen[2] identified the sugar accumulating as dulcitol. No significant amount of sorbitol was detectable. After 3 days, the levels of dulcitol and water appeared relatively constant with the exception of the increase observed at 12 days. This plateau may be more apparent than real since one of the factors not controlled was the amount of galactose ingested. Usually after 5 days on the diet, the rats did not appear to consume as much food. The sudden increase observed at 12 days was caused most likely by the higher intake of food by this group of rats.

The first sign of opacities was observed ophthalmoscopically in the lenses of rats maintained for 5 days on the galactose diet. At this stage of cataract development, the lens had accumulated 2·2 µmoles of dulcitol. This represents a concentration of 72 µmoles of dulcitol per gm. of lens or 117 µmoles per ml. of lens water. The level of dulcitol found here is lower than that reported by van Heyningen[2], who estimated the concentration of dulcitol by paper chromatographic procedures to be 110 µmoles per gm. of lens. The increase of water in the lenses of rats fed galactose for more than 5 days ranged from 4 to 9 mgm., representing an increase of 20–35 per cent over the normal water content of the lens. The effect of the increase in water may be more pronounced than it appears since the accumulation of both water and dulcitol is probably not distributed uniformly but confined to the superficial layers of the lens.

The results obtained here suggest that the hypertonic condition created by the accumulation of dulcitol produces a net increase in the water content, which may be sufficient to lead eventually to the rupture of the lens fibres. Further investigations are being made to determine whether this osmotic change adequately describes the clinical manifestation observed not only in galactose cataract but in the diabetic and xylose cataracts as well. The differences in the course and rate of development of these cataracts may be directly related to the properties of the enzymes involved in the metabolism of polyols.

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Effect of Age and Thymectomy on Urinary 17-Ketosteroid-levels in Male Rats

THE question whether or not there is an endocrine function of the thymus is one that has been much debated. Various investigators have claimed that thymectomy caused retardation of growth and gonadal development[1] and that injections of thymic extracts induced precocious development[2]. Others[3–5] have refuted these claims. Comsa[6] reported that thymectomy of male guinea pigs caused transitory stimulation of the adrenal cortex as evidenced by significant decreases in the levels of adrenal cholesterol and ascorbic acid. The study, however, lacked comparable sham-operated controls. In view of the various claims, we tried in the present investigation to determine whether the acute and chronic effects of thymectomy on adrenal and gonadal androgenic output are reflected by alterations in urinary 17-ketosteroid-levels.

The excretion of urinary androgens has been frequently used as an index of adrenocortical activity[7,8] as well as an index of adrenal and gonadal function[9]. An advantage of the urinary procedure is that it permits total estimation of the daily ketosteroid production of the gonadal and adrenal hormones.

Albino male Wistar rats averaging 50 gm. were matched by weight and divided into 3 groups (thymectomized rats, group *A*; sham-thymectomized rats, group *B*; and normal un-operated rats, group *C*). Thymectomy was accomplished by the method of Farris and Griffith[10]. Identical procedures were utilized in the sham-thymectomized animals except for the removal of the thymus. At the 4th, 12th and 24th weeks, a 24-hr. urine sample was collected from each of the rats. Two ml. volumes were tested for 17-ketosteroid content by the method of Rappaport *et al.*[11].

Table 1 lists the data in terms of urine volume, 17-ketosteroid content/ml. and total daily 17-ketosteroid-levels. It is apparent that, at the fourth week, no significant changes were observed in either urine volumes or ketosteroid-levels when the various group differences were compared by *t*-test procedures[12].

Table 1. EFFECT OF AGE AND THYMECTOMY ON URINARY 17-KETOSTEROID-LEVELS IN MALE RATS

Group	n	Body-weight (gm.)	Daily urine (vol., ml.)	17-ketosteroid (μgm./ml.)	17-ketosteroid (μgm./day)
4 week period					
Group *A* : Thymectomized	11	197·6	4·2	6·3	26·3
B : Sham-thymectomized	11	175·0	4·1	5·4	21·9
C : Normal unoperated	12	188·9	4·0	6·3	23·2
Per cent differences between *A* and *B*		+12·9	+2·4	+16·7	+20·1
P value			0·84	0·35	0·31
Per cent differences between *A* and *C*		+4·6	+5·0	—	+13·4
P value			0·79	>0·90	0·45
Per cent differences between *B* and *C*		−7·4	+2·5	−14·3	−5·6
P value			0·90	0·26	0·76
12 week period					
Group *A* : Thymectomized	20	358·6	6·4	13·2	87·2
B : Sham-thymectomized	18	374·7	7·3	12·4	88·4
C : Normal unoperated	20	349·1	6·3	10·7	64·5
Per cent differences between *A* and *B*		−4·3	−12·3	+6·5	−1·4
P value			0·23	0·70	>0·90
Per cent differences between *A* and *C*		+2·7	+1·6	+23·4	+35·2
P value			>0·90	0·18	0·16
Per cent differences between *B* and *C*		+7·3	+15·9	+15·9	+37·1
P value			0·20	0·32	0·06
24 week period					
Group *A* : Thymectomized	18	469·0	7·3	11·8	86·6
B : Sham-thymectomized	17	484·6	8·2	11·6	95·8
C : Normal unoperated	18	463·4	6·1	11·6	70·5
Per cent differences between *A* and *B*		−3·0	−11·0	+1·7	−9·6
P value			0·12	>0·90	0·56
Per cent differences between *A* and *C*		+1·2	+19·7	+1·7	+22·83
P value			0·03	>0·90	0·3
Per cent differences between *B* and *C*		+4·6	+34·4	—	+35·9
P value			<0·01	>0·90	0·08

However, certain changes do appear at the 12th and 24th week. It is of interest to note that, at both periods, the total ketosteroid-levels in the sham-operated rats were greater than that of the normal un-operated controls and approached the 0·05 level of significance. At the 24th week, both the thymectomized and sham-operated animals had a significantly higher urinary output than the normal unoperated rats. Nevertheless, the 17-ketosteroid/ml. values were essentially similar in all 3 groups. Probably, the total ketosteroid decrease observed in the normal un-operated group could most likely be attributed to the smaller urine volumes collected from these rats. Claims have been presented that 17-ketosteroid excretion-levels are dependent on urine volume[13]. In turn, the ketosteroid and urine volume decreases may possibly be correlated with the body-weight differences.

In general, the 17-ketosteroid determinations did not reflect any short- or long-range effects of thymectomy on either adreno-cortical or androgenic output. Consequently, the data differ from previous claims of gonadal inhibition[1] and reports of transitory adrenocortical stimulation[6]. Certainly, the significant increases observed in the 17-ketosteroid values of the three groups at the 12th and 24th week periods as compared with the 4th week suggest the predominant role the gonads assume at the later periods.

Although Comsa[6] observed that the greatest decrease in adrenal ascorbic acid and cholesterol contents occurred 12–24 days after operation, the sample sizes of the thymectomized guinea pigs killed at the various age-levels were small and the study lacked sham-operated controls. The possibility does arise, however, that species differences may exist as well as a time factor since the present study initiated the 17-ketosteroid analyses at a 28-day post-thymectomy period.

Previous investigations of thymectomy demonstrated an increase in histamine LD_{50}, reflecting an increase in the 'histamine-resistance' function of the adrenals[14,15]. Thymectomy also increased resistance to drowning stress[15], another possible reflexion of adrenal-related work performance. Whereas adrenalectomy significantly reduced rat tolerance to histamine[16], histamine tolerance was raised by thymectomy. The findings of no changes in 17-ketosteroid output following thymectomy suggest a dichotomy between absolute output of adreno-cortical hormones and the physiological effects of that output as influenced by the removal of counter-balancing endocrine organ glands, in this case the thymus.

In conclusion, following thymectomy previous evidence of an increase in adreno-cortical function as shown by histamine tolerance studies was not associated with an increase in 17-ketosteroid output. This increase in histamine tolerance following thymectomy may reflect the removal of an anti-adrenal cortical action on the part of the thymus gland with respect to histamine tolerance; and/or the limitation of 17-ketosteroid determination as a measure of adreno-cortical output. Sackler *et al.*[17,18] have postulated a physiodynamic scheme of hormonal equilibrium consisting of a synergistic and antidyne relationship between the adrenal glucocorticoid hormones on one hand and the thyroid, gonad and a possible thymic hormone on the other.

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ANATOMY

Effect of Testosterone on Œstrogen-induced Bone Formation in Mice

It is now well established that the parenteral administration of large doses of œstrogens to mice results in the deposition of new bone in the marrow cavities. This change is most pronounced in the long bones. Here the new bone is first laid down at both extremities on the trabeculæ of the spongiosa and then, becoming more compact, it extends along the longitudinal axis of the shaft towards the centre of the marrow cavity. Among mammals this response to œstrogens is peculiar to the mouse. It has provided us with an important method for studying the factors which influence bone formation.

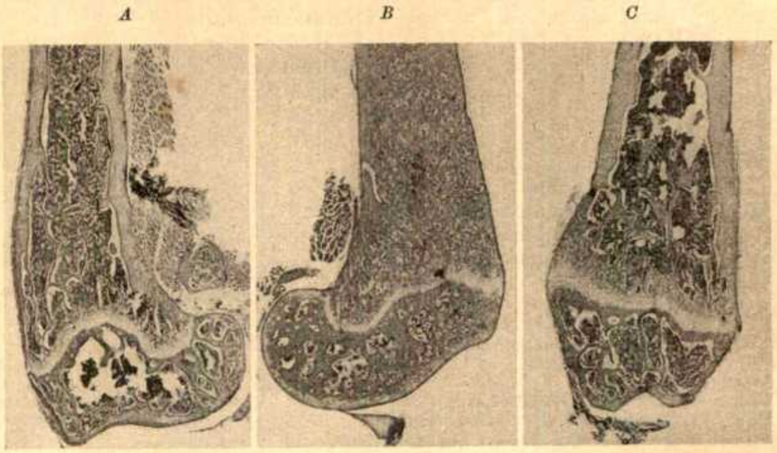

Fig. 1. Longitudinal sections of lower ends of three mice femurs. *A*, Control; *B*, 15-week-old male after 12 weeks treatment with 0·1 mgm. œstradiol monobenzoate per week; *C*, 15-week-old male after 12 weeks treatment with 0·1 mgm. œstradiol monobenzoate and 2·5 mgm. testosterone propionate per week

While many variables, such as the age and the sex of the animals, have been shown to affect the rate of deposition of endosteal bone in œstrogen-treated mice, only the parenteral administration of testosterone propionate has ever been claimed to avert it completely[1]. In 1950 Urist, Budy and McLean[2] stated that in their experiments they had found "Neither an inhibitory nor a synergistic action of testosterone upon the osteogenic action of large doses of œstrogen". In view of the possible bearing of these contradictory observations both on the role of sex hormones in the normal development of bone and on the effects of therapeutically administered sex hormones, a further investigation has been carried out.

10 litters of pure-strain mice (Guy's *C*) were subdivided into 17 litter-mate pairs. There were 10 male and 7 female pairs and each had its litter-mate control. All mice were kept under identical conditions and fed on a standard diet. Both mice in each pair were given weekly subcutaneous injections of 0·1 mgm. œstradiol monobenzoate ('Dimenformenol') while one mouse received in addition a weekly subcutaneous injection of 2·5 mgm. testosterone propionate ('Neohombreol'). Treatment was begun when the mice were between 21 and 28 days old and continued for periods ranging from 4 to 20 weeks. These dosages and the period of their administration are comparable with those used by both groups of workers cited. On completion of the course of injections the pairs, together with their controls, were killed. X-rays were taken of the femurs, which were then decalcified, cut into longitudinal sections, stained with hæmatoxylin and eosin, and mounted serially.

The radiographs of the femurs of mice treated with œstradiol alone showed an increase in the amount and density of bone, first at the distal and later at the proximal epiphysis. This is the characteristic initial response of the mouse skeleton to œstrogens. The amount of bone formation increased with the length of the course of injections. In every case the radiographs of the femurs of those mice treated with both œstradiol and testosterone showed markedly reduced endosteal bone formation when compared with those treated with œstradiol alone. But from comparison with the controls it was apparent that this inhibition of endosteal bone formation was never complete after only 4 weekly injections and not consistently complete until 12 or more weekly injections had been given. A quantitative confirmation of these observations was obtained from measurements made on the longitudinal sections of the femurs. Using a stage micrometer, the distance between the most proximal point on the distal epiphyseal cartilage and the most proximal spicule of bone which was in direct continuity with the cartilage was measured.

These observations show that testosterone antagonizes the osteogenic action of œstrogens in the mouse. This antagonism does not prevent the initial formation of œstrogen bone but appears to involve a renewal of the bone resorption which has been suppressed by œstrogen. These results contradict the findings of Urist, Budy, and McLean[2].

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PATHOLOGY

Role of 3,4-Benzopyrene in Carcinogenesis by Tobacco Smoke Condensate

THAT those engaged in the chemical analysis of cigarette smoke have focused so much of their attention on 3,4-benzopyrene suggests that it has been tacitly assumed that this substance is wholly or mainly responsible for the carcinogenicity of the smoke.

The benzopyrene content of cigarette smoke has been measured or estimated by many groups of workers (for review, see ref. 1). The majority of estimates fall within the range 0·5–2·5 µgm. per 100 cigarettes. In some cases where much higher estimates have been obtained the methods of analysis have been criticized[1]. It has been pointed out elsewhere[2] that the carcinogenicity of the condensate cannot be explained in terms of the carcinogenicity of a benzopyrene content of the order 0·5–2·5 µgm. per 100 cigarettes.

Smoke condensate applied to the backs of mice thrice weekly for 60 weeks such that mice received only 0·005–0·025 µgm. benzopyrene at each application developed benign and malignant tumours of the skin[3,4]. Yet, in experiments in which graded dilutions of benzopyrene in acetone were applied to mouse skin, amounts of benzopyrene well in excess of 0·38 µgm. per application were necessary to obtain a similar carcinogenic response[5].

The discovery that the phenolic fraction of cigarette smoke promotes tumour development in mouse skin following initiation by subcarcinogenic doses of 9,10-dimethyl-1,2-benzanthracene (DMBA) led to a new explanation of the carcinogenic activity of cigarette smoke[4]. According to this hypothesis, the low activity of benzopyrene and other carcinogenic constituents of smoke is enhanced cocarcinogenically by certain irritant phenolic, and possibly other, constituents. This hypothesis is now regarded as at least plausible by several of those working in the field[6,7].

In the experiment which is reported preliminarily here, the part played by 3,4-benzopyrene in carcinogenesis by a condensate of cigarette smoke was assessed by comparing the carcinogenicity of an unadulterated sample of the condensate with that of samples to which benzopyrene had been added in various measured amounts.

Smoke condensate obtained by artificially smoking cigarettes in a special machine was kindly supplied by Dr. F. G. Bock of the Roswell Park Memorial Institute, Buffalo, New York. It was sent from the United States at approximately 6-week intervals packed in dry ice. After arrival it was stored at 4° C. until required. A sample of the condensate was analysed by Dr. A. J. Lindsey of the Sir John Cass Institute, London, and found to contain approximately 0·6 µgm. 3,4-benzopyrene/gm.

One hundred male and a similar number of female mice of a stock albino strain were divided at random into five groups containing 20 mice of each sex. At the beginning of the experiment, and thereafter as necessary, hair was removed from the backs of mice by electric clippers.

Mice of group 1 were given thrice-weekly applications of 0·2 ml. 20 per cent w/v smoke condensate in acetone, that is to say, 40 mgm. of condensate containing approximately 0·025 µgm. 3,4-benzopyrene three times weekly. Mice of groups 2, 3 and 4 were similarly treated except that 3,4-benzopyrene was added to the condensate so as to increase the amounts applied on each occasion to 0·06 µgm., 0·25 µgm. and 1·25 µgm. respectively. Group 5, on the other hand, received thrice-weekly applications of 1·25 µgm. 3,4-benzopyrene dissolved simply in acetone. All treatments were continued for 68 weeks during which period the different groups received totals of approx-

Table 1. EFFECT OF ADDED 3,4-BENZOPYRENE (BP) ON CARCINOGENICITY OF CIGARETTE SMOKE CONDENSATE FOR MOUSE SKIN

Group	Treatment Thrice weekly applications to dorsal skin for 68 weeks	Total dose of smoke condensate (gm.)	Total dose of BP (µgm.)	Survivors at time of first tumour in any group (that is, 39 weeks)	First papilloma seen (weeks)	First malignant skin tumour (weeks)
1	20 per cent smoke condensate in acetone with 'normal' content of BP (0·025 µgm. per application)	8·16	5	33	74	76
2	20 per cent smoke condensate with added BP (0·06 µgm. per application)	8·16	12	30	59	74
3	20 per cent smoke condensate with added BP (0·25 µgm. per application)	8·16	51	34	54	62
4	20 per cent smoke condensate with added BP (1·25 µgm. per application)	8·16	255	33	39	59
5	1·25 µgm. BP in acetone	None	255	30	67	—

imately 5, 12, 51, 255 and 255 μgm. 3,4-benzopyrene respectively. During treatment and after treatment was stopped all mice were examined at fortnightly intervals for benign and malignant tumours of the skin. Malignant tumours were removed at operation under ether anæsthesia when they reached approximately 1 cm. in diameter. They were examined histologically and only recorded as malignant if they showed penetration of the panniculus carnosus muscle.

The results of the experiment are given in Tables 1 and 2.

Table 2. TUMOUR INCIDENCE AT 68 WEEKS AND 84 WEEKS

	Tumour incidence at 68 weeks			Tumour incidence at 84 weeks		
Group	Survivors	Total benign tumours	Total malignant tumours*	Survivors	Mice with malignant tumours	Total malignant tumours*
1	31	0	0	26	3	3
2	21	4	0	15	4	5
3	19	0	1	15	2	2
4	24	17	5	14	9	10
5	21	0	0	14	0	0

* Cumulative total excluding recurrent tumours.

Comparison of the results in groups 1 and 5 indicates that unadulterated smoke condensate is, if anything, more carcinogenic than an acetone solution containing 50 times as much 3,4-benzopyrene. Comparison of the results in groups 4 and 5, on the other hand, shows that benzopyrene mixed with smoke condensate is far more carcinogenic than the same concentration of benzopyrene in acetone. If this were due to simple summation of the carcinogenic effects of smoke condensate and benzopyrene, the tumour incidence in group 4 should have equalled the sum of the incidences in groups 1 and 5: it is obviously well in excess of it. The high yield in group 4, thus, suggests a multiplication rather than a summation of effect. This fits the hypothesis that smoke condensate contains cocarcinogenic substances in the phenolic and possibly other fractions.

Judged by the comparison of tumour-induction times and of tumour incidence, the carcinogenic responses of groups 2 and 3 tended to be intermediate between those of groups 1 and 4.

The clear-cut nature and obvious interest of these results prompted their publication before the completion of the period of observation.

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IMMUNOLOGY

Immunological Identification of a Cryomacroglobulin in a Patient with Acquired Hæmolytic Anæmia associated with a Cold Antibody

PATHOLOGICAL macroglobulins are reported to b antigenically related to normal 19S as well as norma 7S γ-globulins[1,2], although recent work woul indicate that antigenic identity and cross-reactivit between normal 7S and 19S γ-globulins might hav arisen from their imperfect separation[3]. Ultracentrifugal studies indicate that the pathologica macroglobulins[1,4] are composed of 19S and a smalle proportion of 26S γ-globulin, while the pathologica cold agglutinins consist mainly of 19S component an a much smaller amount of a faster sedimenting component[5]. The present work demonstrates evidence o cross-reactivity between a cryomacroglobulin wit cold agglutinin activity and cold agglutinin macroglobulins, which were not cryoglobulins, in the ser of other patients suffering from acquired hæmolyti anæmia associated with cold antibody formation.

Materials were obtained and methods were use according to Mehrotra[6]. The cryoglobulin wa precipitated in the cold and the 19S component wa prepared in the ultracentrifuge from normal huma serum. The cryoglobulin was used as the antigen i the preparation of the specific antiserum.

The isolated cold agglutinin and cryomacroglobuli had the same electrophoretic mobility as γ_1-globulin and this corresponded to the abnormal γ_1-globuli peak in the whole serum.

The results of investigation by double diffusion i agar are illustrated in the figures, and they were th same with the whole sera as with the isolated proteins After absorption of the antiserum with 7S γ-globuli (Fig. 1) (Fraction G4 kindly supplied by the Bloo Products Laboratory, Lister Institute, and prepare by the technique of Kekwick and Mackay[7]), two line developed against the patient's cryomacroglobuli and cold agglutinin. At least one faster componen was developed with the patient's whole serum an this was probably due to the presence of anti-β_1-globulin in the antiserum. The single line develope against the 19S component from normal serum ca clearly be seen to cross-react with the faster diffusin component of the patient's cryomacroglobulin, wit the formation of a single spur. When the antiseru

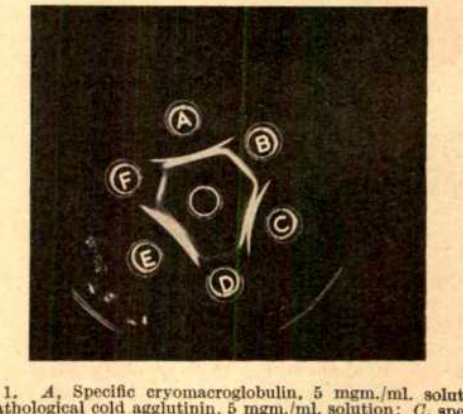

Fig. 1. *A*, Specific cryomacroglobulin, 5 mgm./ml. solution; *B*, pathological cold agglutinin, 5 mgm./ml. solution; *C*, specific cold agglutinin, 5 mgm./ml. solution; *D*, pathological cold agglutinin, 5 mgm./ml. solution; *E*, specific serum, diluted 1 in 4; *F*, normal 19S macroglobulin, 5 mgm./ml. solution. Centre cup, specific antiserum absorbed with 7S γ-globulin

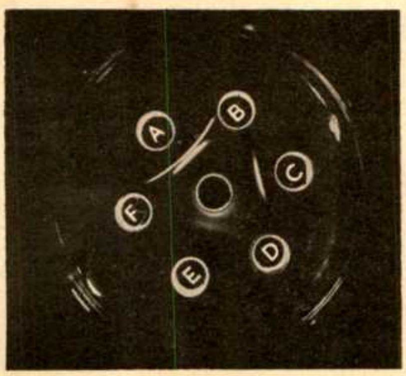

Fig. 2. *A*, Specific serum, diluted 1 in 4; *B*, normal serum, diluted 1 in 4; *C–F*, cold agglutinin serum, diluted 1 in 4. Centre cup, specific antiserum absorbed with $7S$ γ-globulin and an excess of normal serum

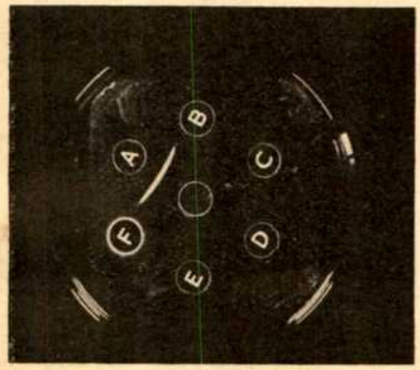

Fig. 3. *A*, Specific serum diluted 1 in 4; *B–F*, cold agglutinin serum, diluted 1 in 4. Centre cup, specific antiserum absorbed with $7S$ γ-globulin and an excess of normal serum and a cold agglutinin serum

was absorbed with an excess of normal serum (Fig. 2), two lines developed against the patient's serum, the faster component cross-reacting with three other sera containing pathological cold agglutinins. The line nearer the antigen cup was patient-specific, and no lines developed against normal serum. In Fig. 3 the antiserum has been absorbed with a mixture of normal serum and one of the cold agglutinin sera, and it is now specific for the patient, producing only a single line.

These results indicate that the cryomacroglobulin has at least two antigenic determinants not present in normal serum. One, in the faster diffusing component by agar-gel diffusion, cross-reacts with several sera containing pathological cold agglutinins (and the isolated cold agglutinins), and the other, in the slower component, is patient-specific.

This finding is in keeping with those of Habich[8] and Kanzow *et al.*[9], using tube precipitin tests. Korngold and Van Leeuwen[2], reporting on the sera of patients with macroglobulinæmia but no cold agglutinins, state that these pathological macroglobulins have at least two antigenic groups not present in normal serum proteins and that they are situated on two components. Both are macroglobulin in nature and the authors presumed that the slower was the $26S$ component, but this is difficult to establish. According to these workers exhaustive absorption of the specific antisera with normal serum proteins rendered them patient-specific, but this is contrary to the present finding. Pathological cold agglutinins have been studied in the ultracentrifuge and shown[5] to contain a major $19S$ component and a small amount of a faster sedimenting one. In an immunological investigation[6], Mehrotra concluded that the cold agglutinins were $19S$ macroglobulins and did not demonstrate any cross-reactivity between them, not shared by normal macroglobulins.

Examination of the present cryomacroglobulin suggests the possibility that the pathological cold agglutinins share cross-reacting antigenic determinants as well as having patient-specific ones, not present in normal serum. One other pathological cold agglutinin studied in the same manner has been shown to cross-react with another cold agglutinin after exhaustive absorption with normal serum proteins, suggesting that the findings presented here were not the result of an isolated exception; but this has to be tested by further work.

We thank Dr. W. d'A Maycock for the supplies of fraction $G4$, Dr. M. E. Mackay and Dr. R. A. Kekwick for the preparation of normal $19S$ component and Prof. J. V. Dacie for advice.

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Inherited Immunologically Atypical Haptoglobin

THE presence of three genetically determined haptoglobin types (*Hp* 1–1; *Hp* 2–1; *Hp* 2–2) for the Caucasian race was first demonstrated by Smithies using starch-gel electrophoresis[1,2]. Only a small number of atypical haptoglobins have since been described[2,3]. Although the three *Hp* types differ with respect to molecular weight and electrophoretic mobility, they have been shown to be immunologically identical among each other[4,5], that is, to possess identical antigenic determinants.

In the course of investigations with a modified agar-gel diffusion technique[5,6] for the demonstration of the three *Hp* types, one serum was encountered which did not give the usual reaction of identity. As shown in Fig. 1, serum B. Wi. showed a distinct spur formation when tested next to normal sera of the three *Hp* types, thus demonstrating only partial antigenic identity with the normal *Hp* proteins. From the position of the spur it could be concluded that the *Hp* of this serum possessed an additional antigenic determinant. Since this patient suffered from cirrhosis of the liver it was thought that this atypical behaviour might be a result of this disease. However, the same spur formation was shown by the serum of one brother (W. Wi.) and of the son (G. Wi.) of the original patient, although these were both healthy individuals. Among each other, the

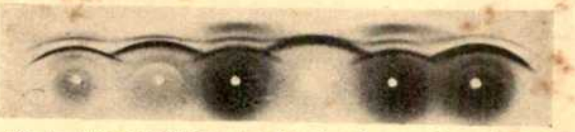

Fig. 1. Agar-gel diffusion test showing the spur formation by the Wi. sera. Antiserum in trough along upper limit of photograph. From left to right: serum B. Wi., normal *Hp* 2–1, serum W. Wi., normal *Hp* 1–1, serum G. Wi., normal *Hp* 2–2. Benzidine stain

three sera Wi. gave a reaction of identity. All three had normal *Hp* serum levels. Two female members of this family had a normal *Hp* 2–1 type.

The spur formation could be demonstrated using several antisera (horse-anti-human-serum-antisera 13412, 13414 and 13415, Institut Pasteur, Paris). One rabbit antiserum with an apparently low titre of antihaptoglobin antibodies (number 4, Behring Werke AG, Marburg/Lahn) reacted with normal *Hp* only, but did not give any reaction with the *Hp* of the three sera Wi.

The atypical nature of the *Hp* proteins of these sera was further demonstrated by absorption of the antisera with a normal *Hp* 2–1 (kindly supplied by Prof. H. E. Schultze, Behring-Werke, Marburg/L.), after which the antiserum still gave a somewhat weaker precipitate with the three sera Wi., whereas with normal sera a benzidine positive precipitate was no longer obtained. On ordinary agar-gel immunoelectrophoresis, the three sera Wi. presented the usual benzidine positive precipitin band in the α_2-region, thus showing that we were indeed dealing with Hp^s. On starch-gel electrophoresis, they showed a constellation similar to type *Hp* 2–1; however, one band in the slower migrating group seemed to be broadened. The immunological identification[6] of this band gave the same spur formation as the whole serum when tested in the same manner. This latter finding suggested that only part of the *Hp* of these sera carried the atypical antigenic determinant. It should be mentioned here that spur formation need not necessarily represent partial identity, if two precipitates of two closely related proteins are superimposed in an agar-gel diffusion experiment[7].

From the preliminary family investigation (Fig. 2) a dominant inheritance appears probable. The direct inheritance observed represents evidence against a recessive transmittance because then both parents should have had the recessive gene. There seems to be no indication for a sex bound inheritance, since female conductors are obviously not involved.

From these findings it would appear that the three sera Wi. contain a rare antigenic determinant in their *Hp* proteins. However, among the human sera used for immunization in the production of the antiser there must have been present at least one seru containing this atypical *Hp*. Another possibili might be a mere quantitative prevalence of th unusual determinant in the investigated famil where one would further have to assume that th determinant would be present in 'normal' sera amounts too small to be detected by the usu technique, failing to give a visible precipitat Further investigations aiming in the isolation of th *Hp* protein are under way.

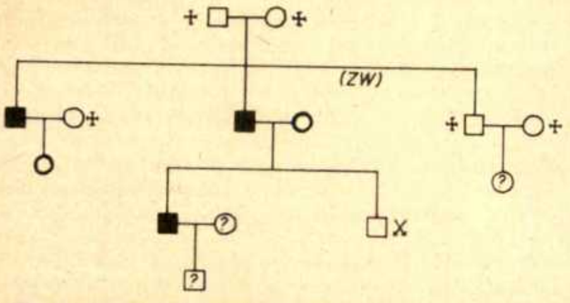

Fig. 2. Pedigree of family Wi. ■ (solid squares), members showing the atypical *Hp*; ○ (heavy circles), two investigated female family members with normal *Hp* 2–1. ? = not investigated

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BIOLOGY

'Egg-dummies' as Natural Releasers in Mouth-breeding Cichlids

THERE are quite a number of African moutl breeding cichlids (*Haplochromis* and related genera the males of which bear very conspicuously yello or orange spots near the base of the anal fin. Th function of these so-called 'anal ocelli' has now bee elucidated in *Haplochromis wingatii*.

The male of *H. wingatii* digs a nest pit and the courts a female and tries to lead her there. A femal ready to spawn will follow the male and soon start to lay eggs in the pit. These eggs, however, ar snapped up into the female's mouth before the mal is able to fertilize them. The male, in his turn, slide over the spot where the eggs have been, fertilizin the empty bottom. During this movement his ana fin is strongly spread and very dark in ground colou displaying the round, orange spots in colour con trast. These spots resemble very precisely the egg of this species in colour, size and form; and indeed the female tries to grasp them with her lips, thereb taking into her mouth the sperm ejected by the male The 'painted eggs' are much more conspicuou against the dark anal fin than are the real eggs in th sand pit; but they are sub-normal objects wit respect to the female's reaction of snapping up eggs

In the literature, these egg-spots are usually calle eye-spots, or ocelli. However, true eye-spots have dark centre and a lighter area around; they are t be found on the opercula of many cichlids, in th posterior part of the dorsal or the upper part of th caudal fin in *Pelmatochromis subocellatus*, and i general they have an intimidating function. Th egg-spots are the negative of an eye-spot, having light or conspicuously coloured centre, normall ringed with dark, and attracting the partner. S both, morphologically and functionally, the latte should be carefully distinguished and not be referre to as eye-spots or ocelli (the term 'annular spot' i suggested for both types).

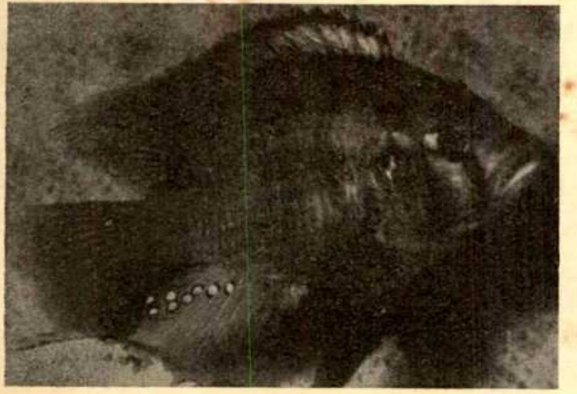

Fig. 1. Male fish of *Haplochromis wingatii*, showing eight annular spots on his anal fin

A second large group of mouth-breeding cichlids in Africa is formed by the genus *Tilapia*. Here, too, this specialization gives the same result: the female takes the eggs into the mouth as soon as they are laid. So these animals have to overcome the same difficulty of getting their eggs fertilized. Here, the females are often seen to nibble at the male's genital papilla, which is bright white or yellow, contrasting well with the dark ventral surface of the male fish. In some species of *Tilapia* the males bear 'genital tassels', long appendages of their genital papillæ, consisting of bifid semi-transparent filaments, festooned with bright orange blobs of tissue. Since in colour and form they resemble the eggs of the respective species, I believe that they function as 'egg-dummies'.

Both types of egg-models are attributes of the nuptual coloration of the male. They seem to be important taxonomic characters, distinguishing the *Haplochromis* group and the *Tilapia* group. Due to their releasing function, the reaction of the female in snapping up eggs is functionally changed into snapping up the (invisible) sperm. Most of the eggs of these species are thus fertilized when in the mouth of the female.

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Weight and Length in the River Lamprey

NORMALLY the length of vertebrates (including fish[1,2]) does not become reduced even during prolonged fasting. This rule does not apply to the cyclostomes. The river lamprey, *Lampetra fluviatilis* (L.) Gray, is known to undergo a considerable shortening (up to 27 per cent[3,4]) during the months between its migration from the sea to the rivers in autumn and the spawning and ensuing death in spring. In this period the animals do not eat, and their gut is reduced.

The reduction in length during fasting of course tends to keep the proportion between weight and length of the animals constant, so it would be interesting to examine to what degree this is accomplished. Therefore the weight and length of single river lampreys were followed in this laboratory from October until spawning. One batch of animals came from Sweden and the other from Denmark. These results were combined with those of Lanzing[5], who measured batches of river lampreys in the Netherlands caught during August–March.

Fig. 1 shows that the data representing the result of fasting fall around a straight line, if plotted on double logarithmic paper. The starved animals do not differ much from the well-nourished ones just coming from the sea, so instead of becoming extremely lean like other severely fasting vertebrates, the river lampreys maintain rather normal proportions (until spawning). The straight line signifies that the reduction in weight and length is allometric (weight = $a \times \text{length}^n$); the line is likely to be identical with the growth-curve of these animals. Zanandrea[6] measured batches of river lampreys caught in sea water while still feeding; their growth is allometric, the growth-curve running parallel to the straight line in Fig. 1. It would be interesting to see how these growing animals become reduced in weight and length if they were submitted to fasting. Presumably they would follow the same curve 'backwards'.

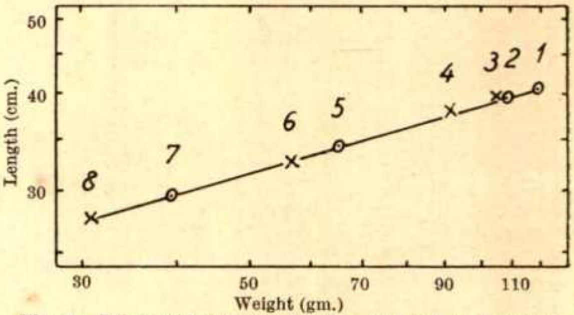

Fig. 1. Relationship between weight and length in *Lampetra fluviatilis*. ○, Recently caught, non-fasting animals; freshwater; ×, fasting for a long time. Place and time of capture and number of animals: (1) Meuse, Netherlands, October, 70 (ref. 5); (2) Meuse, Netherlands, August, 70 (ref. 5); (3) Meuse, Netherlands, January, 70 (ref. 5); (4) Meuse, Netherlands, March, 70 (ref. 5); (5) Varde aa, Denmark, October, 10, measured 1 month after arrival; (6) Varde aa, Denmark, October, 10, measured in February; (7) Dalälv, Sweden, October, 11, measured 1 month after arrival; (8) Dalälv, Sweden, October, 11, measured in February

Under certain conditions the body proportions are altered. Some time before spawning, presumably at the time of ovulation, the females gain in weight and become swollen; but their length is still decreasing, sometimes even faster than before. Moreover, animals disturbed by transport seem to gain in weight by uptake of water (increased permeability of the skin[2]); this is followed by a large decrease in body-weight observed after 2 weeks in the laboratory; these fluctuations in body-weight are accompanied by the usual slow shortening.

The relation between weight and length during the life-time of the river lampreys is thus quite different from that found in other vertebrates. The lack of a skeleton and the nature of the chorda may make the shortening possible. Shortening during fasting was observed in planarians[7] and is probably not uncommon in other animals which do not possess a stiff exo- or endo-skeleton.

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Fossil Dinoflagellates and Hystrichospheres in Australian Freshwater Deposits

During investigations of algae and aplanospores in Holocene peat deposits of south-western Australia[1], fossil dinoflagellates and hystrichospheres have been found; these are the first representatives of these groups to be discovered in non-marine deposits. The dinoflagellates are referable to the genera *Gymnodinium* (Fig. 1*b*), *?Peridinium* (Fig. 1*a*), *Palaeohystrichophora* and to an undescribed genus of the family Peridiniaceae; they are found in assemblages from two localities near Lake Muir, about 40 miles north of Walpole, Western Australia, and from Myalup Swamp, 24 miles north-north-east of Bunbury, Western Australia. The hystrichospheres are referable to six undescribed species of the genus *Baltisphaeridium* (Figs. 1c and 1*d*): they are present in assemblages from the above localities and from deposits at Boggy Lake, 6 miles south-west of Walpole, Western Australia. In no case do these species afford evidence of dinoflagellate affinities.

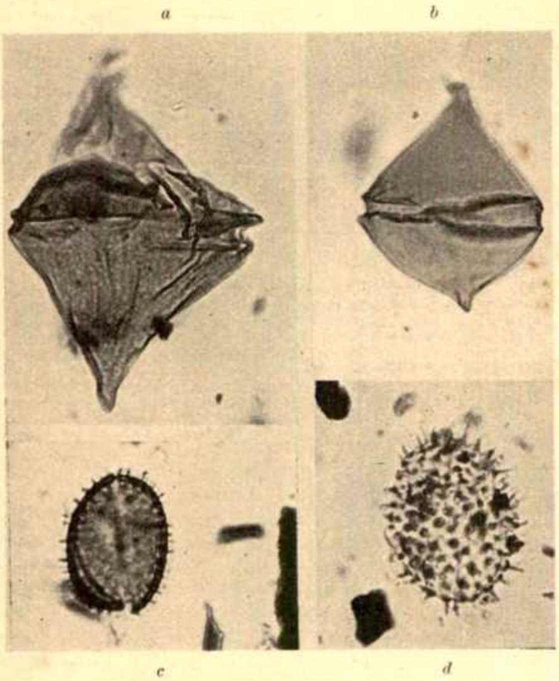

Fig. 1. Fossil freshwater dinoflagellates and hystrichospheres from south-west Australian peats. *a* (?) *Peridinium* sp. West Lake Muir at 30–40 cm. depth; *b*, *Gymnodinium* sp. West Lake Muir at 30–40 cm. depth; *c*, *Baltisphaeridium* sp. *A*, boggy lake at 90–100 cm. depth; *d*, *Baltisphaeridium* sp. *A*, at 20–30 cm. depth. (× *c*. 600)

Since the hystrichospheres have been regarded hitherto as "characteristically marine"[2] and have been considered as potentially reliable marine indices[3,4], their presence in undoubtedly non-marine deposits is of particular interest. Although there was some variation in relative salinity between the localities, in no case were the waters even brackish in terms of their absolute salinity. The assemblages may have originated by wind dispersal of cysts; evidence of the dispersal of aplanospores by this means has already been recorded[1]. The formation of the bogs was not related to any marine incursion, and the microplankton assemblages cannot be regarded as relict marine floras.

Dinoflagellates and hystrichospheres are absent from assemblages from peat profiles at other localities studied in Western Australia. There are no obvious special factors to account for the presence of these organisms in the localities listed and their absence elsewhere.

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Uptake of Phenylalanine by *Tetrahymena pyriformis*

The nutritional requirements of *Tetrahymena pyriformis* are well known, and it can be cultured in a chemically defined medium. Nevertheless, some aspects of its feeding are still not clear. In nature the organisms are believed to feed phagotrophically on bacteria[1]. In synthetic media, however, food vacuoles are rarely seen. Although Seaman[2] found that vacuole formation could be stimulated by the addition of proteose peptone-yeast extract to the medium, the phagotrophic activity induced was not sufficient to supply the energy requirements of the organisms.

Uptake of amino-acids from solution has been reported for both micro-organisms and metazoans[3–5]. In some cases, membrane transport has been implicated. Since phagotrophy does not account for the obvious ability of *Tetrahymena* to thrive on a suitable mixture of small organic molecules, it seemed desirable to investigate this possibility.

Tetrahymena pyriformis B was grown axenically in 1 per cent proteose peptone, 0·05 per cent yeast extract, with added ferrous sulphate ($8{\cdot}95 \times 10^{-5}\ M$) and sodium-ethylenediamine tetraacetic acid ($6{\cdot}87 \times 10^{-5}\ M$). Cells were gathered at the end of the log phase by centrifugation and washed and suspended in $M/100$ phosphate buffer, *p*H 6·4, at a concentration of 10^6 cells/ml. DL-phenylalanine-3-^{14}C was added and samples were taken at suitable time-intervals. Samples were collected by filtering 1 ml. of the cell suspension through a 1-in. *AM*–1 Polypore membrane filter (pore size, 5μ). The filter was then washed with 8–10 vols. of phosphate buffer. Radioactivity in the organisms was determined by measuring the labelled material restrained by the filter using a thin-window Geiger tube. The technique was checked by washing labelled cells with non-radioactive buffer and measuring the radioactivity of samples dried on aluminium planchets. The results presented have been corrected for the greater self-absorption encountered in the filter disks.

At concentrations of about 1 μgm./ml., 40–50 per cent of the DL-phenylalanine was removed in the first 20 min. No additional material was removed if incubation was continued for longer periods. The rate of uptake appeared linear for the first 10 min. of incubation. The relation between the initial rate of uptake and phenylalanine concentration was determined and is presented in Fig. 1. An excellent linear relation is obtained when the reciprocal of this rate is plotted against the reciprocal of phenylalanine concentration. Hence it seemed reasonable to treat the system as formally equivalent to an enzymatic reaction for purposes of analysis. The K_m and $V_{\max}$ were calculated using a Burke–Lineweaver plot of the data. The concentration at which uptake is half-maximal (K_m) is 2·5 ± 0·25 µgm./ml. The maximum rate of uptake ($V_{\max}$) is 0·227 µgm./10^6 cells/min.

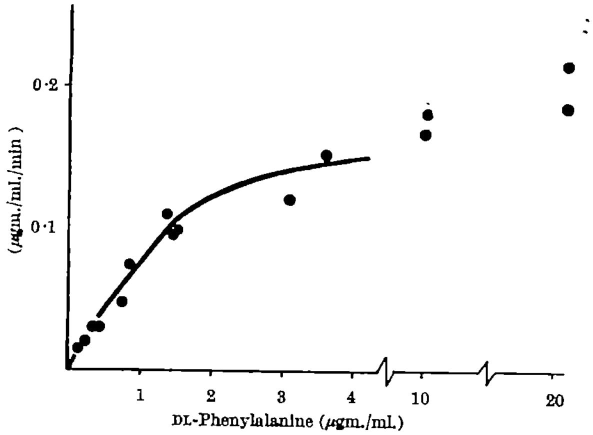

Fig. 1

The uptake process depends on temperature exhibiting a Q_{10} of 2·19 for the temperature-range 12°–20° C. and 1·63 for the range 20°–28°. 2,4-dinitrophenol acts as an inhibitor at pH 6·4. 10 per cent inhibition was observed with 5×10^{-6} M; 86 per cent with 5×10^{-5} M.

Although labelled phenylalanine was available only as a racemic mixture, observations indicate that only the L isomer is involved in this process. The addition of 100 µgm./ml. of L-phenylalanine produces a very marked decrease in the rate of removal of radioactivity from the labelled racemic mixture at low concentrations. Addition of 100 µgm./ml. of D-phenylalanine has no effect. Fresh cells suspended in medium from which radioactive phenylalanine had been previously removed by *Tetrahymena* do not show significant uptake despite the fact that approximately 50 per cent of the labelled material is still present. This residual material is presumably D-phenylalanine.

Seaman[2] determined that cells of *Tetrahymena* washed and suspended in phosphate buffer did not take in materials through the cytostome. Even under his optimal conditions for food vacuole formation, the rate of uptake of phenylalanine at low concentrations vastly exceeds observed rates of vacuole formation.

The rapidity of the uptake process, its kinetics, its stereospecificity, the fact that the process is dependent on temperature, and the inhibition of uptake by 2,4-dinitrophenol all suggest a membrane transport mechanism. Preliminary experiments with other amino-acids indicate that they are also removed from solution. We are now extending this work in an effort to demonstrate accumulation, to study possible interactions between amino-acids, and to determine if such an uptake process might be sufficient to provide for the energy requirements of the organisms.

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Utilization of Ethanol-2-^{14}C by Pea Slices

STIMULATIONS in the growth of algae[1,2] and seedlings[3] by added ethanol solutions prompted Cossins and Turner[4] to report preliminary experiments which indicated that ethanol, accumulated under anaerobic conditions, can be utilized by pea seedlings under subsequent aerobic conditions.

Further experiments have now been carried out to determine the changes in the concentration of the keto and organic acids accompanying ethanol disappearance in pea seedlings. During this period, it was found by means of paper chromatography that large fluctuations in the contents of acetaldehyde, acetone and the acids of the Krebs cycle occurred, which further suggested that the ethanol might be utilized by these tissues.

Clear evidence, however, for such ethanol utilization has now been obtained in experiments where ethanol-2-^{14}C was fed to pea slices. After germination for 48 hr. at 20° C., Kelvedon Wonder pea cotyledons were hand-sliced at 2° C. and 1 gm. samples of the slices were incubated at 20° C. with 1 ml. of ethanol-2-^{14}C with an activity of 12·5 µc. Oxygen was passed over the slices and the gas stream was finally passed into barium hydroxide solution to remove carbon dioxide. At the end of the experimental periods, extracts of the ethanol soluble and the 2,4-dinitrophenylhydrazine derivatives of the carbonyl compounds were prepared and separated by paper chromatography. The radioactivity of the compounds isolated was assessed by scintillation counting using Panax *AC*/300/6 automatic counting equipment with a Panax scintillation counter with an anthracene crystal.

Samples of the slices taken after 15 and 30 min. incubation with the ethanol-2-^{14}C showed that a large number of compounds contained carbon-14, which

Table 1. DISTRIBUTION OF CARBON-14 AFTER 15 AND 30 MIN. INCUBATION OF THE PEA SLICES WITH ETHANOL-2-^{14}C
(Activity expressed as c.p.m./gm. pea slices)

	Incubation for 15 min.	Incubation for 30 min.
Carbonyl compounds		
Keto-acids	120 ± 10	2,100 ± 45
Acetaldehyde	1,200 ± 34	2,280 ± 46
Acetone	2,820 ± 53	3,720 ± 62
Organic acids		
Acetic	120 ± 10	780 ± 27
Citric	0 ± 1	520 ± 22
Malic	0 ± 1	1,440 ± 38
Succinic	0 ± 1	1,080 ± 32
Fumaric	0 ± 1	600 ± 24
Amino-acids		
γ-Aminobutyric acid	0 ± 1	240 ± 15
Arginine	180 ± 13	600 ± 24
Aspartic acid	660 ± 25	780 ± 27
Citrulline	0 ± 1	240 ± 15
Glutamic acid	240 ± 15	660 ± 25
Glycine	120 ± 10	300 ± 17
Methionine	480 ± 21	900 ± 30
Serine	300 ± 17	540 ± 22
Histidine	0 ± 1	480 ± 21
Threonine	0 ± 1	180 ± 13

included those shown in Table 1. No carbon-14 was detected in the barium hydroxide traps.

These results indicate that in the slices of germinating peas, the carbon-14 of the ethanol-2-^{14}C rapidly became distributed among a large number of organic compounds. Furthermore the pattern of this labelling was similar to the changes in concentration of the organic and keto-acids occurring in intact germinating peas during the disappearance of the endogenous ethanol. This would suggest that germinating pea tissues, unlike the mature carrot disks used by Lowe and James[5], are capable of metabolizing ethanol. Therefore there is reason to suppose that at least some of the ethanol accumulated under anaerobic conditions can be utilized during the subsequent aerobic phases of germination in peas.

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Variability of the Niacin Content in Coffee

IT has been found that coffee seeds contain small amounts of certain vitamins, among which niacin prevails in higher proportion. An average consumption of 3·5 cups of coffee per day could supply about one-third of the minimum daily niacin requirement for a normal adult male[1]. In order to determine the possible effect on niacin content of varying environmental conditions under which coffee is grown, as well as of the different methods of processing, two sets of specially prepared samples were analysed.

In April, 1958, four such samples were prepared: two belonged to the Bourbon Vermelho cultivar (*C* 662) and two to the Bourbon Amarelo (Jaú). The samples were derived from sun-dried beans (dried with pericarp), which is the usual system used by the majority of Brazilian coffee farms. One sample of each cultivar was taken from plants grown without shade, while the other was derived from shaded coffee plants. Table 1 shows the amounts of niacin obtained. While the niacin content seems to be similar in both cultivars, the amount of niacin obtained from samples collected from shaded coffee trees was in both cases reduced.

During November 1959 new samples were prepared from coffee plants of the cultivars Bourbon Vermelho (*LC* 408), Mundo Novo (*LCP* 379–19) and Bourbon Amarelo (*LCJ* 30), grown in shaded and unshaded environments. Some of the samples were from sun-dried beans while others were from de-pulped coffee (dried in parchment). The results obtained are given in Table 2.

Table 1. SHOWING THE AMOUNT OF NIACIN OBTAINED

Cultivar	Treatment	Niacin (mgm./gm.)
Bourbon Vermelho (*C*662)	Without shade	0 329
Bourbon Vermelho (*C*662)	Shaded	0 237
Bourbon Amarelo (Jaú)	Without shade	0 332
Bourbon Amarelo (Jaú)	Shaded	0 260

Table 2

Cultivar	Treatment	Niacin (mgm /gm)
Bourbon Vermelho (*LC*408)	Without shade, sun-dried cherries	0·329
Bourbon Vermelho (*LC* 408)	Without shade, dried in parchment	0·400
Bourbon Vermelho (*LC*408)	Shaded, sun-dried cherries	0·271
Bourbon Vermelho (*LC* 408)	Shaded, dried in parchment	0·314
Mundo Novo (*LCP* 379–19)	Without shade, dried in parchment	0·265
Mundo Novo (*LCP* 379–19)	Shaded, dried in parchment	0·226
Bourbon Amarelo (*LCJ* 30)	Without shade, dried in parchment	0·160
Bourbon Amarelo (*LCJ* 30)	Shaded, dried in parchment	0·206

With the exception of the results obtained with samples of the cultivar Bourbon Amarelo (*LCJ* 30), shade again reduced the niacin content. It can also be seen that the de-pulping operation seems to increase the amount of niacin content, although not so markedly as when coffee is grown without shade.

It is, furthermore, noteworthy that the latter three cultivars differ in niacin content, indicating that selection might be effective in isolating strains with higher amounts of this vitamin.

In order to obtain reliable comparative results when investigating the niacin content in coffee seeds, it is therefore necessary to use samples collected from coffee trees grown under identical environmental conditions and also that the samples be processed the same way.

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Biochemical Contributions to the Taxonomy of the Genus *Chlorella*

SINCE Warburg's[1] work, in 1919, green algae of the genus *Chlorella* have been among the experimental organisms most frequently used for plant physiological and biochemical research. However, due to the little morphological differentiation of their more or less spherical cells, the taxonomy of these algae has been rather confused (cf. ref. 2). This holds especially true for the members of the section *Euchlorella* comprising the most common species, that is, *Chlorella vulgaris* Beijerinck, *Chl. pyrenoidosa* Chick, and *Chl. ellipsoidea* Gerneck. The criteria given by Pascher[3] for their identification (size and shape of cells and chloroplasts, visibility of the pyrenoid, etc.) have been shown to be highly variable and ambiguous, and to depend to a large extent on culture conditions and developmental stage of the cells[4]. Therefore, in most cases it is very difficult, if not impossible, to identify these species by morphological criteria. Since the characteristics traditionally used for taxonomy are obviously insufficient in this case, we propose to use mainly physiological and biochemical rather than morphological criteria for the identification of these *Chlorella* species.

The experimental material used in this work was the strains of *Chlorella* available in the culture collection of Prof. Pringsheim, Göttingen. Three biochemical properties known to be present in some, but not all, strains of *Chlorella* were chosen for our work, that is, hydrogenase activity[1], formation of secondary carotenoids under conditions of nitrogen deficiency[4], and presence in the cell and in the cell wall of carbohydrates (probably pectin or related substances) giving a red colour reaction with ruthenium red. For the hydrogenase tests, the algae grown in a medium[7] containing nitrate were suspended in phosphate buffer (4×10^{-3} *M*; *p*H 6·5) and incubated under an atmosphere of pure hydrogen in Warburg vessels (with potassium hydroxide in the centre well) at 20° C. in the dark. After 3, 6, 12, 24, 48, 72, and 100 hr., nitrite (final concentration 1×10^{-3} *M*) as the most suitable hydrogen acceptor[5,8] was poured in and the uptake of hydrogen measured manometrically. Nitrogen-deficient cultures were obtained by replacing 90 per cent of the potassium nitrate of the culture medium[7] with potassium chloride (nitrate concentration 8×10^{-4} *M* instead of 8×10^{-3} *M* in the normal medium). After four weeks of growth at a light intensity of 4,000 lux, the colour of the deficient cultures was either orange or pale greenish-yellow to white. The ruthenium red reaction was carried out by treating algae taken from agar slant cultures with a 0·2 per cent solution of ruthenium red (Merck, Darmstadt) dissolved in hot distilled water. The resulting colour of cell contents and of the empty membranes of mother cells after the discharge of the autospores was then observed under the microscope.

Table 1. BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES OF 25 DIFFERENT STRAINS OF THE GENUS *Chlorella*, SECTION *Euchlorella*

Hydrogenase activity	Colour of N-deficient cells	Reaction with ruthenium red	Strain Nos. of Pringsheim's culture collection	Species
Strong	Orange	None	211-8*b*, 8*c*, 8*e*, 11*m**, 11*n**	*Chl. pyrenoidosa*
Weak	Pale	Weak	211-8*k*, 11*g*, 11*h*. 11*k*	
None	Pale	Strong	211-11*b*, 11*c*, 11*f*, 11*i*, 11*l*, 11*p*, 11*qu*, 11*r*, 11*s*, 11*t*, 8*l*†, 1*a*‡, 1*e*‡	*Chl. vulgaris* Beijerinck
None	Pale	None	211-1*b*, 1*c*, 1*d*	*Chl. ellipsoidea* Gerneck

* *Chlorella* 11*m* and 11*n*, listed in the culture collection as *Chl. vulgaris*, were found to belong to *Chl. pyrenoidosa*.

† *Chlorella* 8 *l*, listed as *Chl. pyrenoidosa*, was found to belong to *Chl. vulgaris*.

‡ *Chlorella* 1*a* and 1*e*, listed as *Chl. ellipsoidea*, were found to belong to *Chl. vulgaris*.

The results of these investigations are summarized in Table 1. It is evident that the 25 strains of *Chlorella* studied fall into four well-defined groups. The first group, which can be identified with *Chlorella pyrenoidosa*, is characterized by strong hydrogenase activity, a deep orange colour of nitrogen-deficient cultures, and no reaction with ruthenium red (cf. ref. 9). The third group, which comprises the most common species, *Chlorella vulgaris* Beijerinck, does not possess hydrogenase, turns pale due to the loss of both chlorophylls and carotenoids under conditions of nitrogen deficiency, and develops a brilliant red colour with ruthenium red. The fourth group is identical with *Chlorella ellipsoidea* Gerneck. This species can be recognized by the strongly elliptical shape (even in agar cultures) of its cells, which do not contain hydrogenase, turn pale fairly slowly in nitrogen-deficient cultures, and do not react with ruthenium red. In addition to these well-known species, there is another group, the second in Table 1, which according to our tests seems to be uniform, showing characteristics intermediate between those of *Chlorella pyrenoidosa* and *Chl. vulgaris*. This group is characterized by rather weak hydrogenase activity, and quite a feeble reaction with ruthenium red; under nitrogen-deficient conditions, these algae turn pale. Future experiments and taxonomic considerations will have to decide whether this group must be regarded as a sub-species of *Chlorella pyrenoidosa* or of *Chl. vulgaris*, or whether it should be given the rank of a new species.

On the whole, our results seem to suggest that the taxonomy of the critical section *Euchlorella* of the genus *Chlorella* can be soundly based on some physiological and biochemical characteristics which can easily be determined. Thus an unambiguous identification of these most common and widely used algae should now be possible.

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Growth and Development of Leaves from Intercalary Meristems in *Phalaris arundinacea* L.

THERE is general agreement[1-4] that the differentiation of phytomer units from the vegetative shoot apex of grasses initially involves the appearance of periclinal divisions in the dermatogen, and that these give rise to a series of alternate distichously arranged ridges which mature basipetally. The blade of the phytomer develops as a result of a combination of marginal growth from periclinal divisions and apical growth from a rib meristem. This eventually transforms the ridge into a crescent, then a collar, and finally a hood over the apex. Shortly thereafter the ligule is formed from the epidermal cells on the upper surface of the blade.

A point of disagreement is whether an active meristem persists at the base of the blade during its subsequent development. Sharman[3,4] and Esau[5] support the idea that the differentiation of the ligule marks the end of meristematic activity in the blade. They describe a basipetal wave of maturation of phytomer tissues that is initiated at the apex of the blade and generates, in turn, the fully formed blade, sheath, and internode. According to this view the enlargement of the blade that occurs after the ligule forms is attributable to the enlargement of existing cells.

Several workers in the field of range management[6-8] have expressed the view that a meristematic zone persists at the base of the blade. Although these reports do not use the phytomer concept, the inference is that this zone remains, or is potentially active after the rest of the phytomer unit is mature.

The objectives of this investigation were to establish the time at which leaf blades of *Phalaris arundinacea* L. (reed canary grass) were fully developed, and to learn whether they retained any meristematic tissue that could be activated by defoliation.

A clone of *Phalaris arundinacea* L. was propagated vegetatively and established as spaced plants in the field as a source of genetically uniform material. Only young, actively growing vegetative shoots from these plants were selected for examination of leaf growth.

In the first experiment the three youngest leaves the blades and ligules of which had emerged completely from the sheath of the next oldest leaf were individually tagged and measured. Blade-length measurements were repeated every week until elongation ceased. Concurrent defoliation treatments were applied to corresponding leaves on similar shoots, to see whether defoliation would stimulate further growth. Some blades were severed just above the ligule, while others were truncated one-third or two-thirds of the distance to the tip of the blade.

From the measurements of blade-length the following conclusions may be drawn: (1) all the three youngest undisturbed blades on the control shoots had reached their maximum length when the ligules were exposed; (2) none of the defoliation treatments caused a renewal of growth in length of any of the leaves so treated.

Thus, once a leaf-blade of reed canary grass has fully emerged from the enclosing sheath of older leaves, it no longer contains any active meristematic tissue, it has ceased to elongate, and it is not stimulated to renewed activity by partial defoliation.

A second experiment was designed to determine whether the leaf-blade was still growing as it emerged from the uppermost leaf-sheath. This was done by marking with ink the exposed section of the rolled blade, measuring its length, and repeating the measurements until the entire blade had emerged. The measurements showed that all emerged portions of the blade had completed elongation.

In order to determine whether this cessation of growth by elongation occurred at, or prior to, emergence of the blade, the length of individual epidermal cells was measured on blades prior to, and after, emergence. However, cell-length was so variable that it was not possible to determine whether growth ceased at or prior to emergence.

The apparent sudden cessation of growth of cells influencing blade-length suggests that a sudden change in their environment is responsible. The emerging blade does in fact experience such a sudden change in environment as it emerges from the protection of the sheaths of older leaves. The apparent influence of this transition on the metabolism of the cells may be another example of a reaction mediated by phytochrome, that is, a red/far-red effect controlling cell development. For the young enclosed blade is receiving only radiation transmitted through the encircling leaf-sheaths, and thus mostly in the far red, whereas on emergence it is receiving more radiation in the red. This transition would also reduce cell turgor, and together with the possible metabolic disturbances may well underlie the cessation of elongation.

These results in general support Sharman's concept of the growth and development of grass leaves. The apparent regrowth of partially defoliated blades reported by other workers may have been due to growth of the concealed basal portion of the phytomer elevating the exposed portion of the blade.

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MICROBIOLOGY

Fractionation of Isotopes in Relation to the Problem of Elemental Sulphur Transport by Micro-organisms

THE mechanism of elemental sulphur transport from the medium into the cells of sulphur-utilizing micro-organisms was investigated by Umbreit, Vogel and Vogler[1]. They concluded that for *Thiobacillus thiooxidans*, the sulphur, on contact with the cells, dissolves in globules of highly unsaturated fat located at the poles of the cells. Knaysi[2] was unable to confirm the existence of polar fat globules and pointed out the difficulties involved in postulating contact between an extracellular particle and an intraprotoplasmic structure. Vishniac and Santer[3] suggested a sulphur-transport mechanism involving reduced glutathione (GSH) or a similar molecule reacting as follows:

$$S^{\circ} + 2GSH \rightarrow S^{=} + GSSG + 2H^{+}$$

the sulphide formed enters the cell in free or bound form and the GSSG is reduced by subsequent intracellular sulphide oxidation. No experimental evidence was presented to support this mechanism. It is apparent that the problem is not yet solved.

During an investigation of biofractionation patterns of stable sulphur isotopes by micro-organisms[4] results were obtained which bear on the problem. The pertinent processes investigated were the oxidation of elemental sulphur by the chemolithotrophic *Thiobacillus concretivorus*[5] and by a photosynthetic purple bacterium, *Chromatium* sp., and the reduction of sulphur to sulphide by *Saccharomyces cerevisiae*. The methods used are given elsewhere[4].

Table 1 presents the ranges of isotopic enrichments observed in various metabolic processes. The reduction of elemental sulphur by *S. cerevisiae* and its oxidation by *T. concretivorus* and *Chromatium* sp. have in common the fact that no significant isotope fractionation occurred. In contrast, there was a very great enrichment of ^{32}S during sulphite reduction by yeast and a significant enrichment during the oxidation of sulphide to sulphate, at least by *T. concretivorus*.

Little enrichment of the light isotope in sulphate was observed during photosynthetic oxidation of sulphide by *Chromatium* sp. This was probably due to difficulties encountered in controlling the very rapid oxidation process, and 15 to 50 per cent of the initial sulphide was oxidized by the time the sulphide samples were taken. That a more significant fractionation might have been observed if the oxidation

Table 1. ISOTOPE ENRICHMENT DURING MICROBIOLOGICAL METABOLISM OF SULPHUR COMPOUNDS

Organism	Starting compound	Range of isotopic enrichment			
		Intermediate		End product	
		Compound	$\delta^{34}S$*	Compound	$\delta^{34}S$*
T. concretivorus	H_2S	polythionate	+0·6 to +19·0	$SO_4^{=}$	−10·6 to −18·0
	S°	—	—	$SO_4^{=}$	−0·1 to +1·4
Chromatium sp.	H_2S	polythionate	+8·4 to +11·2	$SO_4^{=}$	−2·9 to +0·9
	S°	—	—	$SO_4^{=}$	+0·3 to +0·4
S. cerevisiae	$SO_3^{=}$	—	—	H_2S	−34·0 to −41·0
	S°	—	—	H_2S	−0·6 to +0·8

* $\delta^{34}S$ refers to the enrichment (or depletion) of sulphur-34 in parts per thousand relative to the $^{34}S/^{32}S$ ratio of the starting compound.

had been stopped earlier is suggested by the accumulation of an intermediate, most likely a polythionate, which was markedly enriched in ^{34}S (Table 1). The same was observed for *T. concretivorus*.

It is generally believed that elemental sulphur is an intermediate in the chemosynthetic and photosynthetic oxidation of sulphide to sulphate and it is possible that it is also an intermediate in the reduction of sulphite. The question then arises as to why no fractionation occurred during either elemental sulphur oxidation or reduction. Since there is no reason to believe that the same enzymes or the same rate-limiting processes are involved in the oxidation or reduction *per se*, it is suggested that the explanation for the lack of fractionation in both processes lies in the feature common to both, the manner of transport of the elemental sulphur across the cell boundary.

It was observed that *Chromatium* sp. grown on elemental sulphur contained sulphur globules within the cells which disappeared on standing in a sulphur-free medium. This, together with the observed accumulation of sulphur in vacuoles of elemental sulphur-grown *Thiobacilli*[4], suggests that the sulphur was transported into the cell without a change of valence. The absence of significant isotopic fractionation during sulphur metabolism further indicates that groups of atoms are transferred across the cell membrane, eliminating any kinetic isotope effect. The atomic groups involved could be single or multiple molecules of sulphur chains; but the evidence does not permit speculation on the size. It also suggests that all, or the major part, of the sulphur entering the cell is metabolized to sulphate without an equilibrium existing between external and internal free sulphur.

The experimental results thus support the concept of movement of elemental sulphur *per se* across the cell boundary but cast no light on the actual mechanism of transport. The suggestion of Vishniac and Santer[3] that the sulphur is first reduced to sulphide seems unlikely in view of the significant fractionation observed with sulphide as a substrate.

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Tetramethylpyrazine: a *Bacillus subtilis* Growth Factor

WE have found[1] that a strain of *Bacillus subtilis* produced tetramethylpyrazine in synthesized media containing asparagine as nitrogen source.

The strain did not show satisfactory growth in media containing any amino-acid, except asparagine and proline, etc., as nitrogen source.

From the fact, it seems that the amino-acids asparagine and proline participate in the biological synthesis of tetramethylpyrazine, and the compound is a growth factor for the strain. In fact, growth of the strain and *Bacillus subtilis PCL* was considerably accelerated when teramethylpyrazine was added in culture media containing amino-acid, such as alanine, histidine, lysine, tryptophan and glycine, etc. The results obtained are given in Table 1.

Table 1. EFFECTS OF SUBSTANCES ON GROWTH OF THE *Bacillus subtilis* STRAIN AND *Bacillus subtilis PCL*

Substance as nitrogen source	*BS* factor	Degree of growth			
		B. subtilis strain		*B. subtilis PCL*	
		20 hr.	35 hr.	20 hr.	35 hr.
Asparagine	−	+ +	+ + +	+	+ +
	+	+ +	+ + +	+ +	+ + +
Proline	−	+	+ +	+	+ +
	+	+	+ +	+	+ +
Alanine	−	−	+	−	−
	+	+	+ +	−	±
Histidine	−	+	+	−	±
	+	+	+ +	+ +	+ +
Cystine	−	−	+	−	−
	+	±	+ +	±	+
Lysine	−	−	−	−	−
	+	−	+	±	+ +
Phenylalanine	−	−	−	−	−
	+	−	+	−	+
Glutamine	−	+	+ +	±	+
	+	+	+ +	+	+ + +
Tryptophan	−	−	−	−	−
	+	−	+	−	+
Glycine	−	−	−	−	−
	+	+ +	+ +	±	+ +
Arginine	−	+ +	+ + +	−	+
	+	+ +	+ + +	+	+ +
Tyrosine	−	−	+	−	−
	+	−	+ +	−	+
Leucine	−	−	−	−	−
	+	+	+	+	+
Methionine	−	−	−	−	−
	+	−	−	−	−
BS factor	+	−	−	−	−

As shown in the table, it might well be said that tetramethylpyrazine is a growth factor for the *Bacillus subtilis* strain and *Bacillus subtilis PCL*. We therefore suggest the name of *BS* factor (*Bacillus subtilis* factor) for tetramethylpyrazine.

From these results tetramethylpyrazine may be accepted as a growth factor for *Bacillus subtilis*.

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Oxidation of Anthranilic Acid by a Species of *Achromobacter* isolated from Soil

STUDIES of the microbial decomposition of anthranilic acid have been confined mostly to work with various strains of *Pseudomonas* spp.[1-3] which oxidize anthranilic acid to catechol by an unknown pathway. The role of salicylic acid in this conversion is not clear since reported experiments based on the technique of simultaneous adaptation have yielded divergent results[1,4]. Sakamoto *et al.*[4] have further shown that cell suspensions, adapted to oxidize anthranilic acid, are inactive towards 3-hydroxyanthranilic acid and 5-hydroxyanthranilic acid. By contrast, the two last-named compounds have been clearly implicated in the breakdown of anthranilic acid by mammalian tissue since both have been isolated from the urine of animals injected with anthranilic acid or *o*-aminobenzamide[5–9].

I have isolated from the soil an *Achromobacter* sp. which adaptively oxidizes anthranilic acid. The organism may be grown in a simple mineral salts medium containing 0·05 per cent (w/v) anthranilic acid as the sole carbon source; but in practice 0·1 per cent (w/v) yeast extract is also included to give greater yields of active cells. Unadapted cells are grown in a similar medium without anthranilic acid.

Adaptation of the organism to oxidize anthranilic acid does not result in its simultaneous adaptation to oxidize catechol. Both adapted and unadapted cells oxidize catechol after a lag period (90–120 min.) when oxidation occurs at similar rates (12–14 µl. oxygen consumed/30 min./mgm. dry wt. of cells) and to the same extent (50–65 per cent of theoretical oxygen uptake for complete oxidation to carbon dioxide). Experiments using chloramphenicol provide further evidence that catechol is not an intermediate of anthranilic acid oxidation by *Achromobacter* sp. As shown in Fig. 1, the rate of anthranilic acid oxidation by adapted cells is unaffected by chloramphenicol (50 µgm./ml.); the total uptake of oxygen is actually greater in its presence due presumably to its ability to inhibit protein synthesis and hence make a greater proportion of substrate carbon available for oxidation. Chloramphenicol completely inhibits catechol oxidation. In all cases estimations of residual substrate are made by absorbancy measurements in the ultra-violet region using a 'Shimadzu *QR*–50' spectrophotometer.

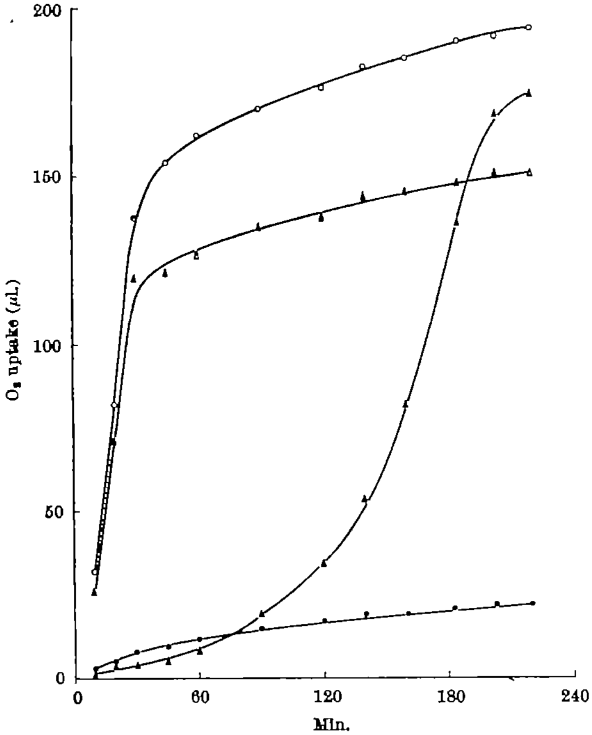

Fig. 1. Effect of chloramphenicol (50 µgm./ml.) on the oxidation of anthranilic acid and catechol by anthranilic acid-adapted cells. Bacterial suspension (= 3 8 mgm. dry wt.) pre-incubated with chloramphenicol for 15 min. before addition to 2µ mole substrate in 2·0 ml. (final volume) 0 05 *M* potassium phosphate, *p*H 6 0. temperature, 30° C. ○, Anthranilic acid + chloramphenicol; △, anthranilic acid; ●, catechol + chloramphenicol, ▲, catechol

Table 1. OXIDATION OF ANTHRANILIC ACID AND RELATED COMPOUNDS BY ADAPTED AND 'UNADAPTED' BACTERIAL SUSPENSIONS

Substrate	Rate of oxidation (µl. O_2/30 min. mgm. dry wt. bacteria) Adapted cells	Unadapted cells
Anthranilic acid	28 2	3·4
5-Hydroxyanthranilic acid	18 5	1·9
3-Hydroxyanthranilic acid	1 0	2 5
2:5-Dihydroxybenzoic acid	17 0	8·4
2:3-Dihydroxybenzoic acid	2 4	1·6
Catechol	0·5	2·2
Salicylic acid	6·3	6·5
Endogenous	1·7	1·6

Cell suspensions incubated at 30° C. in 2·0 ml. 0·05 *M* potassium phosphate *p*H 6 0 containing 2 0 µmole substrate. Substrate oxidation-rates corrected for endogenous cell respiration.

Cells adapted to anthranilic acid are simultaneously adapted to oxidize 5-hydroxyanthranilic acid and 2 : 5-dihydroxybenzoic acid (Table 1). 3-Hydroxyanthranilic acid and 2 : 3-dihydroxybenzoic acid are oxidized slowly after a lag period of negligible oxygen uptake. Salicyclic acid, *o*-aminophenol and the remaining isomers of 2 : 5-dihydroxybenzoic acid are not oxidized by anthranilic acid-adapted cells. The slight uptake of oxygen observed with salicylic acid (Table 1) occurs in the first 15 min. and is not continued; nor is it generally observed with other suspensions of either adapted or unadapted cells. It will be reported elsewhere that salicylic acid actually inhibits anthranilic acid oxidation and on these grounds is further eliminated as a possible intermediate. The results of simultaneous adaptation experiments indicate that an *Achromobacter* sp. oxidizes anthranilic acid, not via catechol, as established for *Pseudomonas* spp., but via 5-hydroxyanthranilic acid and 2 : 5-dihydroxybenzoic acid. Cell-free extracts of adapted *Achromobacter* sp. convert 2 : 5-dihydroxybenzoic acid to maleylpyruvic acid[10]. However, it must be pointed out that neither 5-hydroxyanthranilic acid, 2 : 5-dihydroxybenzoic acid nor any other aromatic derivative has as yet been detected in incubation mixtures containing anthranilic acid and suspensions of adapted *Achromobacter* sp. The pathway of anthranilic acid oxidation by *Achromobacter* sp. differs apparently from that reported for a *Flavobacterium* sp. by Ke *et al.*[11], who have implicated salicylic acid (but not catechol) as an intermediate.

I thank Prof. E. Boyland, Royal Cancer Hospital, London, and Dr. Y. Shibata, Wakayama Medical College, Japan, for gifts of 5-hydroxyanthranilic acid.

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SOIL SCIENCE

Location of Water-Table Level

WITH the increasing use of irrigation in horticulture and agriculture and the developing interest in the effects of evapotranspiration, it is frequently necessary to locate the level of a shallow water-table. This can be done inexpensively and effectively, using a small-diameter observation well and an electric probe.

The observation well is constructed by inserting into an auger hole (2-in. diam.) a zinc mesh tube bound closely with strips of hessian. Zinc is preferable because it does not easily corrode, the mesh allows free movement of water through the wall of the well at any level, and the hessian acts as a filter, preventing the inwashing of fine sand and silt particles which can otherwise rapidly reduce the effective depth of the well. In order to prevent the inflow of surface water, the upper 3 in. of the well casing should be unperforated, and this section should project slightly above ground-level. Finally, precipitation can be excluded by corking or covering the open end of the well.

In an observation well of this size an ordinary dipstick would cause a considerable displacement of water. The level of the water-table can, however, be located by using a simple electric probe, similar to that described by Russell[1], whose circuit is completed by contact with the water surface. Such an instrument, incorporating a 4·5-V. dry battery and a galvanometer, can be easily constructed and obviates the necessity of using more expensive manufactured equipment. Basically, the probe consists of a stainless metal shaft which is graduated in fractions of an inch. When two contacts at the tip are closed by the water-surface the galvanometer needle is deflected and the depth can be read off against the lip of the well casing.

Twelve observation wells and a probe, constructed to the specifications described here, have been satisfactorily used by me, in soils ranging from fine clay to coarse gravel, for a period of eighteen months, in connexion with investigations of the water-table in the Thames flood-plain. It is almost certain, however, that, if necessary, these instruments would give trouble-free service for a much longer period.

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PSYCHOLOGY

Effect of Age on Responses to Dichotic Stimulation

THE responses made by young normal adults to various methods of dichotic stimulation have been investigated and reported by Broadbent[1]. His observations have led him to postulate that both perceptual and storage mechanisms are necessary for the correct sequential recall of such simultaneous stimulation.

I have suggested that the defect of acquisition which has been found in elderly psychiatric patients suffering from memory disorder might be based on a breakdown of the storage mechanism postulated by Broadbent. Such patients should therefore show disturbance of recall in those half-sets of digits reproduced second in the dichotic stimulation situation, relative to the performance of a matched group of patients without memory disorder. Investigations by Caird and Inglis[2] and Inglis and Sanderson[3] show that this appears to be the case. Since increasing age in normal individuals also seems to affect learning capacity and as such impairment may also depend on changes in some short-term storage process it was anticipated that responses to dichotic stimulation might also show interesting associations with age.

Broadbent[1] has suggested that the first half-set of digits recalled from each dichotic span does not involve the storage process. If age primarily affects storage then the reproduction of the first half-set recalled should not be affected by advancing years. If the second half-set recalled must pass through the storage process then the recall of these digits should be affected by age.

Results obtained from a preliminary analysis of the performance of 120 subjects between the ages of 11 and 70 seem to support these expectations. As age increases there is no significant impairment in the ability to recall the first half-spans. Progressive, and significantly greater, difficulty is, however, shown in the reproduction of the second half-spans. Furthermore, the longer the span to be recalled the greater the difference, overall, between the first and second half-spans ($P < 0·001$). It has never before been convincingly demonstrated that short-term storage ability continuously decreases with age. The work described here will be replicated before a full report is published. If these results are confirmed we shall have come to a closer specification of a fundamental process the diminished efficiency of which may produce some of the impairments of age and the breakdown of which may underly the psychosis of senility.

This work was supported by a Federal-Provincial Mental Health grant (project No. 605-5-285). The testing was carried out by Mr. W. K. Caird.

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Transfer between the Hands of a Mirror-Image Tactile Shape Discrimination

A SHAPE like that of the figure *C* is seen as a *C* in both the right and left halves of the visual field by adults. It is not seen as a *C* in, say, the right and as a Ↄ in the left half field. One may ask whether such an identity of the perceived shape in the two half fields is given by the structural organization of the sensory system, or whether it is the result of some learning process. To answer this question would require subjects without experience of shape and object discriminations, and yet able to perform such discriminations. Monkeys were selected as experimental subjects, and tactile discriminations were used in order to minimize the effect of previous experience with shape discriminations. In brief, the animals were trained to discriminate by touch with the left hand between the solid shapes *C* and Ↄ; they were afterwards tested for their preference between the same two shapes with the right hand.

Four immature rhesus monkeys were subjects. All had been housed in the laboratory for eight months, and had been trained to perform five visual discrimination tests. For a period of 10 weeks three of the animals had received tryptophan or glycine supplements to their diet (combined with iproniazid for two weeks), without behavioural effect. The fourth animal (No. 7) had served as an untreated control. Details of the previous training histories are to be found elsewhere[1].

For the experiment reported here all four animals were first trained (using conventional apparatus and procedures) to discriminate in the dark by touch between two objects differing in texture. Forty trials (non-correction) were given each day. Training was continued until the animals were successful in 90 per cent of 150 consecutive trials. During this, their first tactile test, use of the right hand was restricted by attaching a weighted ball to the right wrist. Therefore all animals learnt to discriminate between the textures by touch with the left hand. All animals were then trained to discriminate by touch between the solid shapes *C* and *Ɔ*. The shapes were exact mirror-images of each other. Choice of the shape *C* was rewarded, irrespective of its position to the animal's left or right. Training was continued until 90 per cent of 400 trials were successful. Use of the right hand was again restricted, so that this tactile discrimination between mirror-image shapes was also made with the left hand.

Next, the animals' performance with the previously untrained (right) hand was tested by restricting the use of the left hand. Forty 'transfer trials' were first given for the discrimination between textures. During these (and the subsequent) 'transfer trials', rewards were placed under both or neither test object on alternate trials. There was thus no differential reward for one of the test objects and rapid learning was prevented. After the initial 40 'transfer trials' with the right hand the animals were retrained, still with the right hand, to discriminate by touch between the objects differing in texture. Training was continued until the animals were successful in 90 per cent of 100 trials. All animals were then given 40 'transfer trials' for the discrimination between the shapes *C* and *Ɔ*. After these 40 trials with the right hand the animals were trained, still with the right hand, to make a reversal of their previous choice in the test of tactile shape discrimination: choice of the shape *C* was no longer rewarded while choice of the shape *Ɔ* was now rewarded. Training was continued until the animals were successful in 90 per cent of 100 trials.

Finally, the animals' performance with the first (left) hand to be trained was again tested by restricting the use of the right hand. Forty 'transfer trials' for the discrimination between the shapes *C* and *Ɔ* were given.

The results are presented in Table 1. Three of the four animals performed significantly above the chance expectation when they were tested for 40 'transfer trials' with the right hand for the discrimination between textures. However, all four animals performed below the chance expectation, indicating their choice of the previously unrewarded shape *Ɔ*, when they were tested for 40 'transfer trials' with the right hand for the discrimination between mirror-image shapes. In one animal performance was significantly below chance (two-tailed), in another the two-tailed probability was 0·08, giving a one-tailed probability of 0·04. Moreover, only 0–140 trials (mean of 83 trials) were required to learn with the right hand the reversal of the shape discrimination previously learnt with the left hand. Finally, all animals performed significantly above the chance expectation, indicating choice of the shape *C*, when they were tested for 40 'transfer trials' with the left hand for the discrimination between mirror-image shapes. Continued choice of the shape *Ɔ* (rewarded during reversal training with the right hand) was expected with the left hand on the basis of results for tactile discriminations not involving mirror-image shapes with intact monkeys[3]. The results indicate that for the monkey and our test conditions, a shape *C* palpated with the left hand is treated as equivalent to a shape *Ɔ* palpated with the right hand.

Table 1. RESULTS OF TRANSFER BETWEEN HANDS

Animal Original hand preference	1 Left hand	5 Left hand	6 Left hand	7 Right hand
Trials required to learn *C* vs *Ɔ* discrimination (left hand)	860	310	570	380
Percentage correct in 40 transfer trials · textures (right hand)	98*	63	98*	98*
Percentage correct in 40 transfer trials · *C* vs. *Ɔ* (right hand)	43	40	35†	20*
Trials required to learn *C* vs. *Ɔ* reversal (right hand)	140	100	90	0
Percentage correct in 40 transfer trials · *C* vs. *Ɔ* (left hand)	100*	100*	100*	95*

Original hand preferences were determined in an unrestricted but formal test situation as previously described (ref. 2). Trials do not include criterion trials during which performance was 90 per cent correct or better. Significance levels were established as two-tailed probabilities on the binomial test. * $P < 0{\cdot}01$. † $P < 0{\cdot}1$.

Opposite (reversed) discriminations may thus be concurrently acquired by the intact monkey. Trevarthen[4], also using monkeys but not mirror-image shapes, has shown that such opposite visual discriminations can be learnt separately through the two eyes after sagittal section of the optic chiasma and division of the cerebral commissures. In his experiment each cerebral hemisphere apparently controls one of the two opposite discriminations. However, in our intact monkeys the hemispheres were normally linked. Moreover, there was normal (not reversed) transfer between the hands with the discrimination between textures. We would therefore propose that the structural organization of the neural systems concerned with tactile shape discriminations by the monkey is such as to produce equivalence between laterally reversed (mirror-image) shapes when each is separately palpated with a different hand. It may be suspected that the motor systems contribute to such equivalence. However, no movements are made in certain tactile shape discriminations (graphæsthesia). It has also been shown[5] that total destruction of the motor cortex does not impair the ability of monkeys to discriminate by touch with either hand between the shapes *L* and *⅃*. This evidence does not exclude some contribution to mirror-image equivalence from the motor systems. Generalization of the present findings from the two hands to the two half fields of vision is inadvisable until the role of the motor systems in discrimination performance has been ascertained.

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FORTHCOMING EVENTS

Monday, June 18

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Fain: "Some Problems of Causation".

Tuesday, June 19

Photoelectric Spectrometry Group (joint meeting with the Photobiology Group, in the Large Lecture Theatre, School of Pharmacy, Brunswick Square, London, W.C.1), at 6 p.m.—Dr. C. A. Parker: "Some Aspects of Phosphorescence in Spectrophotometry"; Dr. R. Barer: "Ultra-Violet Television Microscopy"; Dr. Britton Chance: "Applications of Spectrophotometry to Biophysical Research at the University of Pennsylvania".

Wednesday, June 20

Bone and Tooth Society (at 234 Great Portland Street, London, W.1), at 4.15 p.m.—Annual General Meeting, followed by a programme of Casual Communications.

Wednesday, June 20—Friday, June 29

Third Congress of the European Federation of Chemical Engineering (at Olympia, London).

Wednesday, June 20—Saturday, June 30

Second Chemical and Petroleum Engineering Exhibition (at Olympia, London).

Thursday, June 21

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. T. Eayrs. "Endocrine Influence on Cerebral Development" (Special Review Lecture).

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. M. Marstrand· "A Problem on Polynomials".

Society for Endocrinology (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.30 p.m.—Prof. Adolf Butendandt (Munich)· "Bombycol, the Sex-Attractive Substance of the Silkworm, *Bombyx mori*" (Annual Lecture).

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Fifty-fifth Annual General Meeting, followed by Sir Gordon Covell: "Reminiscences of the Campaign in East Africa 1914–1917".

Monday, June 25

British Society for the History of Science (in the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Annual General Meeting and Presidential Address.

Monday, June 25—Tuesday, June 26

Institute of Rural Life at Home and Overseas (at the Department of Technical Co-operation, 6 Carlton House Terrace, London, S.W.1), at 10.30 a.m. on Monday and 10.15 a.m. on Tuesday—Twelfth Annual Conference—"Training the Farming Community at Home and Overseas".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer (graduate in microbiology, agricultural microbiology or some related subject) in Soil Microbiology in the Department of Physiological Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (June 22).

Assistant Lecturer in Pure Mathematics—The Registrar, The University, Sheffield (June 23).

Lecturer in the Department of Biological Chemistry—The Secretary, The University, Aberdeen (June 23).

Lecturer or Assistant Lecturer in Physics at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 28).

Lecturer or Demonstrator in Chemical Pathology, for duties to be divided equally between original investigation and supervision of the hospital laboratory—The Registrar, The University, Leeds 2 (June 30).

Lecturer in Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield (June 30).

Research Demonstrator (with good honours qualifications in organic chemistry) in the Department of Chemistry—The Registrar, University College, Singleton Park, Swansea (June 30).

Research Fellow in the Surface Physics Group, to join a team investigating mechanism of photoelectric emission—The Secretary and Registrar, The University, Southampton (June 30).

Senior Lecturer (with an honours degree) in Physics, and a Lecturer (with an honours degree) in Physics—The Director of Education, The Polytechnic, Regent Street, London, W.1 (June 30)

Chair of Physics in the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 7).

Lecturer or Assistant Lecturer (honours graduate in either oceanography, physics or mathematics, and willing to spend several weeks each year at sea) in the Department of Oceanography—The Registrar, The University, Liverpool, quoting OV/101/T (July 9).

Reader in Science of Materials at Queen Mary College—The Academic Registrar, University or London, Senate House, London, W.C.1 (July 11).

Associate Professor of Medical Biochemistry in the Departments of Medicine and Biochemistry, University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 13).

Lecturers (2) in Pure Mathematics at the University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 13).

Lecturer (with a higher degree in philosophy and preferably with special interest in the theory of knowledge and perception) in Philosophy at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 14).

Chair of Theoretical Physics at the Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 16).

Lecturer in Biochemistry in the Faculty of Medicine, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 28).

Senior Lecturer or Lecturer in Physiology at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 28).

Head of the Department of Civil Engineering—The Registrar, Bradford Institute of Technology, Bradford 7.

Vice-Chancellor of the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

REPORTS and other PUBLICATIONS

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EDUCATION IN AND FOR SCIENCE

IN the spring issue of *Daedalus*, the journal of the American Academy of Arts and Sciences, under the broad title "Science and Technology in Contemporary Society" are presented seven of the papers prepared for the centenary of the Massachusetts Institute of Technology during April 1960. Prof. R. Aron discusses the education of the citizen in industrial society, touching specifically on its bearing on problems of Government. Sir Eric Ashby is concerned more particularly with the education of the administrator, the precise function of administration and the skills it demands, while Aldous Huxley, under the title "Education at the Nonverbal Level", examines the effectiveness of modern education. Dr. L. S. Kubie, writing on the fostering of creative scientific productivity, is concerned with the fostering of creativity in the training of the scientist, while Prof. W. A. Lewis discusses education for scientific professions in the poorer countries, Dr. R. S. Morison the university and technical assistance, and Dean G. S. Brown new horizons in engineering education, including a glance at the creative element as well as the revision of curricula and interdisciplinary research centres.

There is, as might have been expected, a forthright and realistic note in all these addresses which brings the scientist and technologist into continuous contact with the changing world of to-day. However, it is equally worthy of note, though perhaps scarcely surprising, in view of the common element in the general titles, that there should be such a similarity in theme and emphasis between these papers and the eight Oxford lectures which the Conservative Political Centre has recently published under the title *Science and Society**. The first two are both concerned with problems of science and Government: Lord Hailsham under the title "Iconoclasm and Civilization" discusses the organization of research, while Sir Hugh Linstead reviews the working of the Parliamentary and Scientific Committee. Mr. Aubrey Jones, in dealing with the Soviet challenge, is also concerned with the organization of scientific, including industrial, research, and Mr. W. Rees-Mogg, in "The Conquest of Poverty", looks at the challenge presented by the under-developed countries. Sir Frank Engledow deals simply with science and farming, Mr. D. Price with the education of the scientist, and Mr. M. Howard, under the title "Security in the Nuclear Age", with defence. Only Dr. N. St. John-Stevas's lecture on "Science and Faith" goes outside the field of those given at the Massachusetts Institute of Technology, and even this is more apparent than real. Dr. St. John-Stevas, while not beguiled into sociology, has clear views of the function of religion which in his judgment has a vital part to play, by slowly moderating the passions of men and pointing to altruistic ideals, in forwarding the achievement of the human unity which science has made necessary.

Apart, however, from the extent to which once again the problems of the under-developed countries force themselves on the attention of a comprehensive survey of the problems of science and society, the feature of both these symposia is the similarity of outlook in Britain and the United States in regard to problems of science and Government, and the education of scientist or citizen. The solutions suggested are not necessarily the same, where indeed solutions are suggested, for it is fully recognized that the conditions are not the same in the two countries and that the organization of either science or Government and the system of education at all levels must take full account of the traditions of the past as of present-day trends, practices and needs. Moreover, it must be remembered that the addresses do not cover precisely the same ground: Sir Eric Ashby, for example, is more restricted than Lord Hailsham, though to a certain extent he overlaps also with Sir Hugh Linstead.

One valuable feature of Sir Eric Ashby's paper is the picture he draws of the functioning of the administrator in regard to science and from it of particular skills with which such an administrator must be equipped. This is the pragmatic approach; but unless it is grasped that the essential technique of the administrator is that of using other men's minds, we are unlikely to recognize the type of education required for an administrator or even to set up the sort of organization which allows him to practise that technique most effectively. The very term 'red tape' carries the implication that we have failed to do so.

Sir Eric Ashby himself points out that the education of administrators should be such as to minimize failure to elicit appropriate replies from the experts; inability to create a simple and persuasive decision out of the information, or failure to take viable decisions, through underestimating the political component, through overlooking the adage that politics is the art of the possible. It should fundamentally assist him to conduct a traffic in ideas, and no conceivable education could equip the administrator to make from his own knowledge many of the decisions he is called on to make. Nor can the skills required be acquired by formal education alone, though they can be encouraged by certain kinds of instruction. Sir Eric, in fact, regards the administrator as one of the chief channels through which science and society interact: depending on his qualifications he can promote or hinder that interaction and for that reason alone part of the equipment of the administrator must include some understanding of the approach of the scientist, the attitude of the scientist to time and the way he assesses the value of his work.

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* Science and Society: Eight Oxford Lectures. Pp. 94+2 plates. (London: Conservative Political Centre, 1962.) 3s.

Such an understanding is fundamental to the formulation of any strategy of scientific advance and is scarcely less essential in the consideration of tactics. Lord Hailsham, who once again questions the practicability of scientific strategy, is equally at pains to make clear that science is a part of culture and that the relation of science and Government is one aspect of the adaptation of our institutions to change and of changes to our institutions. Science, he points out, is an inseparable and indeed the one characteristic feature of modern civilization, and while he is no less concerned than Sir Eric Ashby with promoting the traffic in ideas, it is to the problem of organization that he primarily addresses himself, and more especially the organization of research. Once again he expounds his conception of the Minister for Science as responsible for promoting the pervasion of science through industry, agriculture, the professions and the universities.

In this Lord Hailsham adds little to what he has previously expounded during the past year or so. It is, however, in his forthright condemnation of the attempt to dictate scientific projects on grounds of national prestige that he becomes less inhibited, and he gives frank expression to the belief that the mounting cost of scientific work may be taking us beyond the scope of national budgets. Lord Hailsham's belief in international co-operation in science is already well known. Here he is frankly dubious as to the extent to which we can afford to devote resources to space research in the face of projects for increasing food production, particularly in the under-developed countries. The production of proteins from fibrous plants, farming from the sea, plant breeding, desert irrigation, fresh water from salt, plastics from wood, and reducing the need for industrial crops are cited as examples for possible international co-operation in research and which may have repercussions on the present political framework, possibly even leading to new political institutions and international loyalties.

Lord Hailsham's clear recognition of the limitations of Britain's material resources is matched by his belief in the intellectual resources and the contribution that Britain could bring to the traffic in ideas. He calls for the wise selection of projects and for the readiness to face change; but he places as much emphasis as Dr. St. John-Stevas on the need for something more than material values, on the ultimates, knowledge and beauty, truth and justice, goodness and God, as part of a satisfying and constructive solution. It is left, however, for Sir Hugh Linstead to touch on the fundamental problems of Government, though even Sir Hugh is content essentially to defend the conception of a Minister for Science and to postulate what is asked of the scientist who wishes to play his part and what the politician must concede to the scientist.

Even so, Sir Hugh emphasizes the vital importance of the scientist retaining so far as possible his independence and freedom from party politics. This is too commonly overlooked. As Sir Hugh rightly remarks, the independence of the scientist should be a matter of professional pride to be jealously guarded, and while science in its search for truth cannot be subjected to the direction of authority nor prostituted to political expediencies, science can only assume its full authority in public affairs when the scientist does assert and maintain his political independence. It is then above all that his advice can be accepted without suspicion.

Nor is this inconsistent with the truth that politics itself is simply a matter of expediency, of what is practicable. Mr. Aubrey Jones's thesis that a civil scientific budget is needed and that the allocation of resources between such activities in the field of Government should be the ultimate responsibility of the Minister for Science confuses the real issue. Some advance on the present arrangements for organizing such scientific activity may well be desirable, in particular possibly something of an adequate intelligence organization, though the value of informal and personal contacts such as exist through the Research Councils and like bodies must not be ignored; to place such responsibility on the Minister for Science could destroy the whole function. He could scarcely at one and the same time satisfy himself that he had obtained and presented to the Government the soundest and most authoritative advice on the scientific and technical aspects of a problem and also undertake personally the responsibility for making decisions on the other aspects involved.

What matters especially to-day is that proper weight should be given to the scientific and technical factors in a problem or situation, not that they of themselves should determine the decision. It is, of course, essential that the scientist and technologist should be enabled to tender advice appropriate to the existing conditions, if that advice is to be appreciated in its true perspective: it is manifest that this did not happen when the University Grants Committee made its recommendations for the next quinquennium, and a good deal of the dissatisfaction with the Government's decision stems from their neglect of this principle. Mr. Aubrey Jones has other suggestions to make for raising the standards of industry—for example, by amalgamating the industrial functions of the Department of Scientific and Industrial Research with the National Research and Development Corporation.

In all this, as is freely recognized by the participants in these two symposia, we are continually facing the problem of the education of the administrator, the ordinary citizen, the scientist and technologist, and Mr. Price's lecture is particularly valuable for its insight and sanity. As Prof. Aron points out, there is a problem of communication which is inescapable and plans for Government as well as plans for education must take account of it. Even with the scientist and engineer, the concern to foster the all-important creative productive powers must not deflect attention from this element of communication. Fuller understanding of the nature of scientific work may ultimately make it easier to provide the conditions which encourage such creative work, but only on condition that there is reasonable communication between the scientist and the administrator and ordinary citizen. It is as much for the contribution which these two symposia offer to that communication and understanding as for the way in which they indicate aspects of the relations between science and society which need careful study that the Oxford lectures and those delivered at the centenary of the Massachusetts Institute of Technology deserve to be studied.

LORD CHERWELL: 'THE PROF.'

The Prof. in Two Worlds

The official life of Professor F. A. Lindemann, Viscount Cherwell. By The Earl of Birkenhead. Pp. 383 + 12 plates. (London: William Collins, Sons and Co., Ltd., 1961.) 45*s*. net.

IN reviewing an earlier book on Lord Cherwell (*Nature*, 184, 1752; 1959) I had speculated that no man in Britain since Francis Bacon had combined such a deep interest in natural science with so high a public office. I would now revise this speculation, having in the meantime read the *Memoirs and Correspondence of Lyon Playfair* (1818–1898), for there are several parallels between Playfair's career and that of Cherwell. Both men studied in Germany, both held science chairs in British universities, both stood for Parliament (Playfair successfully, Cherwell not), both fought for the expansion of education in science and for the development of technology, both were involved in monetary policy (Playfair against bimetallism, Cherwell against convertibility of sterling) and in food policy (Playfair with legalizing margarine, Cherwell with war-time rationing), both achieved ministerial rank (Playfair postmaster-general, Cherwell paymaster-general) and both received peerages.

Careers of such complexity and breadth are a stimulating challenge to biographers; and in Lord Cherwell's case there is the additional challenge of depicting the unusual combination of warmth and reticence in his character. Moreover, any attempt by a biographer to give an impartial assessment is bedevilled by the fact of Sir Charles Snow's partial account of the unfortunate relations between Cherwell and Sir Henry Tizard; happily, Snow to some extent redressed the balance in his recent rectorial address at St. Andrews; but Lord Birkenhead had to complete his biography of Cherwell while Snow's charges stood unmoderated.

Lord Birkenhead has given a good account of Cherwell's work as a member of the Government, which included such administrative measures as resisting the restoration of the convertibility of sterling in 1952 and as disestablishing the Atomic Energy Authority from the Ministry of Supply in 1954, besides much advice on economic matters to Mr. Churchill during the Second World War.

Lord Birkenhead's greatest difficulty has been to give an assessment of Cherwell's work in physics, and in science applied to warfare and particularly to air defence. Here he has had to rely on the evidence of a comparatively few witnesses, including myself; despite this limitation, I believe that the book conveys an accurate impression of Cherwell, both as an individual physicist and as head of the Clarendon Laboratory. His faults are not obscured; but his merits as a physicist, too often overlooked, emerge particularly in the quotations from Sir Cyril Hinshelwood, Sir George Thomson, and Prof. D. A. Jackson. His work in building up the Clarendon is well depicted: it is not easy for any physicist whose experience is limited to after the Second World War to realize the obstacles that any head of a laboratory had to overcome before 1939.

This book gives a good account of the work on recovering aircraft from spins in 1917. This makes it clear that while Lindemann was not the first man to recover an aircraft from a spin, he was the first man to reason how to do so; and his courage in undertaking the practical trials himself amply merits its legendary place in the history of science applied to flight.

As regards Cherwell's work on air matters connected with the Second World War, Lord Birkenhead has given telling evidence to show that this was much more positive than some accounts have suggested. In the attempt to correct the account given by Sir Charles Snow, there is occasional unfairness to Sir Henry Tizard, notably in suggesting that he was liable "to resent the submission of new ideas", which is contrary to my own experience; but the quotations from Cherwell's letters and reports of 1935 and 1936 effectively refute the sensation-seeking charge that Cherwell was anti-radar, and that the Royal Air Force might therefore have lost the Battle of Britain if Winston Churchill had been Prime Minister in 1935.

As regards 'The Prof.'s' character, Lord Birkenhead's account is sympathetically presented, and it rings true. 'The Prof.', by an extraordinary inversion of human nature, exposed his faults and concealed his personal virtues. "No man", said Marshal Catenat, "is a hero to his valet"—but 'The Prof.' most certainly was. Mr. James Harvey was the Prof.'s valet and secretary for thirty years, and Lord Birkenhead most fittingly quotes from Mr. Harvey's tribute: "The world is much the poorer for his passing—only people like myself, whose appreciations are rarely made public, will know how much poorer".

For me, much of the interest in this enjoyable and well-illustrated book is in the light that it throws on the inner life of 'The Prof.', from boyhood onwards. Much of this is new to me, even though 'The Prof.' would sometimes tell me of such personal matters as, for example, his proneness to write rude letters when he had been annoyed, in order to save me from developing similar faults myself. I wish that I could have read this book while 'The Prof.' was still alive.

R. V. JONES

TWO SUB-CULTURES?

The Cell and the Organism

Essays presented to Sir James Gray. Edited by J. A. Ramsay and Prof. V. B. Wigglesworth. Pp. viii + 350. (Cambridge: At the University Press, 1961.) 52*s*. 6*d*.

THIS volume contains contributions from twenty eminent zoologists who are working, or have worked, in the Department of Zoology, University of Cambridge, and it was presented as a mark of their appreciation to Sir James Gray for all that he has done for zoology during the past forty years. It can therefore be considered as a summary of the work of an experimental school which has been for long of paramount importance in the teaching of and research in zoology in Britain. The range of contributions is wide; there is Thorpe's stimulating discussion of the interrelationships between animal behaviour and neurophysiology on one hand, and on the other Picken's thoughtful consideration of molecular biology in relation to the future of zoology. In between these extremes most of the expected contributions will be found; there are, for example, Rothschild on sperm, Wells on lugworms, Swann on cell division and Munro Fox on the various coloured bloods. As would be expected, they nearly all give a clear account of their selected topic with, on occasion, the levity appropriate to the occasion, and it would be presumptuous of me, a not very satisfactory student of this illustrious department, to consider these contributions in detail.

What else is there to be said? Pantin in his introductory essay discusses the development of the experimental approach forty years ago, when morphologists and embryologists, concerning themselves almost entirely with a search for homologies, were dominant in zoology, and he feels, indeed, that the only satisfactory definition of experimental zoology is "those varied and extensive parts of animal biology which certain influential senior zoologists disliked in 1920". Zoology, as an academic subject to-day, seems to be faced with a similar crisis. In addition to the older disciplines, like biochemistry, new subjects, like molecular biology, biophysics and microbial genetics, attract able students and financial support, and part of their attraction is that they combine the promise of immediate usefulness with the synthesis of a wide range of scientific disciplines, while at the same time it is rightly claimed that their findings are of general significance to the older branches of biology. There are other more mundane symptoms; there is a decline in interest in the zoological side, at least, of the only general biological society with an experimental approach, and it is a common experience that meetings on protein synthesis, molecular structure or the genetic code usually command greater support than most of the subjects discussed here.

Must we then conclude that this volume represents a final flowering of a glorious era in biological research? Picken in his chapter argues strongly that this need not be so, and that zoologists and botanists are essential for the future progress of the more analytical subjects. He argues his case on two counts: first, the practical aspect that zoologists and botanists know about animals and plants, and about the vast range of complexity in the organization of their parts. They can therefore provide this information and even keep laboratory animals alive.

Secondly, by constantly keeping the concept of the organism as a whole before the biochemist and biophysicist, the zoologist and botanist can indicate the next step in the hierarchy of cells, tissues and organisms which must be explained by the function and structure of constituent macromolecules. Zoologists might, by their critical presence, hope to curb a facile generalization from simple to more complex organisms.

Good examples of both aspects are found in this book. No one interested in the problems of the control of protein synthesis can fail to be stimulated by Wigglesworth's account of the many changes which occur in insect epidermal cells in response to external and internal stimuli, and he must immediately ask himself whether any of the methods for investigating molecular structure now available could make use of all the detailed information available on these cells. Again, Mitchison considers the several cellular structures, such as the centriole and nucleolus, for which we can at present assign no exact function. They are all of the same order of complexity as mitochondria, the structure and function of which may soon be interpretable on a molecular basis, and it is very proper that we should be reminded that they may have no less importance in the cell's organization, despite the difficulty of isolating them for biochemical or biophysical investigation.

It would seem, however, that to cast themselves as keepers of zoological lore for the benefit of the molecular biologist or as goads to remind him of ever more complex systems to investigate is to cast these eminent scientists in altogether too lowly a role. Perhaps their fault lies in the ready acceptance of the premise, explicitly stated in this book, that zoologists and botanists are, as scientists, a different kind of people from biochemists, biophysicists and physiologists—so different, indeed, that training cannot remedy the matter. Is this so and is there nothing we can do about it? No such differences were present in the early nineteenth century, when, as Pantin points out, biologists were widely concerned with experimental investigations. Adding a little physics and chemistry to overloaded courses will not, of course, bridge the gap between the two sub-cultures, but a unitary subject, biology, could be taught in which the now 'peripheral' subjects would share in teaching and research in an atmosphere of mutual tolerance. Perhaps in this way an era can be initiated in biology as fruitful as that for which Sir James Gray was so largely responsible. P. M. B. WALKER

THE EXPANDING SCOPE OF ANIMAL PARASITOLOGY

Introduction to Animal Parasitology

By Prof. J. D. Smyth. Pp. xx+470. (London: The English Universities Press, Ltd., 1962.) 37*s*. 6*d*. net.

NO aspect of zoology appeals more to the student than that which concerns the intimate relationships between animals and their parasites. There is popular antipathy towards the importunate hanger-on and his cringing pose, but the manifold ways and devious means whereby parasites and their hosts have acquired mutual tolerance during many millennia of close and still closer association is a source of wonderment and a challenge to our understanding. In this small and inexpensive book the author scans a wide horizon, surveying the phyla Protozoa, Platyhelminthes, Acanthocephala and Nematoda, regrettably omitting parasitic crustaceans, insects and molluscs. He uses a 'type' system and deals largely with parasites which can be obtained from common hosts and examined alive, including some human parasites, and he makes a special effort to expound little-known physiological aspects of parasitism. By reducing ponderous morphological and taxonomic data to mere diagnostic criteria and by the use of clever diagrams to expand information concerning life histories, he has been able to introduce physiological data which impart a 'new look' to his subject and at the same time provide a sound basis for its expanding scope. He deals also with the nature of the host's reactions against its parasites, with the important subject of immunity and, at greater length, with the techniques for the *in vitro* culture of parasites.

This has been done with great skill but not without some loss of perspective, historical continuity having been sacrificed to modern developments. There is no mention in this book of Looss or Leiper, Thomas or Leuckart, or Dobell, Fuhrmann, Pintner, Railliet, Sinitsin, Skrjabin and a host of other dedicated pioneers whose classical researches illuminate the subject of parasitology and inspire the student to greater effort. There are sometimes lapses in matters which carry considerable weight of reliable opinion. In the account of ciliated Protozoa, for example, there is no mention of E. Fauré-Fremier and J. O. Corliss, who created a new classification of the Ciliophora during the past decade, and the problem of coccidiosis is discussed without mentioning the researches of E. E. Tyzzer and C. Horton-Smith. This criticism applies to groups other than the Protozoa; the author-index of the book is imposing but the chapter references often fail to indicate classical researches about which the student should know something.

The author's special fields of research are given the emphasis we would expect and they deserve, but the results of physiological researches with parasites are as yet so tenuous as to make unsatisfactory reading because areas of doubt and ignorance remain. There is some information about the chemistry and the mechanism of formation of egg-shell in the digenetic trematodes and the pseudophyllidean cestodes which requires revision, and the function of Mehlis's gland "remains obscure", while "how the operculum is formed remains a mystery". Statements about the blood-feeding habits of *Fasciola*, *Clonorchis* and *Dicrocoelium* are unsound and fail to harmonize with findings for *Paragonimus*, which has been studied in much greater detail. Another lapse is a failure to mention the *in vivo* technique using germ-free hosts, which, like *in vitro* culture methods, holds out great promise for the future. Axenically reared larvæ of *Nippostrongylus muris*, *Nematospiroides dubius* and *Hymenolepis nana* develop to normal fertile adults in germ-free guinea pigs, which are generally regarded as abnormal hosts for these parasites[1]. *H. nana* was more successful in the germ-free host than in conventional hosts, which has interesting implications in the nutrition of this tapeworm. The use of germ-free hosts will also tend to improve the quality of our knowledge about host-specificity.

What can or cannot be included in an introduction to parasitology is a moot point, and the present is catching up with the future now. Apropos of the axenic culture of *Neoaplectana glaseri*, the author states: "a number of media have been used to maintain *N. glaseri*, but these have been of biological origin and little attempt has been made to develop a defined culture medium". This end has been achieved already[2]. A chemically defined medium containing eighteen amino-acids, a fatty acid, urea, Krebs cycle intermediates, purines, pyramidines, B-group vitamins, glucose and also various inorganic constituents has fostered the development of third-stage larvæ of this nematode to the adult stage, with low but consistent level of reproduction. Incidentally, Jackson's aphorism should be memorized by all who enter this field of research: "cultures are as axenic as sterility tests are sensitive; and the media as defined as glassware is clean and chemicals pure".

It should be remarkable if any book were published free of typographical errors, and this book is not remarkable in that respect. A few elegant pages are marred in this way but, apart from mentioning the erroneous spelling of the names Dollfus (pp. 236, 238, 437) and Heyneman (pp. 255, 438), I shall rest content to forward a list to the author. In spite of such blemishes and some other shortcomings, the author is to be complimented on the production of a progressive book which points the way to profitable future developments in the expanding scope of animal parasitology, a book which will do much to assist the progress of a generation of students so long as they do not neglect the finer details of morphology and taxonomy in their quest for physiological knowledge.

BEN DAWES

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[1] Newton, W. L., Weinstein, P. P., and Jones, M. F., *Ann. New York Acad Sci.*, **78**, 290 (1959).

[2] Jackson, G. J., *Exp. Parasitol*, **12**, 25 (1962).

A GUIDE TO ISOTOPES

The Radiochemical Manual

Part 1: Physical Data. Pp. 102. (Amersham: The Radiochemical Centre, 1962.) 15*s*.

THIS handbook presents a great deal of accurate information about the availability and radiation characteristics of those radioactive isotopes, now numbering more than one hundred and fifty, which are in general use to-day. The bulk of the information is contained in a table extending to some 60 pages which lists the isotopes in alphabetical order and gives for each the basic data of half-life, nature and strength of radiation, method of production, intensity available, and chemical form. Subsidiary tabulations summarize and slightly extend the data in special ways (for example, Table 6 lists beta-particle emitters in order of half-life). There are also graphs giving exposure data for the radiation from some isotopes commonly used in radiography.

It is inevitable in such an extensive compilation that there should be a number of slips, typographical and otherwise; these appear to be rather few and trivial. A more important criticism concerns the arrangement of the book as a whole. The order in which the six sections appear does not seem to be very logical; for example, Section 6 on the definition of terms might very well have been placed first and Section 1 on production of isotopes might very well have been combined with Section 3 on special irradiations in reactors. The intervening Section 2 on characteristics and availability of radioisotopes (pp. 3–84) is made rather top-heavy by Table 1 referred to above ("Physical Characteristics and Availability of Important Isotopes"); as one result of this, the explanation for the appearance of some of the isotopes tabulated in Tables 6 and 7 (pp. 78–81) in bold type is to be found on p. 3. Incidentally, the criteria for promotion of certain entries into bold type (importance of the radiation concerned) do not appear to have been consistently applied. The tables of sources of various kinds (Tables 10–14) deserve a place in the list of contents. These slight flaws in presentation of the data are redeemed, however, by a good index.

The *Manual* richly deserves a place by the side of the well-known *Radiochemical Centre Catalogue* on the desk of everyone concerned with the production and use of isotopes. The printing is excellent and the binding adequate.

K. F. CHACKETT

EDWARDIAN SCIENCE AND TECHNOLOGY: THEIR INTERACTIONS*

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As is well known, the history of science views things past with two quite different perspectives. One of these is for the specialist, since it deals with the quiet emergence of new discoveries, in the deliberate seclusion of the study or the laboratory. In most known instances, several years, or even decades, have elapsed before a scientific discovery could be recognized as being really influential and far-reaching in the advancement of natural philosophy. But the other perspective of history deals with the impact of new applications of science to satisfying human needs in the market place. Developments of technology concern a much wider diversity of studies, in economics, in social affairs, and even in international politics. Time-lags are also inevitable in the application of new discoveries of science to practical uses. Under normal conditions, it is interesting to note that the time-lag between the purely scientific discovery of the element chlorine by Scheele in 1774, and its application on a wide industrial basis in bleaching powder by Charles Tennant in 1788, was only fourteen years, not much longer than the reputed average for new discoveries in the chemical research laboratories of progressive modern firms. Time-lags in the interaction between science and technology are obviously amenable to outside stimuli.

It so happens that both perspectives in the history of science show the Edwardian era to have been a period of revolutionary changes, the consequences of which we are still working out in our time. The First World War brought some of the trends to be discussed into sharp and even agonizing focus In the normal developments of science and technology of the nineteenth century this War introduced a deep cleft or Grand Canyon.

It is convenient to deal first with fundamental scientific discoveries. As in other periods of our scientific age, the decade of Edward's reign had its share of new advances in knowledge, too numerous to detail fully. But three of these advances have since proved to be quite cataclysmic. They have completely revolutionized physical theories of the world we observe by measurement. Einstein's theory of relativity was first published in 1905. Planck's quantum theory of radiation first emerged in the scientific literature in 1900. Both these theoretical developments stemmed from active and fertile German schools of theoretical physics. The third revolutionary discovery was experimental and British. It established the spontaneous disintegration of atoms, and was published in 1902 by Rutherford and Soddy as their theory of radioactive atomic disintegration.

In discussing reasons why these three innovations have completely revolutionized physics, the aspects to be viewed obviously depend on the onlookers. For the non-specialist, two facets of Einstein's theory of relativity warrant special comment. One of these is of great interest for the philosophy of time. In the realm of mathematical physics opened up by Isaac Newton, time was a framework common to all beholders. But Einstein postulated that each observer of Nature constructs his own local time-scale. His special theory of relativity based on the velocity of light as a common medium of communication permitted the matching of time-scales at different parts of the Universe. Philosophy is sometimes regarded as of little practical importance, which makes the second facet of Einstein's relativity theory part of the irony in the history of science. This theory first demonstrated the equivalence between energy and mass. In 1905, this equivalence seemed to be a mathematical curiosity, but we now know its fundamental importance for the generation of nuclear energy by the destruction of atomic mass, in atom bombs, and in our nuclear power stations.

Although Planck's quantum theory first emerged during the Edwardian era, its revolutionary importance for modern physics was not fully established until the formulation of Heisenberg's Uncertainty Principle in 1927. For the non-specialist, this innovation is particularly interesting in focusing attention on the nature of physical measurements as a means of acquiring experience about the world we live in. Man has practised measurement for thousands of years and, during the Victorian era, bold new developments were made in metrics. But the quantum theory revealed hitherto unsuspected limitations to the practices of measurement, which are of fascinating philosophical interest. In one sense, this development has corrected a sometimes excessive confidence in the power of metrical science.

Rutherford's theory of the spontaneous disintegration of atoms threw overboard all the inheritance of concepts about indestructible atoms in an everlasting universe of matter, which had attracted speculative thinkers from the time of the Indians and Greeks, and probably even earlier. Already in 1904 Rutherford had observed that large quantities of heat are liberated when atoms such as those of uranium or radium disintegrate spontaneously. At that time, and indeed until Otto Hahn's discovery of a nuclear chain reaction in 1939, there was no means of controlling atomic disintegration from without. But a far-seeing Edwardian scientist, Sir William Ramsay, had already announced the importance of atomic disintegration as a peaceful source of energy in the service of man, at the annual meeting of the British Association for the Advancement of Science in 1911. Ramsay estimated that through various applications of inanimate energy each British family by then had an average of twenty 'helots'—we now call them energy slaves—in its service. He stressed that stores of energy known at that time were not unlimited. The tides, the winds, the internal heat of the Earth, the heat of the Sun, the power stored with water, or in coal, in peat and in the forests all have their foreseeable limits as sources of energy to be harnessed by man. Ramsay's hope, induced by Edwardian discoveries of radioactivity, was to find catalysts for the exceedingly slow radioactive disintegrations which had been observed in naturally occurring elements, with the view of liberating their stored-up energy at a rate which could be utilized practically. We are the somewhat apprehensive inheritors of this Edwardian hope. Its realization in terms of the atom bomb and in terms of nuclear power stations has been accomplished in a way characteristic of so much technological aspiration and prophecy.

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* Substance of a lecture delivered on May 16 at the University of Oxford in the series "Science and Economics in Chemical Technology".

Even this brief mention of some of the striking advances of Edwardian science points to the fact that there was no lack of interest in practical applications of new knowledge among British scientists. But for reasons which it is important to study, this interest was often abortive. Various records show how the Edwardian era marked the culmination of obstacles and frustrations arising from Government inertia in promoting fruitful interactions between science and technology. This situation was particularly galling for those British scientists who could appreciate its dangers, and who were pointing to contrasts in Germany at that time.

Even a very brief survey of Edwardian applied science, or technology, to use that ugly but fashionable word, must include several far-reaching transformations in the material conditions of existence, which first became viable during that brief era. At least two of them have shifted the balance of world power, and world population, but before dealing with these it is pleasant to remind ourselves of some of the less-disturbing advances that helped to make life even more comfortable in those comfortable times. Motor-cars had been adopted by the wealthy before 1900. Kipling, for example, tells us of his first car, a typical gentleman's car hired from a Brighton firm in 1899, together with an engineer at 3½ guineas a week. This was "Victoria hooded, carriage sprung, carriage braked, single cylinder, belt driven, fixed ignition". At times this embryo could cover eight miles in an hour (Kipling), but by 1902 Kipling decided that a single cylinder engine was too easily defeated by the Sussex hills and bought a Lanchester car.

What was new was the advent of motor-cars deliberately produced for the masses. Henry Ford began his production lines in Detroit around 1903. Before the First World War nearly 21,000 Ford cars were in use in Britain in a total car population of about 132,000. The tide was clearly rising, as is shown by the following figures: 1910, 570; 1912, 3,200; 1914, 8,352.

For public transport, motorized vehicles were beginning to displace horse-drawn contraptions in an unmistakable way, both as taxi-cabs and as omnibuses. Five motorized omnibuses were touting for customers in 1897; by 1906 their number had grown to 700. The first motor-driven taxi-cab began to compete with the horse in 1904. One can visualize some of the conflicts between horse and motor in public transport; stirring but unrecorded encounters in a London fog, for example.

The cinema was likewise being developed with clear intentions to provide mass entertainment. Short motion pictures had been projected as part of the traditional variety entertainment since before Queen Victoria's jubilee. Probably the first show of motion pictures for which a special admission fee was charged was given at Olympia in 1896. By 1912 cinemas were available in most of the cities and large towns in Britain and the issued capital of cinema companies was about £3 million. Their bias towards mass-audiences was linked with aims to refine as well as attract the masses, as is shown by the publicity given to amenities such as plush carpets, bevelled mirrors, soft lights and usherettes. Some of the early 'news' to be shown in motion pictures included Derby Day 1896, Queen Victoria's jubilee 1897, Queen Victoria's funeral 1901, and Edward's coronation 1902. Names given to the early picture palaces illustrated the same trend, with glittering specimens such as Olympia, Bijou, Empire, Jewel, Gem, Mirror, Picturedrome, Pallasino and Palace-adium. There may be rarities still to be added to this collection. Cinemas were not at first aiming to attract the wealthy or even the educated classes. It is on record, for example, that Herbert Asquith first visited a cinema in 1911. On this occasion his behaviour must have irritated humbler patrons, with his hearty laughing, and continually making witty comments to his companions.

British enterprise was well ahead in the earliest days of the cinema, when films could still be produced by small organizations, on a scale about comparable to the first motor-cars built by William Morris. For various interesting reasons, which cannot be detailed here, the early British lead was not maintained as films became longer and more consequential. This in itself hampered possible fruitful interactions between science and technology.

British leadership was also prominent in the early developments of wireless telegraphy. The Irish–Italian Marconi took out his first patent in England in 1896. In view of the many other frustrations to be recorded about this era, it is noteworthy that Marconi received important early support from the Post Office engineers, in particular, its chief engineer, Sir William Preece.

German and American competition caught up, and the first passenger steamships to be fitted with wireless were U.S.S. *St. Paul* (1899) and the German *Kaiser Wilhelm der Grosse* (1900). However, it is pleasant to note that wireless was one of the very few technological developments that ever received active support from King Edward. When he was still Prince of Wales, and was confined about the Royal yacht at Cowes by an injured knee which prevented him from joining his "Mamma" at Osborne, it is recorded that he tapped out personally many messages to the general effect that "H.R.H. the Prince of Wales has passed another good night and the knee is in good condition"—less exciting this than Kipling's famous tale, "Wireless", but more comfortable.

Marconi's drive exploited world communication by wireless telegraphy, using what we now regard as a chancy and by no means well-understood effect, based on the coherence of metal filings under the action of wireless waves. The 'coherer' had, in fact, little or no future, the first electronic valve was invented in 1904 by a professor of electrical engineering at University College, London, Ambrose Fleming. Fleming's invention formed the basis of the modern electronics industry, the economic importance of which does not yet even seem to have reached its asymptote.

Motors for the masses, the cinema, and wireless, all became viable in the Edwardian era. Scientific attention had been given to aviation much longer. Even Dr. Johnson mentioned balloons with petulance. While it is true that successful flights with powered machines heavier than air were first made during this era, prominently by the Wright brothers at Dayton, Ohio, during 1903–4, and more spectacularly by Blériot's first crossing the English Channel in July 1909, developments in aviation technology were much more gradual. They cannot be regarded as specifically Edwardian. The deliberate secrecy and small scale of developments in various countries worked against any strong interactions between pure and applied science.

Two developments of applied science which have had a particularly direct influence on world power and world population in different ways illustrate how backward Edwardian Britain was, even by contemporary international comparisons, in public support for scientific education and research. One of these refers to work on tropical diseases, and can be focused by biographical facts about a British leader in this field. Sir Ronald Ross migrated to biological research, starting with a classical education, by way of medicine. His essential contribution to the control of malaria was linked with accurate and tenacious observations of how the malaria parasite was tied up with the habits and life-cycle of the mosquito. It is staggering to find that, for a number of years, this research—the consequences of which were to be so vital for the tropical portions of the British Empire, not to speak of the world—was a spare-time effort on the whole discouraged by the Indian Government Service in which Ross was employed.

Fortunately, Ross was exceptionally tenacious. After he had achieved some success, the physician Sir Patrick Manson wrote to the India Office in 1897 with the aim that Ross be given more time for his work. "To our national shame be it said that few, very few of the wonderful advances in the science of the healing art which have signalized recent years have been made by our countrymen. This is particularly apparent in the matter of tropical diseases in which we should, in virtue of our exceptional opportunities, be *facile princeps*. But even in tropical diseases Frenchmen, Italians, Germans and Americans and even Japanese are shooting ahead of us. We have to get a Kock to find for us the cholera germ, and a Haffkine to protect us from it, a Laveranto to teach us what malaria is . . . ."

Ross was eventually appointed to special duty by the Government of India in January 1898 and was given a laboratory consisting of two rooms, an office and a verandah. The first meeting of a tropical medicine section of the British Medical Association was held in Edinburgh in July 1898. At this meeting the work of Ross was described by Manson, in particular the mode of infection by the mosquito. Practical measures to apply the new knowledge about the life-cycle of the malaria parasite began on an extremely modest scale, at Freetown in Sierra Leone, where Ross and a very small handful of colleagues studied the breeding grounds of mosquitoes and successfully organized ways of suppressing them. Dramatic success was obtained in 1902 at Ismailia, where Ross for a derisory fee showed the administrators of the Suez Canal how to stamp out the spread of malaria locally. In 1903 follow-through work by W. C. Gorgas at Colon first made possible the building of the Panama Canal. Consequences of this backroom work have been particularly great for world population and cannot even yet be fully appreciated.

A second major advance in technology, which almost threatened to become of decisive importance in the conflict between Britain and Germany in the First World War, was the German development sometimes known as the 'fixation of nitrogen'.

Since early studies on agricultural chemistry inspired by the German chemist Liebig, the need for supplementing traditional manuring operations by bulk additions of nitrogen compounds, in order to maintain yields of agricultural crops, was well appreciated. Attention to this requirement to feed rapidly increasing populations had been drawn in a dramatic way by Sir William Crookes at a meeting of the British Association in 1898. Demands on food-producing land could only be met in an acceptable way by the production of synthetic fertilizers. Many of the soil's requirements are restored by good systems of agriculture Components such as potash or lime which are removed by the crops can be restored in the form of mineral fertilizers; but even the most conservative use of manures and organic waste matter does not adequately restore nitrogen compounds in forms that plants can assimilate.

Only three solutions could be seen by Crookes—to accept a diminishing supply of foodstuffs and progressively lower the standard of nutrition, which would not be acceptable in technologically advanced communities; to import most of Britain's main food requirements from countries with abundant land, which was the solution adopted in the latter half of the nineteenth century by Britain. like ancient Rome in this respect; or to supplement any natural manure returned to the fields with other readily assimilated nitrogen compounds added to the soil after each crop. Prior to the First World War. this growing need was met largely by importation of nitrate of soda from Chile, 1,350,000 tons in 1900. Much of this went to Germany and its consumption like other imports was theoretically under threat of interruption in war by the British Navy. But, in addition to their peaceful application for increasing crop yields, nitrates were being used for manufacturing large tonnages of the new high-explosives based on picrite and TNT. It is said the failure of the British to capture 300,000 tons of nitrate of soda in the docks at Antwerp saved Germany's explosives supplies in the early years of the First World War

What was new in German applied science was their discovery of industrial processes for converting the nitrogen in the atmosphere into nitrates and other useful compounds, thereby releasing their explosives industry and agriculture from serious risk of attrition by naval blockade. By 1918, 200,000 tons of synthetic ammonia a year were being made in Germany for conversion into nitric acid and high explosives based on it. The Allies had no factories for this process until after the end of the First World War.

It is important to stress that this note of unfavourable comparison between British and European, chiefly German, scientific progress was being sounded with increasing insistence by leaders of British science throughout the Edwardian era. Presidential addresses to the British Association included strong complaints almost every year of the Edwardian decade. However, unlike the reception given to political agitation about the threat from the German Navy, only small and not very influential groups could understand the repeated warnings by British scientists about the threat from German industry. Since mid-nineteenth century and even earlier, enlightened support for scientific education and research by the German Government was building up a strong technological and commercial empire. In Britain there were frustrations which one would like not to term characteristic of Edwardian complacency.

Particularly full and informative comment about the impenetrability of the governing classes to the importance of science for the nation's welfare was made to the British Association by its president for 1906, Prof. E. Ray Lankester:

"It is unfortunately true that the successive political administrators of the affairs of this country, as well as the permanent officials, are altogether unaware to-day, as they were twenty-five years ago, of the vital importance of that knowledge which we call science, and the urgent need for making use of it in a variety of public affairs. Whole departments of Government in which scientific knowledge is the one thing needful are carried on by ministers, permanent secretaries, assistant secretaries, and clerks who are wholly ignorant of science, and naturally enough dislike it since it cannot be used by them, and is in many cases the condemnation of their official employment. Such officials are, of course, not to be blamed, but rather the general indifference of the public to the unreasonable way in which its interests are neglected. A difficult feature in treating of this subject is that when one mentions the fact that ministers of State and the officials of the public service are not acquainted with science, and do not even profess to understand the results of their importance, one's statement of this very obvious and notorious fact is apt to be regarded as a personal offence. It is difficult to see wherein the offence lies, for no one seeks to blame these officials for a condition of things which is traditional and frankly admitted. . . . The reason, I think, is to be found in the defective education, both at school and university, of our governing class, as well as in a racial dislike among all classes to the establishment and support by public funds of posts which the average man may not expect to succeed by popular clamour or class privilege in gaining for himself—posts which must be held by men of special training and mental gifts. Whatever the reason for the neglect, the only remedy which we can possibly apply is that of improved education for the upper classes, and the continued effort to spread a knowledge of the results of science and a love for it amongst all the members of the community . . . members of the British Association . . . might do a great deal by insisting that their sons, and their daughters too, should have reasonable instruction in science both at school and college . . . the trifling with classical literature and the absorption in athletics . . . is considered by too many schoolmasters as that which the British parent desires as the education of his children."

To explain the more than ordinary deafness of the Government and Civil Service to such warnings, I think we may adduce the almost complete lack of integration of science into the education of Britain's leaders.

By Edwardian times, Britain probably showed the greatest lag relative to Germany in its indifference to the role of science in the education of its leaders, and of the rank and file. For a nation the practical and empirical grasp of realities of which has often been shown in other matters, this deflexion of educational trends is curious. Some light is thrown on it by the famous comment of an eighteenth-century schoolmaster—or should I say educationist ?—on an equally famous seventeenth-century pedagogue—I refer to Dr. Johnson's *Life of Milton*, where he explains how this deflexion began.

Milton's purpose as a schoolmaster, which was: "To teach something more solid than the common literature of schools, by reading those authors that treat of physical subjects; such as the Georgick, and astronomical treatises of the ancients. This was a scheme of improvement which seems to have busied many literary projectors of that age. Cowley, who had more means than Milton of knowing what was wanting to the embellishments of life, formed the same plan of education in his imaginary College", meets with a criticism from one who, like Milton, had tried schoolmastering not very successfully as a way of life. Johnson leaves no doubt about his own very different judgment:

"The truth is, that knowledge of external nature, and the sciences which that knowledge requires or includes, are not the great or the frequent business of the human mind. Whether we provide for action or conversation, whether we wish to be useful or pleasing, the first requisite is the religious and moral knowledge of right and wrong; the next is an acquaintance with the history of mankind and with those examples which may be said to embody truth, and prove by events the reasonableness of opinions. Prudence and Justice are virtues and excellencies of all times and of all places; we are perpetually moralists, but we are geometricians only by chance. Our intercourse with intellectual nature is necessary; our speculations upon matter are voluntary and at leisure. Physiological learning is of such rare emergency, that one may know another half his life, without being able to estimate his skill in hydrostatics or astronomy; but his moral and prudential character immediately appears. Those authors therefore are to be read at schools that supply most axioms of prudence, most principles of moral truth, and most materials for conversation; and these purposes are best served by poets, orators and historians."

In support of Dr. Johnson, it would not be difficult to trace the benefits of prudence, attention to moral truth, and skill in human conversation, in more than one page of British history. But it is not unfair to attribute some of the heavy British losses in the First World War to the inertia of the Edwardian Governments which led to a perilous, but by no means unforeseen, lag in scientific and technological leadership. Prime generations of youth had to pay a bloody price in that War to make up for it. Directly, and by the loss of the contributions this generation might have made, their loss was one of the many factors making the end of the Edwardian era very clearly the end of a period of smooth historical evolution and the beginning of rapid social and economic changes the travail of which is our present experience.

NATURE OF THE BIOLOGICAL CODE

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UNTIL recently the nature of the biological code was a matter for theoretical speculation and a few experimental attempts, themselves fundamentally inexact and inaccurate, to place some limits on the parameters of the code[1-6]. Within the past year spectacular advances in an experimental approach to elucidating the code have been reported. In the first place, an *in vitro* system capable of synthesizing specific proteins on the addition of specific RNA 'messages' has been developed[7]. This plus the capability of synthesizing very simple RNA 'messages' by use of the enzyme nucleotide phosphorylase provide the long-sought direct experimental approach to a solution of the biological code[8]. Secondly, elegant genetic experiments have made it almost certain that the coding ratio (that is, the number of nucleotides corresponding to an amino-acid) is three for all amino-acids[9]. These experiments also demonstrate that the code must be rather highly degenerate.

A great deal of progress has been made in a very short time in obtaining coding nucleotide triplet assignments for the amino-acids[10-13]. The results obtained at first glance seem to pose as many new problems as they answer old ones. For, although the experimentally determined coding assignments invalidate all published theoretical speculations concerning the nature of the biological code, the true code which seems to be emerging from this approach ostensibly is in conflict with what we know concerning the composition of the coding substances, that is, DNA and RNA (ref. 14). However, I feel that this is not the case in actuality, but that it is certainly now possible to develop a theory which will encompass all the existing facts regarding the code, which the present preliminary communication attempts to do.

Consider first the following theoretical argument. Coding is in all probability accomplished in terms of the purine and pyrimidine bases on the nucleic acid. Each of the four naturally occurring bases is uniquely defined by the substituents it carries on the 2 and 6 positions in the ring, adenine (A) being 2-H, 6-NH$_2$; cytosine (C) being 2-OH, 6-NH$_2$; guanine (G) being 2-NH$_2$, 6-OH; and uracil (U) being 2-OH, 6-OH. It appears possible, then, that coding may also be done in terms of the 2 and 6 positions only. (The bases can also be distinguished in part by the fact that two, the purines, have a double, rather than single, ring structure; but I shall disregard this for the present discussion, suggesting only that the main purpose of the double rings may be to bring about the correct electronic configurations necessary to produce desirable tautomeric shifts in the 2 and 6 positions, which are the points of essential interest.) Confining attention to the four major naturally occurring bases one can inquire as to the information content of the 2 and 6 positions. Since only two substituents occur in the 6 position, its information content is 1·0 bits. In the 2 position three substituents occur, making its information content 1·6 bits. When one considers 2 and 6 positions taken together, one defines the four bases; therefore, the information content here is 2·0 bits. Now there are twenty amino-acids in protein to be specified, and probably one must also specify the end (and/or beginning) of a molecule, or at least 21 things, which requires 4·4 bits. The closest approach one can make to 4·4 bits using the purine and pyrimidine bases is 1·0 + 1·6 + 2·0 or 4·6 bits.

This, then, suggests the following coding scheme. Coding is accomplished by a triplet of nucleotides. One member of the triplet codes according to its 2 position on the ring only, a second member codes according to its 6 position only, while the third member codes according to both 2 and 6 positions. A degenerate code composed of 24 distinguishable triplets is thus produced. In other words, when using just the 2 ring position, U and C are indistinguishable so that only three different symbols are possible; when using just the 6 ring position, U is indistinguishable from G and C is indistinguishable from A, so that only two different symbols are possible. The product of 3 × 2 × 4 gives 24 distinguishable triplets.

Before developing this theory further, let us now turn to the experimental facts relevant to coding. The first concerns the DNA composition of various organisms, particularly the micro-organisms. It is known that the DNA of any one species of micro-organism is quite homogeneous in its composition, but different species may differ from one another in G + C content by extremely wide margins[15]. For example, the highest known G + C content of a naturally occurring DNA is that of *M. lysodeikticus* at 72 per cent G + C, while at the other extreme 25 per cent G + C is observed in *T. pyriformis*[16]. Further, the overall amino-acid compositions of a variety of micro-organisms the DNA's of which span the range of G + C contents is known[16]. The second group of facts concerns the 'neighbour frequency' in DNA, that is, the frequency of occurrence of one base adjacent to another base. As before, this type of measurement has also been made on a variety of DNA's which span the range of known G + C values[17]. The third and most important group of facts concerns the composition of coding nucleotide triplets as determined in the above-mentioned *in vitro* system. At present coding assignments have been determined for nineteen of the twenty amino-acids[10-13,18]. Although there are minor disagreements between the two groups active in the field, there is complete agreement between them regarding the fact that for all nineteen of the amino-acids reported there is at least one U in each of the corresponding triplets. Many triplets contain 2 U's. This means that the composition of a coding nucleic acid strand is extremely high in uracil, which would make the coding nucleic acid of a composition that has rarely if ever been observed in Nature. Although this high uracil content at first sight is an anomaly, it is, as seen below, exactly what would be expected on the present coding scheme; and, further, this coding scheme makes the observed coding triplet assignments compatible with the wide variations in G + C content of the DNA of micro-organisms.

Table 1

OBSERVED AMINO-ACID CODING ASSIGNMENTS FITTED TO A THEORETICAL DEGENERATE CODE

The particular observed assignments are in italics

1.	*UUU*, CUU, UUG, CUG	phe
2.	*UUC*, UUA, CUA, CUC	ser (or leu)
3.	*UCU*, CCU, UCG, CCG	leu (or ser)
4.	UCA, *UCC*, CCA, CCC	pro
5.	*UGU*, UGG, CGU, CGG	val
6.	*GUU*, GUG	cys or try ?
7.	*GGU*, GGG	gly
8.	*UAU*, CAU, UAG, CAG	ilu
9.	*AUU*, AUG	tyr
10.	*AAU*, AAG	lys
11.	*AUA*, *AUC*	asn
12.	UAA, *UAC*, CAA, CAC	thr ?
13.	*UGA*, UGC, CGA, CGC	met
14.	*GAU*, GAG	asp
15.	*AGU*, AGG	glu
16.	*GCU*, GCG	ala
17.	GUA, *GUC*	arg
18.	*ACU*, ACG	his
19.	GCA, GCC	—
20.	GGA, GGC	*
21.	ACA, ACC	—
22.	AAA, AAC	*
23.	AGA, AGC	gln
24.	GAA, GAC	—

* Proposed triplets for end-of-molecule.

Table 1 gives the 24 distinguishable coding triplets predicted by the above theoretical code. It is seen that eight are four-fold degenerate, while the remaining sixteen are two-fold degenerate. The table also shows the coding assignments determined by Lengyel, Speyer, Basilio and Ochoa placed in this scheme. Where there are ambiguities as to assigning the experimental unoriented nucleotide triplets to this scheme, we have utilized amino-acid replacement data to make the choice[19-22]. (The assignments given here are to a high per cent compatible with existing replacement data—a point which will be discussed in greater detail elsewhere.) With very few exceptions the present theoretical code accommodates the experimental results very well. The most prominent exception is that either cys or try cannot fit into the scheme. Also, one of the codings for thr, 1U2C, does not fit; but this coding is in doubt (Ochoa, S., personal communication); whereas the other thr coding is not. Further, of the three codings for leu (2U1C, 2U1A, 2U1G) only 2U1C fits the present scheme. (It seems that perhaps the assignment for ser and leu in the present scheme could possibly be interchanged, which would improve the situation.) The coding for gln has not been observed experimentally. Tentatively it has been assigned 1U1C1G[18]. We have assigned it 1A1C1G in order to fit it into the present scheme, a coding which would also account for the fact that the gln code has not been observed experimentally. With the few exceptions noted then, all the experimental coding assignments are compatible with the present theoretical degenerate code, leaving five code places to be accounted for, some of which could be used as the code for end (or beginning) of molecule.

At this point it is interesting to attempt to determine a coding for end (or beginning) of molecule. If the present coding scheme is correct, this should fall into one or more of the five as yet unfilled code places. To determine end-of-molecule it is necessary to find two closely related proteins the chain-length of which differs. The amino-acid in the longer one opposite the termination of the shorter one should give a clue to end-of-molecule, for single mutation in the triplet corresponding to the amino-acid in question should have produced a triplet meaning end-of-molecule. Such a case as we are loooking for occurs in the melanocyte-simulating hormone–adrenocorticotropin series[22]. Here we find two cases of gly opposite to end-of-molecule and two cases of lys opposite to end-of-molecule. Comparing this to the above scheme, it is found that both GG^{A}_{C} (one mutation distant from the gly triplet) and AA^{A}_{C} (one mutation distant from the lys triplet) are yet available to code end-of-molecule, a satisfying result.

We are now in a position to evaluate the present theoretical code in terms of the data on G + C content of various DNA's. As can be seen, due to the degeneracy condition most amino-acids can be coded by a triplet low in G + C as well as one high in G + C. This suggests that the experimentally determined coding assignments can be reconciled with the observed wide variations in G + C. Using the amino-acid composition for *M. lysodeikticus* determined by Sueoka and coding the amino-acids in a form which maximizes G + C, one finds that G + C = 79 per cent, a result which is more than sufficient to account for the observed 72 per cent reported for *M. lysodeikticus*. Using the amino-acid composition for *T. pyriformis* and coding amino-acids in a form which maximizes A + T(U), one finds G + C = 25 per cent, which is the experimentally observed G + C content of this organism. Thus *T. pyriformis* seems to be at (or near) the one extreme of the G + C scale, if the present theory is correct. The wide variations in G + C of the DNA observed in micro-organisms is completely accounted for on the present theoretical code. In the present communication I shall not discuss in quantitative detail the fit of the 'neighbour frequency' data of Josse *et al.* Suffice it to say now that agreement between this set of data and the present code is good at least to a first approximation.

This article, then, outlines a particular degenerate coding scheme derived from an information point of view, which scheme fits to a high degree the existing data on the experimentally observed nucleotide coding assignments, agrees rather well with existing amino-acid replacement data, and reconciles the experimentally determined composition of code triplets with the very wide variations in the G + C content of various DNA's. In further publications I shall attempt to elucidate more fully the points raised here and the question of the molecular basis for the coding scheme.

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ELECTRON MICROSCOPIC OBSERVATIONS ON ROUS SARCOMA VIRUS AND CELL MEMBRANES

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SOME progress has already been made[1] in the elucidation of the ultrastructure of Rous sarcoma virus. On rare occasions structures resembling sub-units were seen, but it was not clear whether these were associated with the external membrane, internal membranes or the nucleoid. Methods were therefore sought to remove the membranes and uncover the nucleoid.

It has long been known that ether and sodium deoxycholate inactivate and disrupt Rous sarcoma virus particles, but neither of these reagents has released recognizable fragments. Rous and Murphy[2] had shown, however, that saponin, in high dilution, inactivates Rous sarcoma virus. This surface-active agent damages the outer membrane of this virus particle in a characteristic manner. It has been suggested[3] that the outer membrane of this virus is similar to the membrane of the cell which produces it. It was not unexpected, therefore, to find that the membranes of Rous sarcoma cells and of chicken liver cells showed a similar pattern of damage by saponin. So, too, did the membranes of human and guinea pig erythrocytes.

Materials and Methods

(*a*) *Rous sarcoma virus preparations.* Rous sarcoma virus, strain *B* (ref. 4), was used throughout. High titre infected culture fluids were prepared in tissue cultures of chick embryo cells using the method described by Crawford[5]. Infected tissue culture medium was clarified by centrifugation at 2,000*g* for 20 min.; the virus was sedimented at 78,000*g* for 30 min. in a Spinco model *L* ultracentrifuge and the pellet suspended in 1/100 the original volume of distilled water. This material was immediately subjected to the various reagents, then either sprayed directly for electron microscopy or diluted in buffered saline for infectivity assay. The virus was assayed by the focus method[6] as modified by Dougherty and Simons[7].

(*b*) *Cell membranes from chicken liver, and from Rous sarcoma, cells.* Membranes were prepared substantially by the method of Herzenberg and Herzenberg[8].

(*c*) *Human, and guinea pig, erythrocyte ghosts.* The washed cells, suspended in isotonic saline, were lysed by pouring into aqueous or saline solutions of saponin, digitonin, 'Triton 165', 'Triton 100', Gurr's '7×' detergent, sodium deoxycholate or 0·01 *M* phosphate buffer at *p*H 7·2 ('control' ghosts). Hæmolysis was completed at 37° C. for 30 min.

(*d*) *Electron microscopy.* Negative staining with 2 per cent potassium phosphotungstate at *p*H 5 (ref. 9) containing 0·05 per cent bovine plasma albumin (to permit spreading of the micro-drops) was used. Virus or cell-membrane preparations in distilled water were mixed directly with this solution. The erythrocyte ghosts were washed in 0·01 *M* phosphate buffer at *p*H 7·2 before adding the negative stain. The preparations were sprayed on grids coated with carbon films made by evaporating carbon on freshly cleaved sheets of mica. A drop of glycerol was placed on the carbon rod point and evaporated on to the mica in order to allow easy flotation of the carbon film. A Siemens Elmiskop 1 electron microscope was used with the double condenser, 200μ centrable condenser aperture, 50μ centrable objective aperture, and was operated at 80 kV. throughout. Micrographs were taken at magnifications of 20,000 and 40,000.

Results

(1) *Saponin treatment of Rous sarcoma virus.* The external membrane of untreated Rous sarcoma virus was intact. Incubation of the virus with 0·001 per cent saponin (37° C. for 60 min.) produced no recognizable change in appearance. At a concentration of 0·005 per cent some holes, or pits, appeared in the outer membrane. The number of these increased with increasing saponin concentration and also varied from particle to particle. Each pit was 80 ± 10 Å. in diameter and, for example, at the periphery of the particle, was some 35–50 Å. deep. With 0·01 per cent saponin the pits were found to be arranged hexagonally (Fig. 1). The pits were each surrounded by a ring, and the 'outer layer' peripheral to the external membrane[1] was not found in those particles which showed pits.

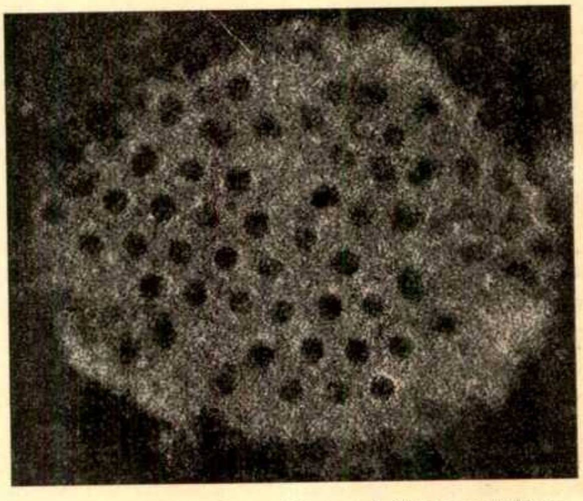

Fig. 1. Rous sarcoma virus particle treated with saponin, 0·01 per cent. The pits are arranged in a hexagonal array, and the edge of the particle shows indentations. The outer layer is not seen in particles showing holes. (× 400,000)

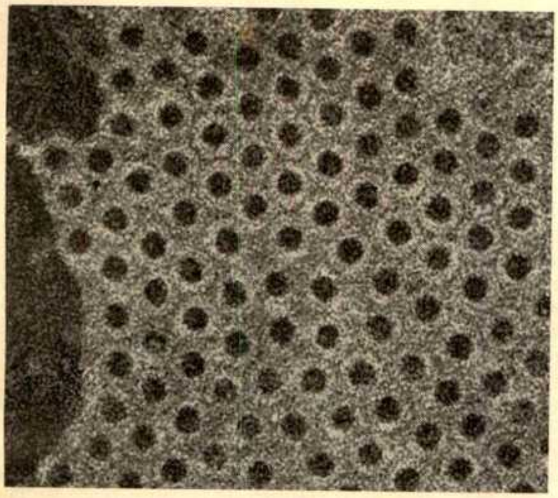

Fig. 2. Rous sarcoma cell membranes treated with 0·05 per cent saponin. There is a single layer of membrane, which shows a hexagonal array of pits surrounded by rings. (× 400,000)

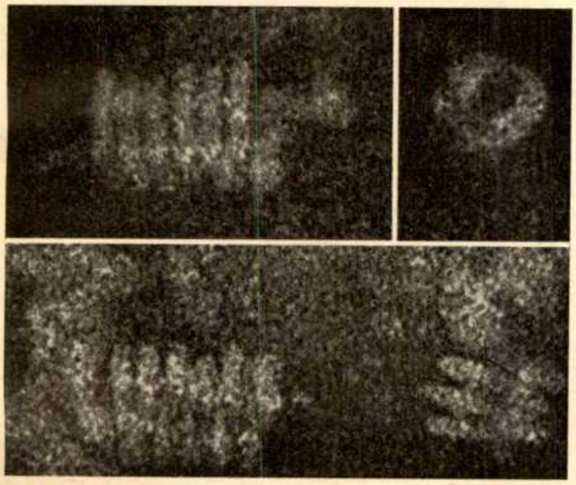

Fig. 3. Chick liver cell membranes treated with saponin. Helices and an individual ring are shown. (× 640,000)

At still higher saponin concentration (0·017 per cent) greater breakdown of the virus membrane led to the formation of a mesh of rings and strands. Saponin at 0·01 per cent at 37° C. for 60 min. reduced the virus titre from $1{\cdot}8 \times 10^8$ focus-forming units/ml. to $3{\cdot}8 \times 10^5$ focus-forming units/ml., that is, by a factor of 500.

(2) *Digitonin treatment of Rous sarcoma virus.* Digitonin reacts stoichiometrically with cholesterol, and also brings about the lysis of erythrocytes. Cholesterol is known to be a component of cell membranes and to be present in Rous sarcoma virus. It was anticipated, therefore, that digitonin, like saponin, might react with the external membrane of the virus.

A Rous sarcoma virus preparation was incubated sequentially in water followed by water (control A); 0·01 per cent digitonin, followed by water (B); water and 0·01 per cent saponin (C) or 0·01 per cent digitonin and 0·01 per cent saponin (D).

The control virus assayed at $6{\cdot}6 \times 10^7$ focus-forming units/ml.; B, 6×10^2, C, $1{\cdot}8 \times 10^5$ and D, $< 2 \times 10^2$. Subsequent treatment with saponin did not change the appearance of Rous sarcoma virus treated with digitonin. It would seem, therefore, that digitonin binds the material with which saponin normally reacts in the virus membrane. Nevertheless, as the titration shows, digitonin inactivates Rous sarcoma virus more effectively than saponin at the concentrations used.

(3) *Saponin treatment of chicken liver, and Rous sarcoma, cell membranes.* Untreated, negatively stained membranes showed an 'outer layer' of material of low contrast with fairly regular projections. No pits were seen. On incubation with 0·05 per cent saponin, some of the Rous sarcoma membranes showed a hexagonal array of pits similar to that observed in Rous sarcoma virus (Fig. 2). The effect of saponin on chicken liver cell membranes and Rous sarcoma cell membranes was the same.

The centre-to-centre spacings were approximately 140–160 Å. At the edges of the membranes the network appeared to be unravelling. Strands were formed, often curled into helices, and separate rings were observed (Fig. 3). The strands had a width of about 50 Å. in one dimension and a thickness of about 40 Å. in the other. Increasing concentrations of saponin increased the extent of the total breakdown of the membranes into strands and helices.

Incubation with 0·1 per cent digitonin (37° C. for 30 min.) appeared to make the membranes more rigid. They were homogeneous with no apparent structure and with slightly curved edges and sharp angles (Fig. 4).

After fixation in Palade's buffered osmium tetroxide, embedding in methacrylate and thin-sectioning, saponin-treated liver cell membranes showed no evidence of pits.

(4) *Saponin treatment of human, or guinea pig, erythrocyte ghosts.* At concentrations of saponin less than 0·03 per cent no changes in the membranes were apparent. At a greater concentration (0·05 per cent) large numbers of pits were seen, and the numbers increased with increasing amounts of the reagent (Fig. 5).

Where there was a single layer of pits they were 85 ± 10 Å. in diameter and the maximum centre-to-centre distance varied between 140–160 Å. At the edges of the membranes the indentations corresponding to profiles of the pits were 40 ± 20 Å. deep. The pits were frequently found in a hexagonal array and

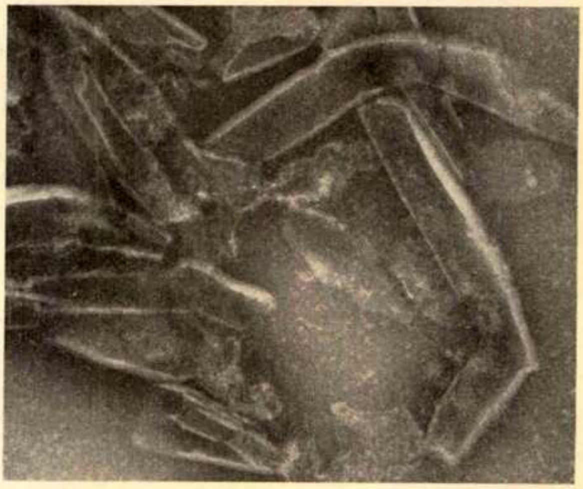

Fig. 4. Chick liver cell membranes treated with digitonin. Note the slightly curved outlines of the membranes. (× 100,000)

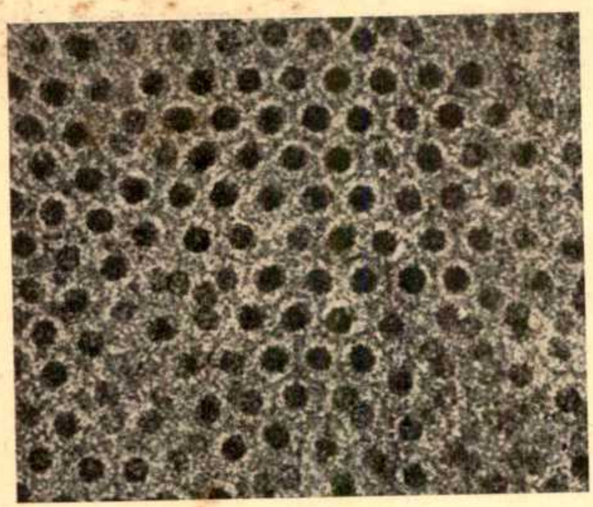

Fig. 5. Erythrocyte suspension in saline treated with 0·05 per cent saponin, then treated with 0·01 per cent trypsin for 1 hr. at 37° C. The surface of the ghost membrane shows a hexagonal array of pits surrounded by rings similar to that seen in Rous sarcoma cell membranes illustrated in Fig. 2. In this picture, two superimposed layers of membranes are seen. (× 400,000)

surrounding each was a ring, 30 ± 5 Å. thick. The rings were hexagonal in outline and separated from each other by a minimum distance of 10 Å.

Larger amounts of saponin (0·09 per cent) caused complete dissolution of the ghosts and the appearance of masses of curled threads and rings. Ghosts prepared by lysing the erythrocytes in hypotonic phosphate buffer had no pits in their membranes.

The protein nature of the residual membrane after saponin treatment was investigated by prolonged peptic and tryptic digestion. The appearance of the ghosts was unchanged—or possibly the micrographs showed more contrast (Fig. 5). Trypsin (or pepsin) treatment of 'osmotic' ghosts did not produce pits (Fig. 6).

(5) *Digitonin treatment of erythrocyte ghosts.* Incubation of human red cell ghosts in 0·01 per cent digitonin in isotonic saline at 37° C. for 30 min. was followed, after washing, by 0·05 per cent saponin at 37° C. for 30 min.

Digitonin prevented the formation of the pits, and the membranes resembled in appearance the chicken liver cell membranes treated in the same way. The inhibition of pit formation by digitonin is quantitative. 1 ml. of packed guinea pig erythrocytes required about 6 mgm. of digitonin to 'block' all the pits against the action of saponin. (One pit was found in one membrane in 5 micrographs magnified 20,000 times.) This again suggests that the digitonin is reacting with a specific component of the cell membrane such as cholesterol.

The biochemistry of the membranes is being investigated by Dr. G. F. Marrian and his colleagues.

(6) *Treatment of human erythrocyte ghosts with other surface-active agents.* Concentrations of 30 per cent '7 ×', 0·8 per cent 'Triton 165', 0·8 per cent 'Triton 100', and 1 per cent sodium deoxycholate were sufficient completely to disintegrate the ghosts. No pits, etc., were observed after treatment with these, or any lower, concentrations of these reagents.

Discussion

The action of saponin on the outer membrane of Rous sarcoma virus on chicken liver or Rous sarcoma cell membranes, and on human, or guinea pig, erythrocyte ghosts is to produce a hexagonal array of pits each of which is surrounded by a more or less distinct circular, or hexagonal rim. With increasing amounts of saponin these membranes are destroyed and masses of strands, or rings, are produced. Prior treatment of the membranes with optimum amounts of digitonin inhibits the action of the saponin. The almost identical appearance of the saponin-treated cell membranes from such disparate species as chicken, guinea pig and man lends credence to a general theory for the structure of the cell membrane. The pauci-molecular theory as first proposed by Danielli and Davson[10] suggested that the cell membrane was made up of a bimolecular leaflet of lipid sandwiched by external and internal layers of protein. By the use of permanganate fixation and thin-sectioning techniques in electron microscopy, it has been possible to demonstrate the existence of a unit structure that is found in cell membranes, endoplasmic reticulum, and mitochondrial membranes[11]. The unit membrane was seen as two dense lines 20 Å. wide, separated by a gap of 25 Å.

Danielli[12] later suggested a mosaic structure of lipid and non-lipid areas, and Parpart and Ballentine[13] proposed an arrangement in which a mosaic of cylinders containing phospholipid and cholesterol were surrounded by a protein meshwork. The diameter of their proposed cylinders was about 90 Å., and the centre-to-centre spacing some 150 Å. In cross-section the lipid-containing areas penetrated the entire thickness of the membrane, while the protein frame-work was conceived to be in two opposed layers. The experimental results obtained here by saponin treatment are in fair agreement with this type of structure. The digitonin inhibition of pit formation suggests, too, that such areas may indeed contain cholesterol.

The similar effect of saponin on the external membrane of Rous sarcoma virus to that on chicken liver cell membranes and Rous sarcoma cell membranes provides evidence for the suggestion that Rous sarcoma virus derives its outermost membrane from the cell's membranes as it is secreted. The sub-units which Dourmashkin and Simons[1] found occasionally in Rous sarcoma virus particles are quite different

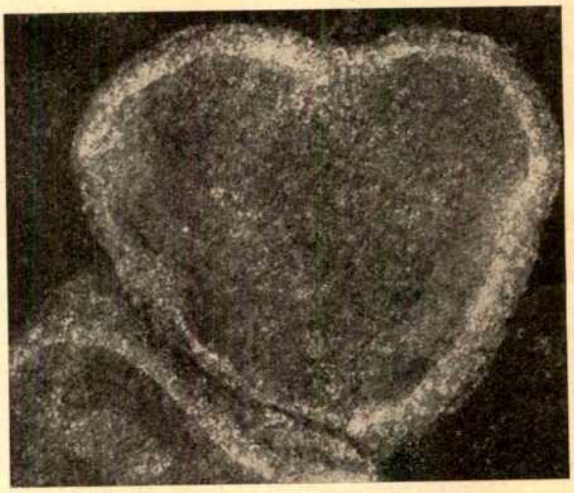

Fig. 6. Control experiment for Fig. 5. Erythrocyte suspension in saline lysed in 0·01 M phosphate buffer at pH 7·2 for 30 min. at 37° C., then treated with 0·01 per cent trypsin for 1 hr. at 37° C. There are no pits in the membrane. (× 200,000)

from those described here. They are smaller, more closely spaced and represent positive rather than negative features, that is, they displace the negative stain. The hexagonal array of pits formed in the external membrane when Rous sarcoma virus is treated with saponin implies another mosaic ultrastructure for this virus. It will be of interest to know whether other viruses with external membranes derived from the cell's membranes behave similarly on treatment with saponin.

It is disappointing that the pits in the cell membranes have not been seen in thin sections of saponin-treated, osmium tetroxide-fixed preparations. It is difficult, otherwise, to decide whether they are, in fact, holes through the membranes or merely pits.

We thank Prof. J. F. Danielli for an opportunity to discuss these results with him, and Miss L. Bates and Mr. A. J. Pringle for technical assistance.

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ROYAL SOCIETY EXPEDITION TO TRISTAN DA CUNHA

PRELIMINARY REPORT

THE aims of the Royal Society Expedition to Tristan da Cunha were to examine the active volcano, to make a geological survey of the Tristan da Cunha group and to investigate the effect of the present volcanic activity on the flora and fauna of the island. The Expedition was composed of twelve members: Dr. I. G. Gass (leader), Dr. P. G. Harris, Dr. R. W. Le Maitre and Mr. P. E. Baker (geologists); Mr. J. H. Dickson (botanist); Mr. D. E. Baird (zoologist); Lieut. Commander A. B. Crawford, S.A.N.R. (meteorologist and deputy leader); Mr. D. Simpson (agriculturist); Staff/Sergeant R. Shaw and Corporal T. McCormack (Royal Corps of Signals); and Mr. Joseph Glass and Mr. Adam Swain (Tristan islanders). Landing on January 29 from the South African Navy frigate S.A.S. *Transvaal*, the Expedition spent seven weeks on the island before embarking on the Royal Navy ice patrol vessel H.M.S. *Protector*.

Fig. 1 is a sketch map of Tristan da Cunha prepared by the Directorate of Overseas Surveys, Tolworth, Surbiton, Surrey, from aerial photographs taken by the Royal Air Force. The stippled area indicates the position of the new volcano and the extent of its lava field.

Geology and Vulcanology

The main geological aims of the Expedition were to study the new volcano and to relate its activity to the geology of the Tristan da Cunha group of islands as a whole.

Previously, the most complete study of these islands was that of Dunne[1]. Since this is mainly a petrographic account it was desirable that the geology should be re-examined with emphasis on structure and volcanic history and mapped in greater detail than before.

Tristan da Cunha consists of a central peak (6,760 ft.) from which the land falls away with diminishing gradient to about 2,000 ft. Below this are almost vertical cliffs, often to the sea. In places, at the foot of these cliffs, are fringing strips of relatively flat land about 100 ft. above sea-level: one such platform is that on which the settlement stands. Local terms for the major topographic features used in this report are: 'the Peak', for the steeply inclined central area above 3,000 ft.; 'the Base', for a gently inclined area between 2,000 ft. and 3,000 ft.; and 'the Main Cliffs', for the 2,000-ft. high cliffs.

The peak and its slopes are dissected by radial valleys or gulches the gradient of which lessens outwards to the edge of the base over which they plunge in a series of waterfalls. At first sight, these pronounced hanging valleys seem incompatible with an origin of the main cliffs by normal marine erosion alone. But since the drainage of the island is almost entirely underground, through permeable ash and lavas, water flows down the gulches for only a few hours after heavy rain. Hence, river down-cutting is very slow and is unable to keep pace with intense marine erosion.

New volcano. The recent volcanic eruption occurred on the settlement plateau, its centre being about 300 yards to the east of the settlement. The eruption was preceded by a series of earth tremors commencing on August 6, 1961. These tremors, sometimes of about force 5 or 6, were highly localized in the settlement area, being very weak at a distance of 4 miles and undetectable 7 miles away. The second phase, on October 9, was the development of rifts 200 yards long and 2 yards apart, parallel to the coastline. The seaward side was uplifted 30 ft. and heavy falls of rock occurred in the cliffs behind, particularly in a well-joined, old, volcanic neck immediately overlooking the settlement. That night, at about 2 a.m., a red glow was observed and next morning a mound of solid incandescent lava had appeared which continued to grow during the next four days, attaining a height of about 250 ft. (summit 480 ft. above sea-level). This mass began to expand northwards, and it is believed that a stream of fluid lava was emitted, carrying with it the seaward wall of the dome which lay in its path. The remnant of the initial dome now formed a horseshoe-shaped crater about 400 yards long by 200 yards wide. Within this crater, a central cone was built up by the pyroclastic activity and from its summit a lava stream poured northwards, lobes extending along the coast to the east, then to

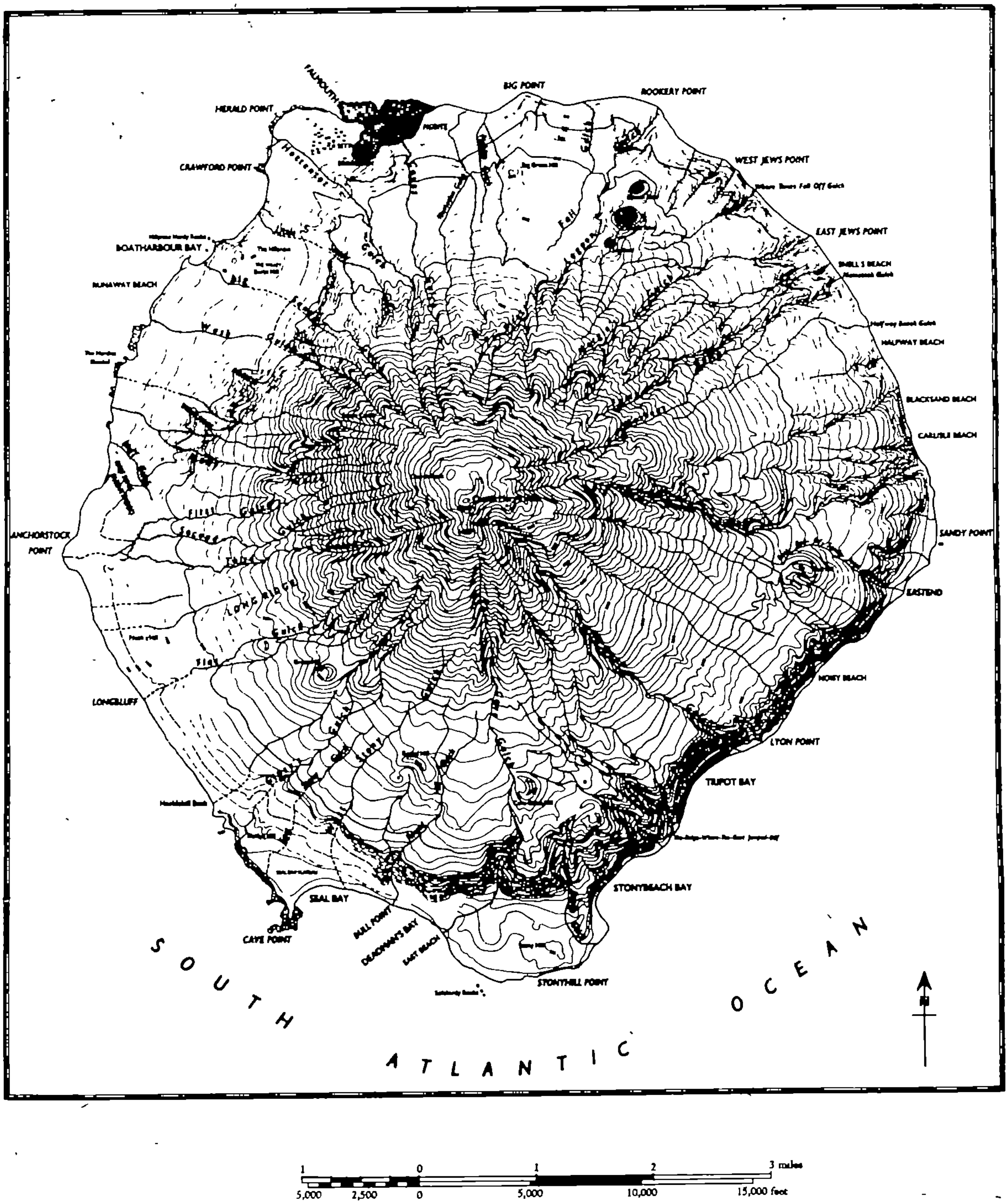
FALMOUTH
HERALD POINT
BIG POINT
ROOKERY POINT
CRAWFORD POINT
WEST JEWS POINT
EAST JEWS POINT
BOATHARBOUR BAY
RUNAWAY BEACH
SHELL'S BEACH
HALFWAY BEACH
BLACKSAND BEACH
CARLISLE BEACH
SANDY POINT
ANCHORSTOCK POINT
EASTEND
LONG RIDGE
LONGBLUFF
NOISY BEACH
LYON POINT
TRIPOT BAY
STONYBEACH BAY
SEAL BAY
CAVE POINT
BULL POINT
DEADMAN'S BAY
EAST BEACH
STONYHILL POINT
SOUTH ATLANTIC OCEAN
1 0 1 2 3 miles
5,000 2,500 0 5,000 10,000 15,000 feet

Fig. 1. Sketch map of Tristan da Cunha showing the position and extent of the new volcano and its lava field

Reproduced by kind permission of the Controller of H.M.S.O. from a sketch map of Tristan da Cunha prepared by the Directorate of Overseas Surveys. Crown Copyright reserved

the west, as well as out to sea. The seaward front of the lava field is at present about 1,300 yards long, is about 900 yards from its source and in places as much as 500 yards beyond the former coastline. It has a thickness of up to 80 ft. The flows are mantled with partly rounded, bruised and abraded lava fragments, with massive undisturbed lava appearing only in the lower part of the seaward margin and near the origin of lava streams.

When the Expedition arrived on January 27 the emission of fluid lava from the central cone appeared to have ceased. Sulphurous vapour was still being emitted from the cone and from a subsidiary crater lying behind the main cone on the rim of the outer wall of the volcano. The spasmodic bursts of vapour and dust, which in December[2] had occurred at about 10 minute intervals, had now become very infrequent and ceased altogether after a few days. At night, a red glow was emitted from the central cone and from cracks in the congealed lava mass which had flowed from it. Ephemeral glowing patches were revealed when blocks fell from this region. These features had ceased by the end of February.

After January, the chief change was the pushing up of a large dome of solid lava about 260 yards long, more than 100 yards wide and up to 100 ft. above the surrounding lava. The long axis of the dome followed the former course of the lava from the cone. The formation of the dome was accompanied by the peeling of sheets and breaking of blocks from its flanks, often exposing red-hot material beneath. This updoming appeared to have ceased by mid-March and the emission of vapour had been greatly reduced. Red-hot rock, formerly visible in deep fractures at the summit of the dome, had cooled by mid-March.

Intensive erosion of the lava along its seaward edge has already occurred, particularly during strong winds in March. The north-western corner of the field has retreated about 20 yards and was cut back about 5 yards during the first day of heavy seas. During the same period, the north-eastern corner underwent a very marked change, and a boulder and gravel spit now extends south-eastwards from this point, running into the coast below Pigbite and encloses behind it a shallow lagoon approximately 140 yards long.

Fumaroles and steam vents occur on the lava field and in the vicinity of the crater. Their activity is diminishing especially in the latter area, and the formation of encrustations of sulphur, common around the second vent, has been curtailed.

General geology of Tristan. For the most part, the island consists of alternate layers of lava and pyroclastic material derived largely from the central conduit. The rocks appear to be chiefly of basaltic composition, though nearer the centre the proportion of trachytes increases. Scoria also becomes more abundant towards the centre. This may be attributed, in part, to the presence of secondary scoria cones on the slopes of the Peak.

In common with most volcanoes, the dip of the flows diminishes away from the central vent. This may be due either to the increase in the proportion of pyroclastic material near the central vent, or to the fact that the more viscous trachytic lavas, flowing relatively short distances, formed steep slopes near the summit crater, while the more mobile basalts flowed longer distances and over a wider front to build the lower slopes.

Dykes are numerous and usually radiate from the centre of the island. They are well exposed in the Main Cliffs, in some of the gulches and in the vicinity of the Peak, where they often stand up as walls up to 40 ft. high. Also exposed in the cliff sections are necks and conduits which have characteristic jointing and are sometimes associated with irregular sills.

Superimposed on the flanks of the central volcano are more than twenty secondary cones, principally of pyroclastic origin though often with thin flows interbedded with the scoria. Most of these cones have summit craters often breached on the seaward side and sometimes with a small crater lake. Usually, in cases where the crater has been breached, a hummocky lava field could be identified beyond it. Centres entirely of effusive origin are limited to Stony Hill and the new volcano.

The absence of weathering and paucity of vegetation on Stony Hill strongly suggest a very recent origin, perhaps 200–300 years ago. The form of this volcano and its lava field, and the nature of the lava itself, bear a striking resemblance to the new volcano. The present eruption is therefore believed to be the second of its type on the island, suggesting perhaps that Tristan is in a new phase of secondary effusive activity, as opposed to the secondary explosive activity which hitherto had been characteristic.

The present volcanic eruption appears to be merely another episode in the long volcanic history of the island. The well-preserved forms of the scoria cones and of Stony Hill indicate that there have been repeated eruptions from widely distributed centres within the past few thousands of years. With only 200–300 years having elapsed between the eruptions at Stony Hill and the present volcano, it is possible that further eruptions from new centres could occur at intervals, probably of a few hundred years.

Botany

Vegetation before the eruption. The only detailed study of the flora of Tristan da Cunha was carried out by the Norwegian Scientific Expedition (1937–38), many of the results of which remain unpublished. The sole comprehensive description of the vegetation so far published is by Wace and Holdgate[3]. These authors recognize a series of clearly defined altitudinal zones; the lowermost is characterized by the 'island tree' *Phylica arborea* Thouars and extends upwards to about 2,000 ft. Next, on the edge of the Base at 2,000–2,300 ft., comes a belt dominated by the small tree-fern *Blechnum palmiforme* (Thouars) C.Chr. and then zones of *Empetrum rumbrum* Vahl, grasses and alpine moss communities.

Early accounts confirm that the settlement plain was formerly covered in the normal low-level *Phylica arborea* scrub, together with tussock grass (*Spartina arundinacea* Carm.) and *Blechnum palmiforme*[4]. Since human colonization in 1810, burning and grazing have led to the replacement of this vegetation by a short, heavily grazed grassy sward largely dominated by introduced species, of which the chief are *Cynodon dactylon* Pers, *Holcus lanarus* L., *Agrostis* spp. and *Plantago lanceolata* L. Vegetation of this type adjoins the new volcanic cone. Immediately to the west and north, *Cynodon* is the only dominant of the grassland, while to the east for about 300 yards there is an area of blown sand sparsely colonized by this species. The Main Cliff immediately behind the volcano has a patchy covering of *Phylica* (formerly cut by the islanders for fuel) with *Empetrum rubrum, Blechnum penna-marina* (Poir) Kuhn, and *Polystichum adiantiforme* (Forst.) J. Sur. conspicuous in the field layer.

Effects of the eruption. Only the lowermost vegetation zones, artificial grassland, *Phylica* scrub and *Blechnum palmiforme* vegetation have been affected by the eruption. The lava itself has covered about 20 acres which were once grassland, and ash has been deposited between the cinder cone and the Main Cliffs. Apart from this direct covering of the ground, the eruption has affected the vegetation in three ways: (1) Numerous minor landslides have cleared strips of vegetation and soil from the Main Cliffs near the volcano. (2) Pyroclasts have set fire to small areas of *Phylica* bush on the cliffs immediately behind the cinder cone. (3) Sulphurous gases, produced in large quantities during the early stages of the eruption, have done considerable temporary damage to the vegetation over a wide area, especially behind and to the east of the volcano. This damage extends about 6 miles eastward around the cliffs, but only 2 miles westward. This asymmetry is due to the prevalence of westerly winds.

The damage is made evident by the browning and withering of the foliage and is accompanied by the cessation of growth. Besides the dominants, *Phylica* and *Blechnum palmiforme*, *Acaena sarmentosa* Carm., *Blechnum penna marina*, *Elaphoglossum succisifolium* Moore, *Empetrum rubrum*, *Scirpus thouarsianus* Schult. and the cultivated New Zealand flax *Phormium tenax* are obviously affected. The damage declines with distance from the eruption, and it was noted that on the Base only *Phylica* and *Blechnum palmiforme* are seriously affected.

Very few, if any, of the *Blechnum palmiforme* plants have actually been killed. New unharmed fronds are now growing on almost every plant examined. On the other hand, it is difficult to assess the full nature of the damage done to the *Phylica* trees. Although a great number are completely brown and have every appearance of being dead, the trees may still survive, as this species has the ability to grow shoots from the widely spread roots.

Recolonization. Even in the most affected areas of the Main Cliffs, where the majority of the trees may be dead, recolonization is likely to be rapid. *Phylica* seedlings have already been discovered growing through 1 cm. of ash on the lower slopes behind the cinder cone. Shoots of *Cynodon* have already pushed up through as much as 15 cm of ash and are spreading over the surface and over volcanic rubble. Moreover, the first plant colonization of the new lava flow has occurred. Where a stream cascades over the former sea cliffs to the west of the new flow and splashes on to the fresh lava, filamentous green algae are growing.

Permanent quadrats have been set up close to the periphery of the volcano in order that the development of vegetation on and around the fresh lava may be recorded.

Zoology

The effects of the volcanic activity on the fauna of Tristan fall into two classes: the direct effect in the form of physical damage and the indirect effects resulting from the evacuation, with consequent decontrol of pests and domestic animals and cessation of fowling. No signs of the last can be expected to appear in so short an interval of time.

(1) *Direct effects*. Some 650,000 sq. yards of mostly invertebrate habitat have been covered by the volcano and its flows. Observations of a comparable site of eruption at the southern end of the island suggest that soil and vegetation will not be re-established for two to three hundred years, during which period the fauna of the area will probably remain impoverished. A further small area has been covered by falls of rock and ash deposits; sulphurous fumes have damaged the vegetation over a wider area. Beyond a few hundred yards from the volcanic centre, soil invertebrates remain fairly numerous: phytophagous species have probably suffered more severely. Plant recolonization is proceeding rapidly and these peripheral effects are likely to be short-lived. Birds and mammals are affected by the eruption to a surprisingly small degree.

(2) *Indirect effects*. (i) *Decontrol of pests*. Principal invertebrate pests during habitation were the noctuid *Peridroma margaritosa*, the aphid *Myzus persicae*, and the calliphorid *Lucilia sericata*. None of these was a serious pest, and none was restricted to the settlement area, but control measures were in force against them all. Of the three, only *L. sericata* appears to have increased, and this may be temporary resulting from the abundance of refuse and carrion which followed the evacuation. In the absence of the potato crop, *M. persicae* is particularly numerous on *Rumex obtusifolius*, and *P. margaritosa* is widespread and numerous on a variety of food-plants. The flea *Pulex irritans* has survived the three months absence of man. Among vertebrates, the only serious pest is the black rat *Rattus rattus*. In the absence of man it has not increased in the settlement area, where it was formerly most abundant, though whether this is due to predation by cats or loss of food supply is not certain. There is no reason for supposing any change in status on other parts of the island.

(ii) *Domestic animals*. There are wild cattle at Stony beach and Cave point, and domestic cattle at Sandy point (non-breeding) and in the settlement area, only the last having been affected. Out of more than a thousand acres, the volcano has deprived them of only 20–30 acres; but their breeding success was severely cut by the dogs. All the cattle are in good condition and should over-winter well in absence of competition from sheep. The sheep have been drastically reduced from more than 700 to less than 50; many appear to have been chased over cliffs by the dogs. Numbers of donkeys are about the same as before, though no young animals have been added; again, probably due to dogs. Fifteen dogs were found, concentrated in the settlement area. The effect of evacuation on these had been to allow them free access to almost all parts of the island and undisturbed predation on other animals. Some damage was done to moulting penguins *Eudyptes cristatus* and to albatrosses *Diomedea chlororhynchos* (and possibly *Phoebetria fusca*); feather and bone fragments were found in the dog-fæces. About twenty cats have survived in the settlement; twelve were found dead around the houses and more may have migrated to swell the number of feral animals on the Base. The hens were found to have suffered little setback, but there was no trace of more than fifty geese which had been left on the island.

(3) *Control measures taken*. On the basis of obvious and deduced damage to wild and domestic animals, it was decided to destroy the dogs. Fifteen were found and killed. A possible sixteenth, an adult male, may have survived on the Base. All bulls and bull-calves in the settlement were killed or castrated, which, even allowing for some *in utero*, will provide a temporary check to any increase in number of the cattle, while the future of the island is undecided.

The results given in this report are based solely on field observations as specimens collected have not yet been subjected to laboratory examination. The scientific members of the Expedition are responsible for the sections of this report covering their own fields of study and will publish detailed accounts elsewhere in due course.

Undoubtedly, the success of the Expedition was largely due to the invaluable assistance it received from many quarters, especially from the South African Navy and the Royal Navy, to whom members of the Expedition extend their sincere thanks.

[1] Dunne, J. C., *Det. Norske Videns. Akad. Oslo* (1946).
[2] Harris, P. G., and Le Maitre, R. W., *Nature*, **193**, 719 (1962).
[3] Wace, N. M., and Holdgate, M. W., *J. Ecol.*, **46**, 593 (1958).
[4] Holdgate, M. W., and Wace, N. M., *Polar Record*, **10**, 475 (1961).

INTAKE OF IODINE-131 FROM MILK IN ITALY DURING THE PERIOD OCTOBER–DECEMBER 1961

By DR. A. CIGNA and DR. C. POLVANI
Comitato Nazionale per l'Energia Nucleare, Roma

DURING the last months of 1961, following the renewal of the nuclear tests, some determinations of the concentration of iodine-131 were carried out on cow's milk distributed to the population of various cities, representing one-fifth of the entire Italian population (Fig. 1). Measurements were not numerous and systematic in the first weeks of October (therefore those results are scarcely representative); but later many more were carried out following a well-defined programme. Daily samples were taken in six cities; weekly samples in seven (Fig. 1).

Fig. 2 gives the pattern of the atmospheric precipitation and of the monthly radioactive fall-out. It must be emphasized that the amount of atmospheric precipitation is usually high in Italy during autumn; for the 90 days under examination the rainfall amounted to 400 mm., out of 872 mm. recorded throughout 1961.

In Italy at the end of the three months under consideration the milk cattle are fed a high percentage of dry fodder as compared with other months, when fresh grass prevails. This change of diet occurs earlier in northern Italy (November) and later in southern Italy (December); however, at least in some areas of northern Italy, the administration of a certain amount of fresh grass is maintained even in December.

The course of rain and fall-out and the change in the diet influenced the concentration of iodine-131 in the milk. The results of the measurements carried out, averaged at intervals of three consecutive days, are given in Table 1 and Fig. 3. (The correction for radioactive decay of iodine-131 was effected with reference to the second of the three consecutive days.) A peak can be observed at about half-way through November : this follows, after a few days, the rainy period which occurred everywhere in Italy between the third and the fifteenth day in November. It must be realized that the figures reported are the arithmetical averages of the determinations on samples collected in various cities; on single samples, in the same period, values of 300 and more pc./l. of iodine-131 were observed.

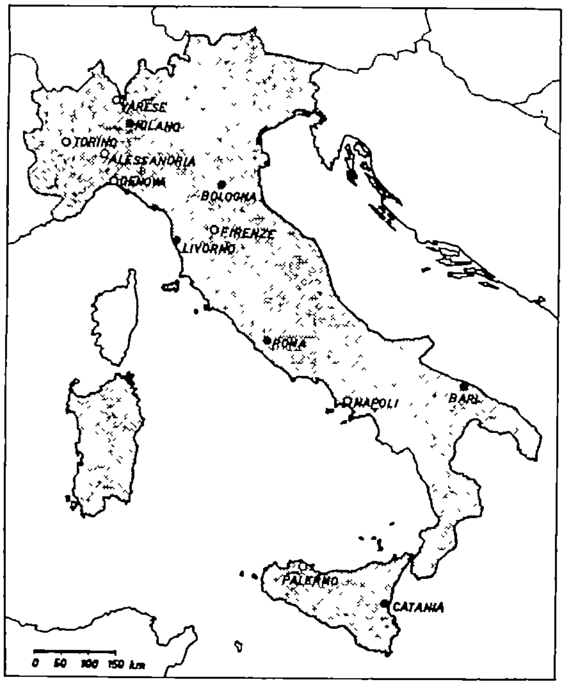

Fig. 1. Map of Italian cities and towns, where the samples of milk were collected. ●, Daily samples, ○, weekly samples

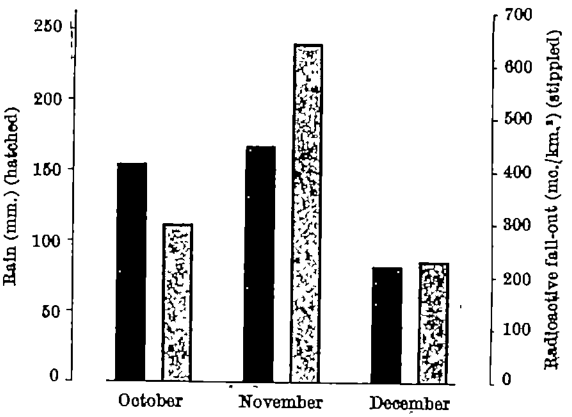

Fig. 2. Monthly atmospheric rainfall (ref. 3) and radioactive fall-out (ref. 4). The data of radioactive fall-out were corrected for self-absorption and radioactive decay (with reference to the fifteenth day of the collection month); the correction factors are roughly approximate

Table 1. CONCENTRATION OF IODINE-131 IN THE MILK DISTRIBUTED DURING THE PERIOD OCTOBER–DECEMBER 1961

Days	^{131}I (pc./l.)	Days	^{131}I (pc./l.)	Days	^{131}I (pc./l.)
Oct. 1–3	(153)	Oct. 31–Nov. 2	80	Nov. 30–Dec. 2	99
4–6	(148)	Nov. 3–5	79	Dec. 3–5	80
7–9	(139)	6–8	84	6–8	71
10–12	(138)	9–11	90	9–11	63
13–15	(119)	12–14	108	12–14	53
16–18	103	15–17	125	15–17	42
19–21	97	18–20	155	18–20	37
22–24	91	21–23	158	21–23	41
25–27	83	24–26	139	24–26	(39)
28–30	84	27–29	117	27–29	(28)
				30–31	(28)

The values in the first half of October are in brackets on account of their lesser significance (see text).

The values of the last days of December (also in brackets) must be taken as representing a maximum concentration.

Gamma spectrometers with sodium iodide (thallium) crystals (5 cm. diam., 5 cm. in height) were used for measuring the iodine-131 content of the milk samples (1 litre each). The resolution of the photoelectric peak of iodine-131 (at 0·360 keV.) was about 11 per cent, and the gamma efficiency for the same photopeak 4 per cent. The lowest concentration of iodine-131 which could be measured in such conditions was 10 ± 2 pc./l., when the time of measurement ranged from 3 to 4 hr. The channel shift was not higher than 20 keV. a week.

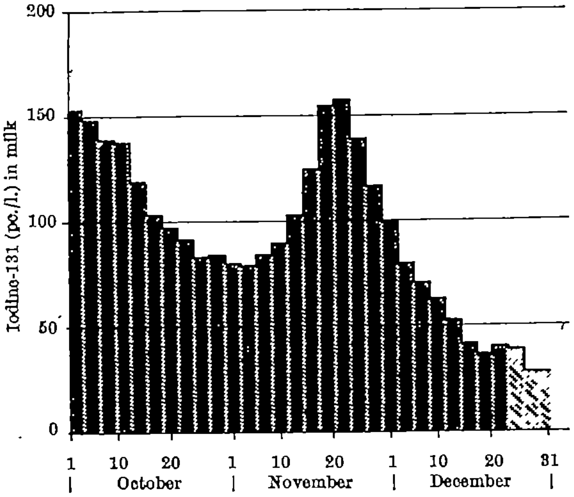

Fig. 3. Concentration of iodine-131 in milk distributed in the period October–December 1961. The values corresponding to the first half of October are shown with a dashed line owing to their lesser significance. The histograms concerning the last days of December are dashed and must be regarded as representing a maximum concentration

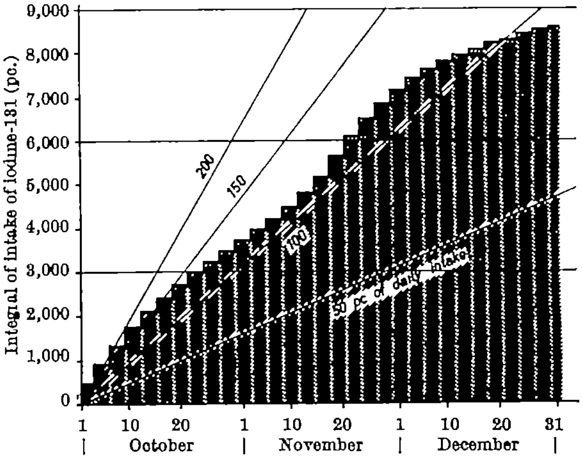

Fig. 4. Graph of the integral, during the months of October, November, December 1961, of the intake of iodine-131 (persons fed with 1 litre of milk a day)

If we consider the babies fed with about 1 litre of cow's milk a day (this figure is the maximum), it is possible to draw the integral, during the months under consideration, of the amount of iodine-131 ingested. A diagram of the integral is given in Fig. 4. It can be seen that the value of the integral of the intake actually occurred in the babies fed with the sampled milk lies for a long period between the straight lines representing the integrals of the assumed intakes of 100 and 150 pc. a day. At the end of the considered period the mean intake amounted to 8,600 pc. of iodine-131.

Even taking into consideration the poor significance of the measurements of the first weeks of October and the limiting factors involved in the sampling, these figures may represent the mean intake of iodine-131 in Italy during the last months of 1961, for the persons fed with 1 litre of milk per day. Considering the dispersion of the observed values in the different cities, it is most likely that in some areas the intake over the quarter exceeded the value of 10,000 pc. of iodine-131.

The C.N.E.N. (Comitato Nazionale per l'Energia Nucleare) authorities organized the measurements over the country; the measurements were carried out by the C.N.E.N. Laboratory for Environmental Radioactivity; those measurements for Livorno were carried out by Centro Applicazioni Militari Energia Nucleare, while those for Bologna were made at the Institute of Physics, University of Bologna.

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[1] Cigna, A., and Polvani, C., Paper presented at FAO/WHO/IAEA Seminar on the Agricultural and Public Health Aspects of Radioactive Contamination in Normal and Emergency Situations. Scheveningen, 1961 (in the press).

[2] Malvicini, A., and Polvani, C., *Notiziario C.N.E.N.*, **8** (4), 19 (1962).

[3] *Boll. Mens. Statistica (Roma)*, **37** (2), 7 (1962) (see data on climatology).

[4] Data on environmental radioactivity collected in Italy (July–December 1961). *C.N.E.N., Doc. BIO*/06/62 (see data of the stations of the Military Air Force-CAMEN and of the stations of CNR-CNEN).

NEWS and VIEWS

Nuclear Fuel Director at Atomic Energy Authority, Risley: Dr. N. L. Franklin

Dr. N. L. Franklin has been appointed nuclear fuel director in the U.K. Atomic Energy Authority Production Group at Risley. Dr. Franklin, who was formerly technical director, will be responsible for the Group's commercial activities and for technical aspects of the Group's work on civil magnox fuel elements. Dr. Franklin was born in Leeds and was educated at Batley Grammar School and the University of Leeds. In 1945 he joined the staff of the British Coke Research Association as a technical officer. From 1948 until 1955 he was assistant lecturer and later lecturer in chemical engineering at the University of Leeds. In 1955 Dr. Franklin joined the Technical Policy Branch of the U.K. Atomic Energy Authority and in 1958 was appointed assistant director of technical planning. In 1959 he was made technical manager, Chapelcross, and in 1960 appointed deputy technical director of the Production Group at Risley. In 1961 Dr. Franklin was appointed technical director, Production Group.

Responsibility for the Production Group's technical and development work on chemical processes, isotopic separation, reactors and fuel other than magnox, and for planning and programming, will remain with the Group's Technical Directorate. Mr. C. Allday, the deputy director (technical), will be responsible for this work.

Industrial Sociology at Northampton College of Advanced Technology: Mr. R. G. Stansfield

Mr. R. G. Stansfield has been appointed reader in industrial sociology in the Department of Management and Social Science, Northampton College of Advanced Technology, London. Mr. Stansfield took the Natural Sciences Tripos at Cambridge in 1936, obtaining first-class honours in Part I and second-class in Part II physics. He worked in the Cavendish Laboratory during 1936–39 and gained a B.Sc. (London) in special physics with first-class honours in 1936 as an external student. During 1939–45 he carried out operational research with the Royal Air Force. During 1945–48 he was a senior scientific officer with the Ministry of Works and was technical officer to the Human Efficiency Panel; then for a year he was with the Board of Trade on a special research unit. In 1950 he became a principal scientific officer and worked with H.M. Treasury for a year as assistant to the chairman of the Panel on Human Factors. In 1951 Mr. Stansfield transferred to the Department of Scientific and Industrial Research, and until 1959 was leader of a team concerned with human sciences in industry. In 1959 he was made head of the Human Sciences Section of the Warren Spring Laboratory at Stevenage.

Clinical Nutrition at Beirut : Prof. D. S. McLaren

DR. D. S. MCLAREN has been appointed research professor of clinical nutrition and director of the nutrition programme in the American University of Beirut, Lebanon. This new appointment and the development of research and research training activities in the Schools of Medicine, Public Health and Agriculture at this University in collaboration with the Institute of Nutrition Sciences at Columbia University, New York, have been made possible by a grant of a million dollars over a period of five years from the U.S. National Institutes of Health. Dr. McLaren graduated in medicine at the University of Edinburgh in 1947 and after five years in India gained the M.D. of that University in 1955. Since then he has specialized in nutritional diseases of the eye, was awarded the Ph.D. of the University of London in 1957 and has published more than thirty papers in this and other related fields. He is Oliver Hawthorne, Ciba Foundation and Barraquer Institute prize-winner, and has acted as World Health Organization consultant in Indonesia and Central America. Previously he was for four years on the staff of the East African Institute for Medical Research in Tanganyika.

Industrial Research and Development in the United States

INDUSTRIAL firms in the United States, according to a survey recently published by the National Science Foundation, devote by far the largest share of their research and development funds to development. Only 4 per cent of the total is spent on basic research and 20 per cent on applied research, whereas 76 per cent goes on development. This heavy concentration on applications of science to production techniques reflects the heavy costs required to translate basic and applied research findings into practical means of producing new products and processes. Development frequently involves large numbers of scientific, technical and other supporting personnel as well as expensive materials and equipment in the construction and testing of models or prototypes. Increased expenditure on research and development has been undertaken by firms not merely as a means of holding their competitive positions in domestic and foreign markets but as a means of promoting long-range economic growth by developing new and improved products or introducing more efficient techniques of production. Some firms, especially smaller undertakings, find it financially advantageous to contract their research and development projects to outside organizations such as commercial laboratories, educational institutions and non-profit research institutions. The survey estimates that some 11,800 industrial firms undertook research and development work in the United States and that funds for these purposes amounted to 9·6 thousand million dollars, of which 41 per cent came from industry's own resources and the remaining 59 per cent from the Federal Government. In addition, a further 3,000 million dollars was spent on research and development by the Federal Government, colleges and universities and other non-profit-making organizations (*Surveys of Science Resources Series*, NSF 62-3. *Funds for Research and Development in Industry* 1959—*Performance and Financing*. Pp. xi+109. Washington, D.C.: Government Printing Office, 1962. 60 cents).

Science in Parliament :
Engineering and Nuclear Power

IN answering a series of questions in the House of Commons on May 28, the Parliamentary Secretary for Science, Mr. Denzil Freeth, said that the Council for Scientific and Industrial Research has delegated to the National Engineering Laboratory the power to place research and development contracts of up to £2,500. Larger contracts in the Laboratory's field are placed by the Department's headquarters and technically supervised by the Laboratory. Regarding the Dounreay Experimental Fast Breeder Reactor, completed during 1959, he said the final capital cost of the reactor and of its associated fuel element fabricating and re-processing plants was £12·5 million. The present delay in raising the power in the reactor to about 30 MW. had been caused by the difficulty of reducing the oxide impurity in the coolant to the desired 4 p.p.m. Mr. Freeth also said that owing to increasing requirements for funds for nuclear physics and in particular for the CERN programme, it has been necessary to review the prospective needs of nuclear physics research as a whole before reaching a decision on the recommendation, in July 1961, of the National Institute for Research in Nuclear Science for provision in Great Britain of a 4-GeV. electron synchrotron to supplement the work of *Nimrod* now under construction.

Coal and Petroleum

AT present, no research is in progress in Government laboratories into the microbiology of coal or petroleum, but the Department of Scientific and Industrial Research will consider on its merits any application for a grant for such research.

Meat Research

ON meat research Mr. Freeth said that in carrying out its plans for the establishment of the Meat Research Institute, the Agricultural Research Council is giving first priority to finding a director-designate of meat research so that he can take as full a part as possible in the detailed planning. A decision whether and where sub-stations should be set up is unlikely to be taken until after his appointment. The Council considers that the organization of meat research in this wider sense will call for a considerable degree of correlation in the programmes of agricultural research institutes: the basic studies of dead meat, which will mostly be done at Bristol, must be related to what is being done elsewhere on breeding nutrition and management. Consequently, the Council's plans for meat research in general must involve Scottish stations, such as the Animal Breeding Research Organization at Edinburgh and the Rowett Research Institute. The Advisory Committee on Meat Research will be appointed by the Agricultural Research Council, and it is intended that its membership will include meat producers, processors and scientists.

Training and Research Grants

On April 1, 2,648 training awards of the Department were currently held, of which a small number are being held overseas; of those held in Great Britain, 10·1 per cent were held in Scotland. Of 724 grants for special researches awarded in the year ended September 30, 1961, 81 were in response to applications from Scotland.

Scientific Manpower

Concerning manpower, Mr. Freeth reported that the third triennial survey of scientific manpower is in progress, and in the course of this the Committee on Scientific Manpower will be able to take into account any changes in the estimated output of the universities since its last review. The Department of Technical Co-operation obtains advice on scientific matters from a wide range of experts, including scientists from the Department of Scientific and Industrial Research and the Nature Conservancy. An important way in which this advice is provided is by making the staff of these organizations available for membership of advisory committees of the Department of Technical Co-operation.

Promoting Civil Science

Mr. Freeth said that a Committee had been set up under the chairmanship of Mr. B. St. J. Trend to review the organization for the promotion of civil science by Government agencies. The other members of the Committee are: Sir Keith Murray, Sir Thomas Padmore, Lord Todd, Mr. F. F. Turnbull, Prof. C. H. Waddington and Dr. E. G. Woodroofe, with Miss K. Whalley as secretary. The terms of reference are to consider: (1) whether any changes are desirable in the existing functions of the various agencies, for which the Minister for Science is responsible, concerned with the formulation of civil scientific research, and whether any new agencies would be created for these purposes; (2) what arrangements should be made for determining, with appropriate scientific advice, the relative importance in the national interest of the claims on the Exchequer for the promotion of civil scientific research in the various fields concerned; and (3) whether any changes are needed in the existing procedure whereby the agencies concerned are financed and required to account for their expenditure.

Industry and Space Research

In a written answer on May 28, Mr. Freeth stated that British industry will continue to benefit from work carried out under contracts for the development and construction of experimental equipment for use in scientific space research and also for the development and construction of satellite launchers. The technological benefits to be derived from this work will strengthen the competitive power of British industry, particularly in electronics and the technology of new materials. It was proposed to hold an inter-governmental conference in the middle of June, at which the European Space Research Organization Convention and its associated protocols would be open for signature.

D.S.I.R. Developments

Both branch offices of the Department of Scientific and Industrial Research in Edinburgh and Cardiff have been strengthened recently, and six regional technical information centres are now operating, in Newcastle, Liverpool, Manchester, Birmingham, Bristol and Portsmouth. A seventh is being established in Leeds.

The Esso Review

The Esso Review for 1961 is a model of how a big company can present information about its activities for the benefit of its employees scattered in many countries. The growth of the oil industry is well described in pictorial form and shows how world crude oil production reached a total of 1,120 million tons, some 70 million tons more than in 1960. This 6·5 per cent increase was only slightly less than the average growth-rate of the past ten years, the outlook being for continued growth. The Middle East produced as much oil in 1961 as was produced in the whole world in 1939. In Europe, the increase in oil consumption was particularly marked, although the rate of growth in the United Kingdom was less than the European average. The *Review* shows that United Kingdom energy demand was about the same in 1961 as in 1960 because of the small rise in economic activity and above-average temperatures, but that demand for oil in the United Kingdom rose by about 7 per cent and oil contributed 27 per cent of the total energy needs. There are also many illustrations of Esso's activities inside Great Britain, including the construction of the pipeline from Fawley to Severnside.

Canadian National Parks

The Geological Survey of Canada has issued two attractive brochures describing various geological features of two Canadian national parks (*Miscellaneous Report No. 2*: *Rocks and Scenery of Fundy National Park*. Pp. iii+32. Ottawa: Queen's Printer, 1962. 50 cents. *Miscellaneous Report No. 3*: *Prince Edward Island National Park—The Living Sands*. Pp. 56. Ottawa: Queen's Printer, 1961. 50 cents). They have been written by David M. Baird, who, in the first, explains the part played by the rocks of Fundy National Park in determining its scenery. In the second brochure he shows how the waves of the Gulf of St. Lawrence have evened out the irregular lines of the north shore of Prince Edward Island to form long sandy beaches and bars across the headlands and the mouths of bays; the latter have been set aside as the Prince Edward National Park. Both brochures are illustrated and add to the value of well-written publications which should give pleasure to many visitors to the parks.

Birds Around Winchester

A short account of the birds of the Winchester district has been prepared from the records of the Winchester College Natural History Society during the past ten years (*Birds of the Winchester District*. Pp. 39 (5 maps). Winchester: Winchester College Natural History Society, 1962. 5*s*.). The gaps in observations resulting from school holidays, particularly those relating to the early migrants, have been largely filled from the records of the Hampshire Field Club and Archæological Society. The publication is not intended to be a guide to bird identification. Rather it is an attempt to bring together. within a small compass, information about birds to be found at different times of the year within a ten-mile radius of Winchester. The various maps included with the text are sufficiently detailed for the identification of most of the bird habitats described. The School's Natural History Society is to be commended for a publication which should be well received by all bird lovers.

Sound—Its Uses and Control

Noise Control, the bi-monthly publication of the Acoustical Society of America devoted to the discussion of the problems of noise and methods of combating noise, has now been discontinued and replaced, as from the beginning of 1962, by a new bi-monthly magazine entitled *Sound—Its Uses and Control* (American Institute of Physics, 335 East 45th Street, New York 17. 5 dollars for Acoustical Society members and 8 dollars for non-members). The editor is Dean R. Bruce Lindsay and the managing editor H. A. Erf, of the H. A. Erf Acoustical Co. Associate editors include members of Bolt Beranek and Newman, Inc., the National Bureau of Standards, K. W. Johnson and Co., Inc., and the Baldwin Piano Co. In his editorial to the first number (1, No. 1; January–February, 1962) Dean Lindsay states that the new magazine is intended "to provide an appreciation and understanding of the importance of sound in all its aspects in the life of man among the widest possible audience, and practical information on the uses and control of sound unencumbered by the heavy mathematical analysis of the conventional research paper".

Biennial Oscillations of the Equatorial Stratosphere

The apparent 26-month oscillation of the wind field at 50 mbars and above over equatorial latitudes continues to mystify meteorologists (*Nature*, 189, 791; 1961). J. K. Angell and J. Korshover, of the U.S. Weather Bureau, have confirmed the results of Ebdon and Veryard, of the Meteorological Office, concerning the existence of this unusual period of 26 months between maximum westerly and easterly winds. The oscillation decreases in amplitude at levels below 50 mbars, becoming almost undetectable at 100 mbars. The oscillation appears to be in phase at all longitudes, but there is a phase variation with height, with the maximum westerly wind taking about 4 months to work down from 25 to 50 mbars and another 6 months to go from 50 to 100 mbars. There is evidence that the maximum westerly wind occurs about 6 months earlier at 30° N. and moves towards the equator. Angell and Korshover suggest that the biennial oscillation may be first noted at between 40° and 50° N., from whence it propagates northward and southward. A great deal more wind data must be analysed before a rational explanation of this unusual phenomenon will be forthcoming.

Psittacosis in Japan

The recent discovery of psittacosis in various avian species in Japan led to an extensive survey on human infections with the virus, utilizing the complement fixation and hæmagglutination inhibition tests. In the survey, a number of psittacosis cases was found and evidence was obtained showing widespread dissemination of the virus in the general population in the areas investigated. A paper by Toshio Goto *et al.*, of the Sagamihara National Hospital, Sagamihara, Kanagawa Prefecture and the Institute for Infectious Diseases, the University of Tokyo, describes clinical observations in forty-two serologically diagnosed human cases (*Jap. J. Exp. Med.*, 31, No. 4; 1961). The cases were diagnosed in a survey of acute respiratory infections carried out at the Sagahimara National Hospital near Tokyo during 1956–58. Of the forty-two cases, thirty-three showed a clinical picture of primary atypical pneumonia, and seven that of acute bronchitis. The remaining two cases showed middle-lobe syndrome of Graham *et al.* Analysis of the clinical findings in each case supported the serological diagnosis and most of the cases were mild with no complications. In one of the pneumonitis cases a transient transparent area was observed in a pulmonary infiltrative shadow, and in another case acute transient middle-lobe disease was revealed by X-ray photography. Spontaneous recovery took place in most mild cases. In severe cases, penicillin, chloramphenicol and tetracycline were administered with favourable effects and no fatality occurred.

Batshioko (Tshokwe) Negro Decorative Art

This recent monograph by Dr. Marie-Louise Bastin, of the Centre National d'Ethnologie des Peuples d'Outre-Mer at Tervuren, is likely to remain for some time the authoritative work on Batshioko (Tshokwe) decorative art, and all those interested in African Negro sculpture will be grateful to the Diamond Company of Angola for its publication in such a sumptuous manner. It forms No. 55 in the Dundo Museum's series of scientific and cultural monographs and consists of two large volumes (Companhia de Diamantes de Angola (DIAMANG) Servicos Culturais. Dundo–Lunda—Angola, Museu do Dundo. Publicacoes Culturais No. 55: Subsidios para a Historia, Arqueologia e Etnografia dos Povos da Lunda. *Art Decoratif Tshokwe*. Vol. 1: Pp. 267. Vol. 2: Pp. 273–394+277 planches. Lisboa: Companhia de Diamantes de Angola, 1961). The first gives a brief historical and ethnographical account of the Tshokwe and a more detailed analysis of their decorative art, supported by 125 plates, 2 maps, 3 tables and numerous sketches and drawings. The second contains 275 pages of excellent photographs by M. A. Oliviera of representative examples of Tshokwe art from the collection in the Dundo Museum. They are preceded by 120 pages of detailed notes on each of the objects photographed, which range from baskets and pots through weapons, musical instruments and chairs, stools, and other domestic utensils to masks and statuettes. The carvings illustrated in plates 153–275 are particularly impressive and apart from some of the masks have never been previously published.

Feldberg Foundation Prizes

Prof. K. J. Ullrich, director of the Physiological Institute of the Free University of Berlin, and Prof. D. Whitteridge, professor of physiology in the University of Edinburgh, have been awarded the Feldberg Foundation Prizes for German–English scientific exchange for experimental medical research (set up by Prof. W. Feldberg, of the National Institute for Medical Research, Mill Hill, London). A prize is awarded each year to one German and one British scientist, and in recognition of this gift the German recipient gives a lecture in Britain, and the British recipient a lecture in Germany. Prof. Whitteridge gave his lecture at the Free University of Berlin on May 28 on "The Visual Pathway". The details concerning Prof. Ullrich's lecture will be announced at a later date.

The Textile Institute: Elections

Dr. J. G. Martindale (Scottish Woollen Technical College) has been elected a vice-president of the Textile Institute by Council. Dr. Martindale fills the vacancy caused by election to the presidency of Prof. J. B. Speakman (see *Nature*, 194, 435; 1962). Dr. A. R. Urquhart (the Cotton, Silk and Man-Made Fibres Research Association) has been re-elected as chairman of Council for the forthcoming year. Vice-chairman elected is Mr. A. W. Bayes (Ashton Bros. and Co., Ltd.), in place of Mr. J. A. Nasmith (Leesona Holt, Ltd.), who has been elected honorary treasurer in place of Mr. A. F. W. Coulson. Mr. J. C. H. Hurd (head of the Department of Textiles, Leicester College of Technology) has been re-elected honorary secretary. Lord Barnby, immediate past-president of the Textile Institute, has been elected to be an honorary life member.

Index Medicus and the U.S. National Library of Medicine

THE note on *Index Medicus*, appearing in the March 31 issue of *Nature*, p. 1236, unfortunately fails to give credit to the National Library of Medicine, U.S. Department of Health, Education and Welfare, Public Health Service, Washington, D.C., which publishes *Index Medicus* each month. Not only does the National Library of Medicine publish the *Index Medicus* but also it renders valuable service to references in medical literature.

The Night Sky in July

NEW moon occurs on July 1d. 23h. 53m. U.T., full moon on July 17d. 11h. 41m. and new moon on July 31d. 12h. 24m. The following conjunctions with the Moon take place: July 5d. 07h., Venus 0·6° N.; July 18d. 13h., Saturn 0·7° S.; July 20d. 18h., Jupiter 2° N.; July 27d. 00h., Aldebaran 0·9° S.; July 27d. 04h., Mars 4° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Regulus on July 12d. 00h., Venus being 1·1° N., and Mars with Aldebaran on July 23d. 13h., Mars being 5° N. There is a penumbral eclipse of the Moon on July 17 and an annular eclipse of the Sun on July 31, both invisible at Greenwich. Mercury is a morning star, but is not favourably placed for observation. Venus is an evening star, visible in the west after sunset. It sets at 22h. 20m., 21h. 55m. and 21h. 15m. on July 1, 15 and 31, respectively; its stellar magnitude is −3·6. Its distance decreases during the month from 109 to 88 million miles and the visible portion of the apparent disk decreases from 0·757 to 0·642. Mars rises at 1h. 30m., 0h. 40m. and 0h. 10m. at the beginning, middle and end of the month, respectively. It is at a distance of 177 million miles from the Earth on July 15, and its stellar magnitude is +1·4. Jupiter rises at 23h. 00m., 22h. 00m. and 21h. 00m. on July 1, 15 and 31, respectively. It is in Aquarius, its stellar magnitude is −2·3 and its distance from the Earth in the middle of the month is 400 million miles. Saturn is in Capricornus, rising at 21h. 50m., 20h. 55m. and 19h. 50m. at the beginning, middle and end of the month, respectively. Its stellar magnitude is +0·4 and its distance from the Earth on July 15 is 835 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: July 15d. 21h. 35·5m., μ Sgr. (*D*); July 21d. 00h. 30·4m., ψ^1 Aqr. (*D*); July 21d. 01h. 35·2m., ψ^1 Aqr. (*R*); July 21d. 02h. 22·0m., ψ^2 Aqr. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Delta Aquarid meteors are active at the end of the month, the maximum being on July 29; the radiant is at R.A. 22h. 36m., Dec. −17°, and conditions are favourable. The Earth is at aphelion on July 4, its distance from the Sun being 94·6 million miles.

Royal Society Appointments

MR. R. W. J. KEAY has been appointed deputy executive secretary to the Royal Society of London. Mr. Keay is at present director of the Federal Department of Forest Research in Nigeria.

Dr. J. N. Murrell, Department of Chemistry, University of Sheffield, has been awarded a Mr. and Mrs. John Jaffé Donation Research Fellowship to enable him to continue his work on theoretical chemistry at the University of Sheffield.

Announcements

MR. P. H. EVANS, of Consolidated Beryllium, Ltd., has been appointed the fifth Robert Horne Memorial Lecturer of the Society of Chemical Industry. His lecture, entitled "Production Problems associated with the Element Beryllium", will be delivered in Swansea on January 18, 1963.

THE following officers of the Nutrition Society have been elected: ***President***, Dr. D. P. Cuthbertson; ***Hon. Secretary***, Miss D. F. Hollingsworth; ***Hon Treasurer***, Dr. A. E. Bender; ***Ordinary Members of Council***, Mr. A. E. Boyne, Dr. G. N. Jenkins, Miss J. W. Marr, Mr. D. S. Miller and Dr. J. A. B. Smith.

A MEETING of the Biochemical Society will be held in the Department of Biochemistry, University of Cambridge, during July 12–13. The programme will include a colloquium on "Control of Protein Biosynthesis—the Role of Nucleic Acid". Further information can be obtained from Dr. H. R. V. Arnstein, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7.

A CONFERENCE on "Preparation of Case Studies for Education and Training in Administration and Management", arranged by the Welsh College of Advanced Technology in collaboration with the Comparative Administration Trust, will be held at the College during July 5–7. Further information can be obtained from Z. M. T. Tarkowski, Comparative Administration Trust, 30 St. George's Street, London, S.W.1.

THE fourteenth conference of the British Occupational Hygiene Society on "Control of Airborne Particles and Fumes" will be held in King's College, Newcastle upon Tyne, during September 19–20. Topics under discussion will include: the work of an occupational health service in environmental control; some observations on asbestosis; control of the vanadium hazard in two industries; an experimental Diesel-exhaust filter; some problems in the large-scale handling of beryllium; dust and fume control—the implementation of current legislation. Further information can be obtained from Dr. J. Steel, Department of Industrial Health, Medical School, King's College, Newcastle upon Tyne.

ERRATUM. Due to an oversight in proof, Dr. J. B. Birks and L. G. Christophorou state that the following changes should be made to their article "Excimer Fluorescence of Aromatic Hydrocarbons in Solution" which appeared on p. 442 of the May 5 issue of *Nature*: Table 1. 3 : 4-Benzpyrene, $D_1 = 514$ mμ, $\nu'(M_1 - D_1) = 5{,}350$ cm.$^{-1}$ (values for specimen of higher purity); Table 2. Anthranthrene, $D_1 = 594$ mμ.

INJURY, INFLAMMATION AND IMMUNITY

THE first section of an international symposium on "Injury, Inflammation and Immunity" was held on May 5, at the Miles-Ames Research Laboratories, Stoke Poges, under the chairmanship of Sir Howard Florey.

This section covered the anatomical, physiological and pharmacological aspects of inflammation.

The symposium was opened by Prof. H. Ruska (Dusseldorf) with a paper on the structure of capillaries and response to chemical injuries, discussing the electron microscopical study of capillaries. He said that capillaries from different tissues varied greatly in respect of the presence or absence of a basement membrane, vesiculation or pores. The presence or absence of these structures appears to be correlated with the permeability of the vessels.

The effect of various chemical agents on capillary permeability was correlated with the changes in capillary wall as assessed by the electron microscope.

Dr. R. Robineaux (Paris) showed a film of the microscopical appearance under phase contrast of cells in spleen explants using the 'Cellophane'-strip tissue culture technique, between the eleventh and twenty-first day of culture. Up to the sixteenth day of culture lymphocytes and plasma cells were seen in close cellular relation to macrophages and reticular cells. Plasma cells were seen in mitosis. After 21 days the culture was transformed into macrophages which absorbed the other cells. Explants were from guinea pig spleen and plasma cells developed under the antigenic influence of chick embryo extract in the culture fluid.

Prof. G. Asboe-Hansen (Copenhagen) discussed the connective tissue response to injury, especially emphasizing mast cell degranulation and the accumulation of chondroitin sulphate and other mucopolysaccharides in ground substance as a result of tissue injury. The increase in chondroitin sulphate was demonstrated by the uptake of sulphate labelled with sulphur-35 in the ground substance.

The effect of various hormones (for example, thyroid-stimulating hormone and corticoids) on the repair mechanism were discussed with reference to their effect on mast cells and the accumulation of acid mucopolysaccharides in the ground-substance.

Dr. W. G. Spector (London), in a paper on endogenous inflammatory mechanisms in the rat, described experiments on the pharmacological content of the turpentine-induced pleural effusion in the rat. He described two phases: an early phase associated with the presence of histamine and serotonin lasting 1 hr. was succeeded by a delayed phase associated with pharmacological agents resembling *SRS–A* and bradykinin. The early phase was retarded by antihistamine drugs without affecting the development of the later phase.

The role of peptides in the first stages of inflammation was the subject of the next paper, by Dr. G. P. Lewis (London), which discussed some of the protein-breakdown products responsible for the effects of inflammation. Among these he laid particular emphasis on bradykinin, a nonapeptide produced by the action of certain enzymes on plasma globulin. Bradykinin was capable of producing all four of the cardinal signs of the inflammatory response—vasodilation, increased capillary permeability, pain and the accumulation of leucocytes. He also discussed the enzymes capable of breaking down globulin to the active peptide and enzymes, kininases, which were in their turn capable of breaking down and controlling the concentration of these plasma kinins. Histamine was shown to play a part in activating plasma-kinin-forming activity and also enhanced the passage of these peptides into the tissues by increasing capillary permeability.

Prof. C. de Duve (Louvain) discussed lysosomes and cell injury. Lysosomes are small cytoplasmic sack-like structures surrounded by a lipoprotein membrane containing a number of enzymes. These enzymes, including cathepsin β-glucuronidase and acid phosphatase, are those which take part in all autolysis but are inactive so long as the lipoprotein membrane remains intact. They could be shown to be released under a number of pathological conditions, that is, in the liver after ligation of a lobe, or in the comatose animal, in the thymus after treatment with cortisone or in the spleen after X-irradiation. They also accumulated in states of physiological autolysis, for example, the regression of Mullerian ducts in the male chick embryo and in the regression of the tadpole tail. He postulated four possible mechanisms of the action of lysosomal enzymes in tissues: (1) the intracellular release of enzyme attacking the cell; (2) diffusion of these enzymes out of the cell, attacking other cells; (3) selective retention of phagocytes rich in lysosomes; (4) colonization of the tissue with phagocytes rich in lysosomes.

The final two papers were on the inflammatory and biochemical aspects of burns. The first was on inflammatory changes in burned skin by Dr. S. Sevitt (Birmingham), who discussed three types of increased capillary permeability, an immediate transient type which was due to release of histamine and was abolished by antihistamine drugs, an early form which was more severe leading to stasis and a delayed form occurring around and deep to the early response. He found that the critical temperature at which capillaries leaked protein was 41°–44° C. and this correlated with the critical temperature for formation of bradykinin. The critical temperature for capillary stasis was 47°–48° C.

The last paper was by Dr. J. Kohn (London) on biochemical and immunological aspects of human burns, discussing the electrophoresis of enzymes in blister fluid, the increased concentration of histamine and a bradykinin-like substance in the urine of patients with burns. Following the reports of the beneficial effect of convalescent serum in cases of severe burns attempts were made to demonstrate precipitating antibodies in the serum of patients with burns against antigens derived from the burned tissues, but these had met with very little success. However, he had found an unusually high level of precipitating antibody against staphylococcal antigens in the serum of patients with severe burns.

The second section of the symposium was at the Instituto Miles de Terapeutica Experimental in Mexico City during May 10–11, and the final section at the Miles Laboratories, Inc., Elkhart, Indiana, during May 15–16.

J. L. TURK

SHELTER RESEARCH IN AGRICULTURE AND HORTICULTURE

MANY agricultural improvers of the eighteenth and early nineteenth centuries attached considerable importance to providing sheltering woodlands for exposed farmland, while horticultural enterprises in mild but windy coastal areas of the United Kingdom have long appreciated the need for shelter. The actual benefits of shelter have, however, remained difficult to evaluate.

On the reconstruction of the Agricultural Improvement Council in 1952, the problems of shelter were raised and in 1955 its Land Management Committee decided that more information was needed about the nature of shelter itself and its effects on stock and crops. The Shelter Research Planning Group was formed to assess these problems and indicate the order of priority with which these might be investigated. It was clear at the outset that many aspects requiring investigation encroached on different spheres of scientific activity and cut across existing fields of scholarship and study: biology, meteorology, aerodynamics and even engineering.

During 1952–53 the Forestry Commission financed research at the University of Edinburgh into the influences of shelter-belts on microclimate, with the ultimate object of designing appropriate types of windbreak for different purposes in agriculture, horticulture and forestry. In the spring of 1953 pilot investigations of horticultural shelter were begun at selected experimental horticultural stations in conjunction with the Agricultural Branch of the Meteorological Office. Afterwards, on the Agricultural Improvement Council's recommendation, the Ministry of Agriculture sponsored projects concerned with livestock shelter at the University College of Wales, Aberystwyth, University College of North Wales, Bangor, and King's College, Newcastle.

A good deal of research, directly and indirectly relevant to the question of shelter, continued to be pursued in separate but allied fields, and in 1959 the Shelter Research Planning Group appointed Dr. J. M. Caborn, University of Edinburgh, to keep in touch with this work and act as a focal point to which information might be sent or inquiries addressed.

It was also realized that, in a comparatively new field of study, much time and effort were inevitably duplicated, particularly during the 'teething troubles' of devising experimental techniques and equipment. Any concerted or co-ordinated attack on the basic problems could only come from closer communications between research workers in the various fields involved. Accordingly, a symposium on shelter research was organized recently at Aberystwyth by the Shelter Research Planning Group.

After the opening by Mr. R. G. A. Lofthouse, chairman of the Group, the first session was devoted to "The Shelter Needs of Horticulture, Methods of Providing Shelter and Plant Response". Mr. F. W. Shepherd, deputy director, East Midland Region, National Agricultural Advisory Service, and former director, Rosewarne Experimental Horticulture Station, examined the case for shelter based on long experience of the horticultural areas of south-west England and presented results of trials of tree and shrub species for shelter hedges. Mr. W. H. Hogg, senior meteorological officer, attached to the N.A.A.S., Bristol, outlined the experiments with horticultural shelters which have continued at Rosewarne and other centres, reviewing Continental experience in this field and concluding with a description of wind survey techniques derived to assess the need for further shelter, other than that contributed by topography.

Shelter for livestock occupied a major part of the programme, and the discussion was opened by Dr. K. L. Blaxter of the Hannah Dairy Research Institute, Ayr, with a paper on "The Reactions of Cattle and Sheep to the Stress of Cold Environments". This dealt with precision studies of physiological and nutritional responses of animals exposed to controlled conditions in calorimeters and preliminary extension of these techniques to outdoor conditions. He concluded that, while quantitative expression can now be given to the effects of still-air environments on the metabolism of sheep and cattle, similar information is urgently needed for the effects of wind and rain. It was also emphasized that the separation of the direct stresses of climatic environment from the indirect ones, for example, lack of food, while instructive in laboratory studies, does not occur under field conditions. Prof. J. E. Nichols, University College of Wales, discussed the more pragmatic approach to the problem, for example, continued observation of individual and flock response to adverse weather conditions under normal systems of management.

A third session heard a paper by Dr. R. W. Gloyne of the Meteorological Office on "Meteorological Techniques in Shelter Research", especially in relation to livestock in hill areas. This included details of instrumentation available for measuring weather phenomena, illustrated by an account of methods applied for investigating long-term influences of introducing a series of shelter plantations on an exposed hill at Bangor. Mr. R. Lines of the Forestry Commission followed with an account of certain shelter experiments on tree growth, and described the technique of assessing comparative exposure by means of observing the rate of tattering of unhemmed linen flags. Finally, Dr. J. M. Caborn spoke on "Assessing the Efficiency of Shelter", dealing with methods of measuring the shelter performance of shelter-belts and windbreaks and discussing the value of shelter on the farm. Although there seemed little prospect of being able to forecast shelter effect on arable crop production from the basis of particular levels of wind, a plea was made for preliminary investigations into the effect of existing shelterbelts on the yield of field crops under Britain's climatic conditions.

Closing the proceedings, the chairman referred to the considerable exchange of information, ideas and experiences made possible by this meeting, in both formal and informal discussion. It was generally felt that the whole subject had gained momentum and that various sections of activity, though independent, promised to dovetail in the future and present a more composite picture of this very diffuse question of shelter.

J. M. CABORN

THE WORLD HEALTH ORGANIZATION

THE communicable diseases are still, for most countries of the world, the greatest obstacle to the achievement of higher standards of health. Developments in the struggle against these diseases, and in many other aspects of the work that is directly assisted or co-ordinated by the World Health Organization are discussed in the annual report of its Director-General for 1961*.

Perhaps the best known of the Organization's activities is the world-wide malaria eradication campaign. During 1961 the campaign grew in scope and complexity. By the end of the year operations ranging from preliminary pilot schemes to the surveillance of vast areas already cleared of infection were proceeding in 85 countries and territories. The Organization's assistance took many forms: it included, for example, special courses for the training of staff, the production of manuals on various specialized techniques of value in the campaign, advisory services to Governments and evaluation of the efficiency of their programmes, and trials on the dosage and frequency of application of insecticides. Research was continued on new insecticides to replace those to which mosquitoes have become resistant and on other problems such as that of resistance to anti-malarial drugs. Through meetings of technical experts and contacts with research centres receiving grants, the Organization continued to stimulate, guide and co-ordinate such research.

In the report on other communicable diseases many advances are described and some setbacks noted. Many countries participated in the world-wide campaign to eliminate smallpox. In Asia, where the highly endemic areas still exist, intensified control measures, pilot projects and mass vaccination campaigns were carried out. Progress was reported from certain countries of Africa and from the few in Latin America where endemic foci are still to be found. In the work on tuberculosis, pilot projects were started in order to find the best application, under different epidemiological and socio-economic conditions, of recent advances in the control of the disease. The continued increase in venereal diseases gave concern, as did outbreaks of cholera, yellow fever and sleeping sickness in countries where they had not apparently been present for some time. Good progress was made in the control of yaws, endemic syphilis, leprosy and trachoma. Poliomyelitis, which is still increasing in countries where mass vaccination has not yet been applied, also received much attention, as did a wide range of other virus diseases.

In its work on environmental sanitation the World Health Organization has been paying particular attention to the improvement of community water supplies. Assistance to Governments has included guidance on the financing, management and organization of water and sanitation schemes, as well as in their design and operation. Other topics dealt with in the same chapter include work on air pollution, as well as the varied activities on insect and vector control, the development of new insecticides research into insect resistance, and studies of the toxicity of pesticides to man.

The accounts of recent developments in many fields of activity provide numerous examples of the benefits derived from medical research. The Organization's assistance to research during the past year has covered a wide range of subjects, including comparative studies of leukæmias and effect of smoking, air pollution and occupation on lung cancer, controlled field trials of anti-leprosy drugs, testing of insecticides, a five-year co-ordinated programme on onchocerciasis in West Africa and Latin America, research into the ætiology of iron deficiency anæmia, and comprehensive field trials of new methods for controlling bilharziasis—a disease of growing importance in newly irrigated areas. These are co-operative activities, co-ordinated by the Organization, and in which institutes and, in some cases, industrial concerns in many different parts of the world take part.

Lack of qualified medical and auxiliary staff is still a major obstacle to progress in health in many parts of the world, and it is clear from the report that education and training is, directly or indirectly, an integral part of nearly all the work of the World Health Organization. Details are given of training courses organized in many parts of the world on almost all aspects of health work, of the provision of professors and teaching staff to medical, nursing and other training schools and of the 1,731 fellowships awarded by the Organization during the period covered. Although the policy is to award fellowships for short periods of study, the special conditions of countries with no medical schools, and where the shortage of doctors is particularly acute, have made it necessary to grant some fellowships for undergraduate medical studies.

Much of the Organization's work is carried out in co-operation with other international agencies working in allied fields, and the report contains references to joint programmes being undertaken with one or several other organizations. Thus, for example, it is working with the Food and Agriculture Organization on nutrition and milk hygiene and on the zoonoses; with the International Labour Organization in occupational health and on the health of seafarers; with the International Atomic Energy Agency on the disposal of radioactive waste and on other aspects of radiation protection; with Unesco on cell biology, training programes for newly independent States, and health education in schools. Work with the United Nations itself covers a wide range—from such traditional activities as the control of addiction-producing drugs, to work in the Congo (Leopoldville) and co-operation in programmes for economic and social development, including the Expanded Programme of Technical Assistance, programmes financed from the United Nations Special Fund and from the United Nations Children's Fund. The report also contains many instances of work with regional inter-governmental agencies and with non-governmental organizations concerned with health.

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* Official Records of the World Health Organization No. 114: The Work of WHO 1961. Annual Report of the Director-General to the World Health Assembly and to the United Nations, covering the period 1st October 1960–31st December 1961. Pp. 194. 6 Swiss francs; 10*s*.; 2 dollars. (H.M.S.O., 1962.)

APPARATUS FOR TEACHING SCIENCE IN SCHOOLS

IN the second part of his articles on "Science Education Today"[*], J. L. Lewis of Malvern College examines what has been done by the Esso Loan Service of Scientific Apparatus to Preparatory Schools. This has been applied only in the limited field of preparatory schools in Britain, but the influence it has had suggests that the methods could be applied more widely with remarkable effect.

In the past only a small number of preparatory schools taught any science: boys from most schools went on to their public schools at the age of 13 and began science there. For various reasons it was felt that science should be introduced into preparatory schools. The problems appeared insuperable: there were no laboratories, no apparatus, no scientists to teach. The Esso Scheme was tried as an experiment in about thirty schools. An existing series of science books was chosen; the schools were supplied with quantities of these, together with units of apparatus to enable every experiment described in the text to be performed by the teacher. In addition, it was assumed that many of the schools would not get the services of a qualified scientist to teach science, so that experienced teachers were invited to write a teacher's guide, specially to help the non-scientist in the presentation of the subject.

Such was the initial enthusiasm that it was soon developed and supplied, without any cost at all, to more than 150 schools. It has since been offered to every Associated Preparatory School in Britain. The result has been remarkable. From September next. more than 400 schools, which formerly were teaching no science at all, will be teaching it. Units on *Weather and the Earth*, *Hearing and Seeing*, *Energy and Engines*, *Electric Currents*, *Freshwater Life*, *Earth and Universe*, *Heating and Cooling*, *Air and Water* will be going to all parts of Britain to remain in a school for a term, enabling a course to be run until another unit arrives to take its place.

Another service has been rendered by Esso's Senior Loan Service of Scientific Apparatus to Schools This, too, has grown from small beginnings to something much valued in the educational world Under this, the Company has made available certain items of apparatus, usually of the more expensive type, which is often beyond the means of many schools, such as pH meters, double-beam oscilloscopes, ratemeters, signal generators, optical pyrometers and viscometers. These are loaned to a school free of charge for one term. The scheme has enabled pupil and teacher alike to become familiar with less-usual apparatus; it has enabled more sophisticated experiments to be carried out, and even small research projects.

A most important development of the Loan Service is now planned, a development likely to be of the greatest value, as its influence will ultimately be of benefit to all schools. Another helpful service is the proposed film on the teaching of modern physics which the Esso Film Unit is planning.

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[*] *Esso Magazine*. Spring 1962 (see also *Nature*, 194, 141, 1962)

HEART DISEASE IN THE BANTU

THE annual report of the South African Institute for Medical Research[*] is noteworthy, besides a number of other interesting features, for a series of investigations bearing on coronary heart disease in the Bantu.

Studies by Dr. F. W. Fox on two rural Bantu groups, namely, Pedi Bantu (Sekhukhuniland region) and Tswana Bantu (south-west Bechuanaland) indicate that, in view of the extremely low incidence of myocardial infarction found among both rural and urban Bantu, it would seem that intensive nutritional and other investigations should be concentrated on that fraction of urban Bantu living closest in diet and manner of life to South African Whites; and that fraction of very poor Whites whose environment factors conform in measure to those of the Bantu.

The present belief that diets very high in animal fat are necessarily promotive of atherosclerosis and mortality from ischæmic heart disease may not be valid irrespective of the dietary setting, and it is suggested that investigations of indigenous nomadic groups in north-east Africa, who subsist largely on milk from camels, goats and sheep, may be rewarding from the point of view not only of their very high intake of animal fat but also of animal protein and calcium.

Electrocardiograph studies have been carried out on groups of rural Pedi and Baralong Bantu; among 1,100 subjects only four gave evidence of infarction. all of whom were more than 75 years of age. Of Urban Bantu, among 385 Johannesburg pensioners (virtually all more than 60 years), one positive was found, aged 63 years. Apart from the foregoing study, there is little doubt that the results of electrocardiograph observations on Bantu populations at risk are in complete harmony with the conclusion reached from several large series of necropsies on Bantu undertaken at different centres in the Union, that is, the occurrence of, and mortality from, acute episodes of coronary heart disease among these people is virtually nil. The critical population next to be considered is the most sophisticated group of Bantu available. If the latter conforms to the reaction of Yeminite immigrants in Israel, and Japanese immigrants in California, then an increasing incidence of myocardial infarction would be expected.

Reports from Durban suggest that local Indians are as prone as local Whites to die from coronary heart disease and, incidentally, diabetes. Mortality from this disease, however, appears to be much lower among Indians in India. The situation regarding Durban Indians is unexpected; electrocardiograph, blood pressure, dietary and other investigations are being carried out by Indian research assistants on Indian population groups in Johannesburg and Pretoria.

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[*] The South African Institute for Medical Research. Annual Report for the year 1960. Pp. 191. (Johannesburg: The South African Institute for Medical Research, 1961.)

Death certificates suggest that South African Whites are probably as prone as overseas White populations to die from coronary heart disease. To give perspective to the electrocardiograph investigations on the Bantu, and to learn something of the incidence of myocardial ischæmia in representative local White populations with the co-operation of the authorities, electrocardiograph and other investigations have been carried out on 248 White persons. Assessment of tracings is still in progress. Permission from the relevant authorities has been obtained to carry out an investigation of 'total heart disease' in a group of workers—a study which will require intensive short-term co-operation from several physicians and other workers.

Other investigations have also shown the commonness of hypertension in the Bantu, a feature associated with a high incidence and mortality from cerebral vascular disease, but with virtually no mortality from coronary heart disease. In endeavours to throw light on hypertension in these people, a number of investigations have been carried out. Blood pressure and other investigations have been undertaken at schools previously visited for nutritional and biochemical studies in the Rustenburg, Hartebeestpoort and Witkoppen regions. It is apparent that blood pressure at one particular school is significantly lower than that of the others. Investigations on blood pressure have also been carried out on groups of mineworkers from different regions. Some differences are apparent; for example, hypertension occurs less among Xhosa compared with Shangaan and Tswana Bantu.

Some Bantu groups are habituated to very soft water; others add plant ashes of variable composition to their food. There is one Bantu group, however, habituated to an unusually high intake of salt, the sole source of water (wells) having a salinity of about a quarter that of sea-water. This group, at Kukong, Southern Kalahari Desert, is being studied from the point of view of blood pressure, electrocardiograph, blood biochemistry and other aspects. Observations will be compared with those on another group dwelling nearby, but habituated to soft drinking-water. This possibly unique natural situation should shed light on the influence of an unusually high intake of salt on hypertension in the Bantu.

EURYHALINE EUGLENOID FLAGELLATES

A RECENTLY published pamphlet* is Part 8 of a series which, when completed, will be the first attempt to give a comprehensive account of the unicellular, mostly motile, algae occurring in British coastal and estuarine waters. It is the second part to appear, Part 1, containing the general introduction to the series, definitions, bibliography and the taxonomic section on Chlorophyceae having been published in 1959. Part 8 consists of the taxonomic section on euryhaline Euglenineae. Since the series will deal with organisms of great importance for fishery and sea-food investigations, and since a taxonomic account of these organisms is not otherwise available, it is to be regretted that the pamphlet under review contains so many inaccuracies that its use cannot be recommended.

In the short description of the class Euglenineae with which the pamphlet begins there are omissions, misconceptions and several factual errors. For example, "Flagellum one or two" is incorrect for the Euglenineae, modern cytological studies having established the taxonomically important fact that all euglenoid flagellates have two flagella. Apart from the genera with two externally emergent flagella (for example, *Distigma, Eutreptia, Peranema*), most genera (for example, *Astasia, Colacium, Euglena, Lepocinclis, Phacus, Trachelomonas*) have one long flagellum which is emergent from the canal and functional in swimming, and a second flagellum which ends within the reservoir. This short flagellum is an independent organelle though its tip may be apposed to the longer flagellum, an arrangement which led to the old, erroneous description of a single flagellum bifurcate at the base. Other points needing correction are that emergent flagella with unilateral mastigonemes have been demonstrated for more genera than those quoted, notably *Phacus* and *Trachelomonas*; colonial euglenoids are known, for example, *Colacium mucronatum*, which forms extensive dendroid colonies; members of the Euglenaceae and Astasiaceae are described as having "simple bilateral symmetry" whereas one of the major taxonomic features of the euglenoid cell is its helical asymmetry; organisms in the Peranemaceae are said to have "complex dorsiventral organization" without the rider that such organization is found in species of most genera and is superimposed on the underlying helical asymmetry; the statement that "*Euglena gracilis* becomes colourless if grown in the dark" is misleading in its context, since permanent apochlorosis equivalent to the condition in *Astasia* is produced only if treatment with high temperature, ultra-violet irradiation, antihistamines or streptomycin is also applied; finally, *Distigma* cannot be said to be "the colourless counterpart of *Eutreptia*" in the way that species of *Astasia* might be for species of *Euglena*, or *Hyalophacus* is for *Phacus*.

Mistakes in the opening section are repeated in the key to the genera of the Euglenaceae (the only family dealt with in the pamphlet) and in the descriptions of genera and species. For example, the erroneous concept of species with only one flagellum appears several times and is carried to greater lengths for *Euglena obtusa* and *E. vermiformis*. *E. obtusa* is described as having "complete absence of a flagellum" when in fact it has two perfectly good flagella. It is true that in this species both flagella terminate within the reservoir at about the level of the eyespot. Dr. Butcher mentions a flagellar "vestige inside the canal" without apparently realizing its significance. As a practical point of distinction in the specific key it would be correct to say for *E. obtusa* "neither flagellum emergent" or, possibly, "no locomotory flagellum".

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* Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series IV: An Introductory Account of the Smaller Algae of British Coastal Waters. Part VIII *Euglenophyceae = Euglenineae*. By Dr. R. W. Butcher. Pp. v+17+8 plates. (London H.M. Stationery Office, 1961.) 12*s*. 6*d*. net.

The main text of the pamphlet contains new inaccuracies and misconceptions of which only the most obvious can be noted here.

It is undesirable to re-introduce Schiller's genera *Ottonia* and *Chlorachne* into the Euglenaceae when most authorities now exclude them entirely from the Euglenineae, especially since the descriptions of these organisms are merely repeated from Schiller's original records from the Adriatic.

Secondly, there is confusion in the section on *Eutreptia* where Dr. Butcher describes a new species, *Eutreptia scotica*, from material which I collected at Millport and gave him as a fresh isolation of *Eutreptia viridis*. Re-examination of my cultures of the Millport isolation shows this species to be cytologically identical with Prof. E. G. Pringsheim's isolation of *E. viridis* in the Cambridge Culture Collection, even to the extent of having the same chromosome count of 44. On the other hand, cells of *E. pertyi* supplied from Prof. Pringsheim's Sammlung von Algenkulturen, Göttingen, are considerably larger and cytologically more luxuriant than cells of *E. viridis* and the chromosome number, 90 ± 2, is approximately twice that of the smaller species. Yet Dr. Butcher finds his material of *E. pertyi* almost indistinguishable from the Cambridge *E. viridis*. From all this it seems probable that Dr. Butcher's "*E. pertyi*" is really *E. viridis*, as is also his "*E. scotica*", presumably described from Millport material in poor condition. The new species, *E. scotica*, cannot therefore be accepted.

Thirdly, an important error occurs in the section on *Lepocinclis*, where the reserve material is listed as "leucosin". Since the generic key states for *Lepocinclis* "2 ring-like paramylum grains always present" (itself not a true statement for all species of *Lepocinclis*), the introduction of the reserve material characteristic of the Chrysophyceae is presumably a slip of the pen, though it does occur five times.

Fourthly, descriptions of pyrenoids, particularly important in the classification of species of *Euglena* and *Trachelomonas*, are incorrect. The description of the double-sheathed pyrenoids typical of the Catilliferae group of the genus *Euglena* is inadequate, and there is nothing in Dr. Butcher's description to indicate that this pyrenoid structure occurs also in *Trachelomonas hispida*. The pyrenoids of *T. volvocina* are described as simple when they have, in fact, the taxonomically important structure referred to by Prof. Pringsheim as "inner pyrenoids", and *T. zorensis* is stated to have "pyrenoid absent" whereas it has good double-sheathed pyrenoids. The *Trachelomonas* species listed could certainly not be identified from the key and descriptions given.

Finally, the term "plastidome", strictly meaning the entire chromatophore complex of a cell, is wrongly used for the paramylum centres ("Paramylonherde" of Mainx) in *Eutreptia*, *Euglena viridis* and "*E. schmitzii*" (= *E. geniculata*).

The illustrations consist of a plate of paintings of cells and two plates of poor photographs which show nothing apart from the size and shape of the organisms. Surely what is needed in a taxonomic work provided with keys is a series of large drawings which can be aids to identification. The final photograph, purporting to show mastigonemes on flagella of *Eutreptia pertyi*, is unintelligible.

One appreciates the work involved in producing this taxonomic account of marine and brackish Euglenineae, and its major errors are listed above in the hope that future pamphlets in the series will achieve a higher standard of biological accuracy, especially since they are appearing as official publications of H.M. Stationery Office and will be read with this in mind by marine biologists throughout the world.

GORDON F. LEEDALE

THE ISRAEL SOUTH RED SEA EXPEDITION

By O. H. OREN*

Director, Sea Fisheries Research Station, Haifa, and Deputy Leader of the Expedition

MANY of the waters covering the globe have been extensively explored. One of the less explored and underexploited is the Indian Ocean. This fact and the desire to continue international and co-operative efforts and achievements during the International Geophysical Year led the United Nations to propose an International Indian Ocean Expedition. Many nations accepted the proposal, formed special committees and submitted national programmes for the exploration of this Ocean.

The Red Sea was not specifically included in the original programme. At a meeting of the Special Committee on Oceanic Research held in Copenhagen in summer 1960, the Israel representatives requested the inclusion of the Red Sea, as a part of the Indian Ocean, into the framework of the International Indian Ocean Expedition. This proposal was accepted provided that a National Programme for Red Sea exploration be submitted to the Special Committee on Oceanic Research.

The reason for Israel's request was based on its more than ten years experience in marine biological and fisheries research in the northern Red Sea. Israel has explored its 12-km. coast of the Gulf of Eilat (Gulf of Aqaba). During 1956–57, two parties of marine biologists had the opportunity of exploring the entire western coast of the Gulf of Eilat, Tiran and Sanaphir Islands, and around the Sinai Peninsula to Tor.

During 1957–58 exploratory fishing, leading to commercial fishery in recent years, was conducted from Massawa (Eritrea) in the north and south Massawa Channels and in the vicinity of Kamaran Island by an Israeli fisheries research vessel and scientific personnel. Observations were made and vast material collected, part of which was examined and the results published in a special series entitled "Contributions to the Knowledge of the Red Sea" in the *Bulletin of the Sea Fisheries Research Station, Haifa*.

Considerable interest in the exploration of the Red Sea was also shown by foreign scientists. By the time the Israel South Red Sea Expedition 1962 materialized, the original team of nine Israeli scientists, led by Prof. H. Steinitz, was reinforced by four American, two Dutch and one Ethiopian specialists. The leader of the American team was Dr. E. Clark, and that of the Dutch team, Dr. L. B. Holthuis.

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* Compiled from notes by the following members of the Expedition: A. Abulafia, A. Ben-Tuvia, E. Clark, L. Fishelsohn, T. F. Goreau, L. B. Holthuis, Ch. Lewinsohn, Y. Nir, O. H. Oren, G. F. Papenfuss, F. D. Por, A. S. Rogers, H. Steinitz, J. H. Stock and S. A. Wainwright.

The members met in Massawa on March 5, 1962, and the Expedition set its camp on Entedebir Island on March 9 to explore this island and its vicinity during five weeks of intensive field work. After a short interval one group of the expeditionary team continued field work in the mangrove swamps of Melita Bay while the other turned inland to make hydrobiological and geological investigations of various freshwater bodies.

The organization of the Expedition was made possible by grants and assistance of the Government of Israel, the Hebrew and Tel Aviv Universities, the U.S. National Science Foundation and the U.S. Office of Naval Research and the Netherlands Organization for Pure Scientific Research.

The Expedition thanks the Imperial Ethiopian Government, and especially the Imperial Ethiopian Navy, its deputy commander, Commander Alexander Desta, for his personal and keen interest in the work of the Expedition; to the naval officer in charge, Captain S. Braathen, who put the naval vessels at the disposition of the Expedition for the preliminary survey of the working area, for carrying the team and equipment to the camping place, for a cruise to Harmil and Romia Islands, for several days of stay in Zula Bay and for the frequent visits of the cutters to the camp site supplying water and provisions to the camp on Entedebir Island.

Physical Features of the Area

Entedebir, an island of an area of 1·5 km.^{2}, is situated 50 km. east of Massawa on the western approach to the island Dahlak Kebir. It is a coral island of irregular shape built mainly of fossil coral reefs having a general east–west trend with steep cliffs on the northern and southern sides. The main rock of these ridges is a rich coralline limestone with a great number of other fossils, most of them in very good condition.

Recent sediments, mainly calcareous white sands, fill the valleys of the island and its five bays. The island was formed probably during the Pleistocene and was uplifted with the entire Dankalic uplift, still taking place. This can be seen all over the archipelago up to Farasan Island off the coast of Yemen.

The submarine investigations included sampling of representative bottom sediments, and sonar sections showing a variety of features to be interpreted at a later stage.

The climate of Entedebir during the stay of the Expedition (March 9–April 9) was more pleasant than that of the nearby coastal town of Massawa, having lower relative humidity, lower air temperature, northern winds starting at noon time, relieving the midday heat, and cool and breezy nights.

The sea surrounding Entedebir and vicinity is already characterized in the early spring by temperatures as high as 29° C. while in the shallow bays they reached more than 37° C. At a depth of 125 m., north-east of Entedebir, the temperature measured was nearly 27° C. The salinities are higher in the enclosed and semi-enclosed basins, delimited by islands, sills and channels, than those in the open Red Sea. A typical enclosed basin is Ghubbet Mus Nefit, enclosed by Dahlak Kebir and Nocra, and connected with the open Red Sea by two shallow channels. The deepest part of this Ghubbet is depleted of oxygen. This is not the case in the semi-enclosed basin north of Entedebir, which is connected with the open sea over a sill 29 fathoms deep on its north-west side. Both these basins were investigated.

Marine Plants

Entedebir proved to be rather poor in algal growth except for encrusting corallines, the brown algae *Pocockiella*, *Padina*, *Dictyota*, *Sargassum*, *Turbinaria* and *Cystoseira*, and the green alga *Valonia*. *Padina* especially forms extensive growth in quiet bays.

Romia, a mangrove island at the north-east extreme of the Dahlak Archipelago west of Harmil, has a typically tropical dense upper sub-littoral and littoral algal flora with the genera *Boergesenia*, *Turbinaria* and *Hydroclathrus* especially abundant.

The usually ubiquitous *Codium* was found only in one restricted locality at Cundabilu, a small island west-north-west of Entedebir. *Galaxaura* was represented sparsely by only one species, and *Liagora* was unexpectedly absent.

Caloglossa, a genus thought to be absent from the Red Sea, was found on the aerial roots of the mangrove trees of Melita Bay.

The area was found to be poor in marine angiosperms, except at Harmil.

About a half of some 200 species of algae known from the Red Sea are at present in the Expedition's herbarium.

Birds

In the area investigated by the Expedition's ornithologist complete records were made of the birds of Entedebir: those having chosen the island as their domicile as well as those visiting and/or passing it. Naturally, those birds living in close relation with the sea were most closely investigated. Observations and collections made on Dahlak Island and on a number of smaller islands of the Dahlak Archipelago supplied important additional material.

Since biological production was very much in the mind of the Expedition, observations regarding feeding and food were carried out regularly: time of feeding and preference for feeding sites were recorded; stomach contents were analysed in order to find out the role of sea birds in the production cycles of this part of the Red Sea. Breeding season, maturity and nesting habits were studied as closely as possible. Salt glands and kidneys of various birds were preserved for microscopic examination at a later stage.

Among the birds observed and collected were pelicans, various gulls and terns, egrets and herons (including the Goliath heron), tropic bird (*Phaethon*), sooty booby (*Sula leucogaster*), stork (*Sphenorhynchus*), spoonbills, crab plover (*Dromas*), and the osprey (*Pandion*).

Internal and external bird parasites were collected for detailed examination later on.

Fish

More than 280 species of fishes, including new species and new records for the Red Sea were collected by 'Rotenone' poisoning, beach seining, trammel net, trolling lines, or obtained from commercial fishermen. Collecting methods were directed towards obtaining larger samples of certain systematic groups for speciation studies. Elasmobranchs, syngnathiform and apodal fishes were collected for biological population studies. In addition, special attention was given to Erythrean species of the genera *Hemiramphus*, *Caranx*, *Siganus*, *Atherina* and *Sphyraena*. The circumtropical *Auxis thazard* will be studied to see whether the Red Sea population is a taxonomic entity by itself.

An attempt to determine the composition of the fish fauna in a specific area covering 2,250 m.^{2} of coral, sand and rock bottom resulted in an estimated population of at least several thousand individuals of more than 100 species.

Species and individual numbers of blennioid and gobioid fishes, scarids, labrids, pomacentrids and apogonid fishes were found to be abundant in the vicinity of Entedebir whereas chætodontids, plectognaths and certain scorpaenid fishes (*Pterois*), and large sharks were poorly represented. The spectacular large forms commonly encountered in the area were *Manta* rays, *Chanos chanos* and *Bolbometopon muricatus*.

Decapods

The decapod and stomatopod crustaceans of the area were closely examined and collected extensively in all accessible habitats, 'Rotenone' poisoning being one of the more important collecting methods. Despite the relatively poor coral fauna a fair number of shrimps, crabs and stomatopods were obtained from corals, care being taken to record species associations. In colonies of coral *Galaxea* two species of shrimps were found which seem to be restricted to this habitat, one characterized by extreme lateral flattening, the other by marked dorso-ventral flattening. Commensal Pontoniinae were found associated with sponges, ascidians, molluscs and starfish. The special faunas of the rocky cover above the waterline, the sand and sandy mud areas were extensively studied by ecologists of the Expedition. Among such algae as *Cystoseira* many interesting Majid crabs were found. Seining in eel grass and algae provided unusual forms; in this way the only Crangonids were obtained.

A most interesting shrimp belonging to the genus *Antecaridina* was found in a land-locked salt-water pool with tidal fluctuations. This genus was previously known only from a similar habitat in Polynesia and near Madagascar.

Colour patterns of decapods and stomatopods were especially noted because it was recognized that natural colours could be used for field determination without resorting to dissections. Many decapods from the Red Sea were described by Forskal in 1775. Forskal's descriptions, apart for the account of the colour, often provided very few morphological details. For this reason some of his species, hitherto unidentified could now with the help of the life colours be determined with certainty.

Copepoda

As very little is known about the meiobenthos of the Red Sea, the extensive collections made by the Expedition are expected to add significantly to the knowledge of the meiobenthic animals. Various biotopes were examined, the algal growth on the corals in shallow water and the muddy bottom of the mangrove swamps were rather poor in species although the fauna of the mangrove mud was composed of *Enhydrosoma* of the Harpacticoida as well as of interesting Amphipoda, Ostracoda and Tanaidacea. The fauna of some level bottoms is, on the contrary, surprisingly rich both quantitatively and qualitatively. This is especially the case in the coralline gravels at depths of 5–16 m., which are also remarkable for the abundance of lancelets. Deep muds from 50 m. downwards, again, are poor. The harpacticoid characterizing the deepest inhabited muds, down to some 100 m., is the genus *Typhlamphiascus* found at similar depths in all the other areas studied. From 100 m. downwards in the deep basins of the Dahlak Archipelago life progressively disappears with oxygen decreasing and hydrogen sulphide content rising.

Very little systematic research of associated copepods was previously carried out in the Red Sea region. At attempt was now made to collect as many hosts as possible. To this end more than 300 potential hosts were thoroughly examined; about 50 per cent of them proved to be infested. Associates were mostly found on invertebrates although several fish parasites were also obtained. A considerable part of the material presumably belongs to new species and/or genera, or will represent new host and distribution records. Especially striking was the abundance of ascidicolous copepods (formerly called Notodelphoida) in the region covered.

Corals

In the area investigated by the Expedition corals occur in patches, not in structural reefs. The coral patches usually have a dominant substrate-forming genus. They occur in water from sub-tidal limit in a broad area surrounding every island visited by the Expedition (depths beyond 7 m. could not be explored). About half the coral species known from the Red Sea have been found: almost all on Entedebir, which is by far the richest site for number of coral species seen. The results proved to be very significant for the geographical distribution of several common Indo-Pacific coral genera.

Cirripedia associated with corals are abundant, and occur in 90 per cent of the coral genera collected.

The fossil coral reefs show every sign of having been very rich indeed.

Physiological Work

Physiological relationship of the calcification process and of the photosynthesis was investigated by calcium-45 and carbon-14 methods in hermatypic scleractinia (9 species of 6 genera), hermatypic algae, (7 species of 6 genera), a hermatypic hydrocoral, a foraminifer, and two species of *Tridacna*. These experiments provided the first opportunity to extend to reef-building organisms of the Indo-Pacific region, physiological observations of a type that hitherto were carried out only on calcareous biota of west Atlantic reefs. Of the species investigated in the Red Sea three species of algae have also been studied in the Caribbean.

The abundance of young *Tridacna* in the immediate neighbourhood of the Expedition's camp at Entedebir provided an unusual opportunity to investigate, by means of isotope labelling technique, the turn-over rate and metabolic pathways of the photosynthetically fixed carbon in this interesting organism.

Similar studies were also carried out on the small scleractinia coral *Fungia*. Both these organisms contain large numbers of zooxanthellæ. It was of interest to compare *Tridacna* and *Fungia* since the former appears to utilize its symbiotic algae as food whereas the latter does not appear to do so.

Intertidal Ecology

Three different intertidal habitats were investigated for littoral biotic associations, two of them situated conveniently for study on Entedebir, but also represented on other islands of the Dahlak Archipelago. The habitat consisting of almost vertical surfaces or rocky shore was very typical, and most widely distributed. Erosion of the intertidal surface of the rocks formed deeply undercut cliffs and mushroom-like formations scattered around the islands. The second habitat was the gradually sloping bottom of sandy bays. These two biotopes showed different communities of algae and animals. Boring cirripeds and molluscs were prominent on vertical rock surfaces, along with small amounts of filamentous algae, and blennies were the principal fishes. Contrary to this, in the shallow sandy bays are found large communities of various decapod crustaceans, digging holothurians and various gobies.

The third intertidal habitat investigated was the monospecific mangrove of Melita Bay (Gulf of Zula). In the area covered by the mangrove two distinct zones were noted; an outer zone of mangrove trees facing the sea, and an inner one facing a swampy lagoon, the two separated from one another by a piled-up sand barrier. This barrier was narrow enough to allow for the tidal oscillations of the lagoon to coincide with those of the open bay. A marked difference of bottom structure was observed on the two fronts, that of the bay front being much more sandy with a certain amount of mud appearing more in the sub-surface layers of the bottom. The bottom of the lagoon front was made up by a deep layer of mud in which decomposing organic substances were predominant. The lagoon front had a population of animals much denser than that of the bay front. Among the various animals, digging crustaceans and polychætes were outstanding. *Periophtalmus* and their nests were also very abundant.

In these investigations measurements were made of exposure times during the tidal cycle; diurnal temperature fluctuations were recorded, bottom samples were taken from various layers, and attention was given to movements of animals during tidal oscillations. Some of these animals, such as large chitons, were marked so that their movements could be analysed.

Limnological Investigations

The limnological party of the Expedition consisted of five members who covered about 2,000 km. on their inland trip. The results of their work have still to be evaluated.

Biological samples were taken at the hot springs of Ailet. about 50 km. west of Massawa.

Collections were made by the group from the artificial lake Balaza, 10 km. north of Asmara, and at the nearby spring of Mai-Mariam. While proceeding to Gondar, the party collected in the Mareb Valley and River Takazze, a rather large tributary of Atbara River (Nile system). At the mountain pass of Walkefit, some 200 km. north of Gondar, springs and small waterfall were investigated.

Of Lake Tana both the northern shore (near Gorgora) and the southern one (near Bahar Dar) were sampled. No proper pelagic plankton could be taken, but both plankton and benthos were collected in the littoral zone. The small River Arno, an eastern affluent of Lake Tana, provided fairly rich samples. Proceeding southward the party investigated several localities on the Blue Nile: (1) near the outflow from Lake Tana; (2) above the small falls; (3) below the latter falls. Lack of time prevented a study of the large falls of the Blue Nile.

Some geological samples were taken in the Lake Tana region, mainly for the examination of pollen and heavy minerals. These samples will be compared with sediments from the Lower Nile Valley, and from the Eastern Mediterranean Sea close to the Nile Delta.

Conclusions

The presence of a number of experts of certain animal and plant groups among the members of the Expedition naturally resulted in more complete information and more extensive collection in their respective fields than in others. This lack of balance has, however, certain advantages. A short-termed expedition of this kind cannot be expected to give a comprehensive, well-balanced and at the same time much-detailed picture of the nature of the area visited. But specialization will certainly pay in that it will enable us, after collections have been worked up, to draw conclusions as to the fish life, to the decapod fauna, to the occurrence on algae, the distribution of corals and whatever additional groups have been particularly investigated. Thus, an important contribution can be made to the knowledge of the region. Faunistic and floristic features will be co-ordinated with hydrographic observations, and also in part with geological results, to render sound background and to strengthen the conclusions reached. Moreover, all this can and, indeed, will be made a stepping-stone towards a scientific comparison of this southern part of the Red Sea with the northern one and particularly with the Gulf of Eilat, which has been made an objective of extensive exploration over several years in the past.

ION WAVES IN A BOUNDED PLASMA

By P. F. LITTLE

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EXPERIMENTS have been carried out on the transmission of low-frequency ion waves in the direction of the electron drift along a mercury arc column[1]. Measurements were made with and without an axial magnetic field. The waves were excited by compressing the plasma over a small region with the magnetic field of a short exciter coil wound round the column, or by modulating the ionization-rate locally with the electric field between a pair of grids across the column. The wave-length was observed to

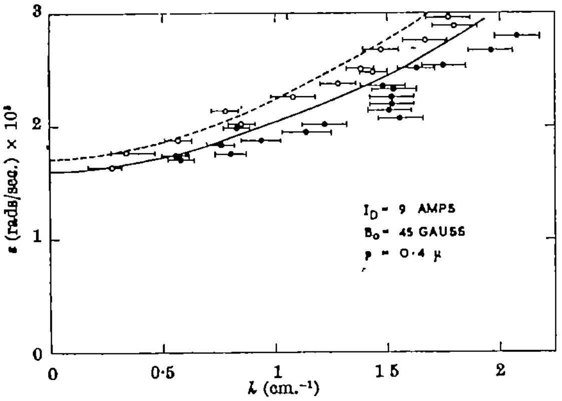

Fig. 1. Observed values of the wave-number $k = 2\pi/\lambda$ for various propagating frequencies ω excited by a short external coil in two different experiments with discharges carrying 9 amp. in a magnetic field of 45 gauss. The theoretical curves are explained in the text

increase strongly as the frequency was reduced, and below a critical frequency no waves appeared. Near this lower limit the phase velocity is greater than the electroacoustic wave velocity in an infinite medium. This is of interest in considering the growth of instabilities when currents flow in a plasma.

The arc was maintained between a mercury pool cathode and a water-cooled anode in a 5-cm. diameter glass tube about 1 m. long. In a fairly narrow range of vapour pressures between 0·1 and 1μ, ion waves could be propagated toward the anode. Under the same discharge and magnetic field conditions there was some weak, irreproducible propagation of cathode-directed waves, critically dependent on the axial position of the exciter coil. The modulation in density was detected by a photomultiplier, the light emission in such a discharge being proportional to the local electron density.

To achieve a tolerable signal-to-noise ratio it was essential to use a phase-sensitive detector[2], which compares the photomultiplier signal v_0 with a reference voltage wave from the c.w. oscillator feeding the exciter. The output from the detector is proportional to $v_0 \cos \varphi$, where φ is the phase difference between the signal and the reference wave-form. A chart recorder displayed the detector output. The photomultiplier assembly was moved steadily along the discharge tube by a synchronous motor, so that the chart record displayed directly changes in amplitude and phase along the column. At the longest wave-length only half a cycle could be traced with certainty, and at the shortest about thirty complete cycles were observed.

Langmuir probe measurements at one point in the tube gave direct confirmation that density modulation was indeed occurring.

Dispersion plots are shown in Fig. 1 for the discharge in a magnetic field, using average wave-lengths measured over about 30 cm. of the column. Two different experiments are shown by the circles and dots. Apparently the discharge conditions were not identical, possibly because in the interval between these measurements (about two months) some contamination had occurred. Below $\omega_0 = 1{\cdot}6 \times 10^5$ rads/sec. no waves propagated, although modulation of the light at the coil still occurred. The theoretical curves are discussed here.

In Fig. 2 the results in zero axial magnetic field are given. The circles represent measurements with a coil exciter, the triangles show results from the grid modulation. The signals were weaker without the axial field and at low frequencies heavily damped, making errors larger there, but both types of excitation give points on a common curve. The approach to a cut-off frequency is not so clear as before. Propagation ceased below 10^5 rads/sec., but the scatter of the points above this frequency is large. It seems possible that with the coil exciter two different modes of propagation can occur, and that these modes have different lower limits for propagation.

From the two-fluid treatment of waves in a plasma[3] such as that examined in these experiments the dispersion relation is:

$$\omega^2 = (k^2 + k_c^2)V_s^2 \tag{1}$$

where

$$V_s^2 = \varkappa T_e/m_i \tag{2}$$

is the electroacoustic velocity predicted by Tonks and Langmuir[4]. For the arc $T_e \sim 3$ eV. and $V_s \sim 10^5$ cm./sec. This treatment holds if $T_i \ll T_e$, $V_s \ll V_a$ the Alfven speed, and if $\omega \gg \Omega_i$, the ion cyclotron frequency. The radial dependence of the density modulation is of the form $J_m(k_c r)$.

For the lowest mode with a pressure node at the plasma boundary[5] R, we have $k_cR = 2{\cdot}40$. Langmuir probe measurements in the discharge conditions of Fig. 1 gave $T_e = 5{\cdot}3 \times 10^4$ ° K., which from equation (2) fixes $V_s = 1{\cdot}5 \times 10^5$ cm./sec. The radius R measured photographically is $2{\cdot}1 \pm 0{\cdot}2$ cm. From equation (1) then the lower bound for:

$$\omega_0 = k_0 V_s$$

is $1{\cdot}7 \times 10^5$ rads/sec., in good agreement with the measurements.

The broken line in Fig. 1 follows from equation (1) with these constants when the drift of the centre of mass of the plasma (6×10^3 cm./sec. approximately, equal to the ion drift velocity) is taken into account. The theoretical dispersion curve would fit better to the experimental points if R were taken as 2·0 cm. and T_e as $4{\cdot}2 \times 10^4$ ° K., 20 per cent lower than the probe temperature. This yields the full line shown.

In Fig. 2, the two lowest modes for $m = 0$ with $k_0R = 2{\cdot}40$ and 5·52 are shown by the broken line, using the tube radius 2·5 cm. as the boundary and $T_e = 2{\cdot}4 \times 10^4$ ° K. from probe characteristics taken in a separate experiment. Again the effect of the plasma's motion has been taken into account. The predicted cut-off frequencies and phase velocities

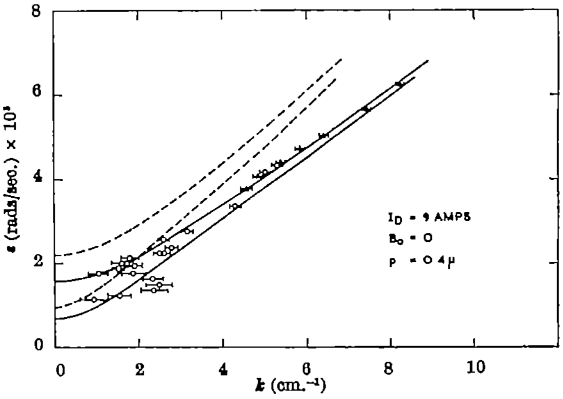

Fig 2. Observed values of the wave-number k for various frequencies ω in discharges carrying 9 amp. in zero magnetic field. The circles show results using a coil exciter; the triangles those obtained with a pair of grids across the column. The theoretical curves are explained in the text

are too high. The electron temperature must be reduced by 40 per cent and the radius R taken as 2·8 cm. to obtain the full-line curves, which give a good fit between experiments and the theoretical dispersion curve for the second mode. The predicted dispersion curve near ω_0 for the lowest mode is in rough agreement with the experimental points, which are subject to large errors in this frequency range because of the strong damping.

The agreement between observed and calculated values of ω_0 and between the electron temperatures measured by the probe and from the dispersion curve when a small axial magnetic field is applied is enough to establish that these are indeed ion waves of higher phase velocities than in an infinite medium. In zero axial field the experimental errors are larger but there is some indication that such waves exist. They may be regarded as the sum of waves moving at an angle to the column and reflected at the boundaries to form a waveguide mode. The existence of this branch of the dispersion relation reduces the maximum electron drift velocity permissible in a plasma where $T_i \sim T_e$ to about ten times the ion thermal velocity if growing waves are not to arise[6].

In my experiments $T_i \ll T_e$ and the electron drift velocity always exceeds the limit for stability so that growing waves would arise in the absence of damping by collisions and by losses to the wall[7]. Since only damped waves propagate with the electrons, the damping must predominate at the frequencies used here. This is consistent with the irreproducible and heavily damped character of cathode-directed waves.

I thank Dr. R. J. Bickerton for much helpful discussion and also Mr. B. E. Avis and Mr. H. G. Jones for their assistance in the experiment.

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PARTICIPATION OF DEOXYRIBONUCLEIC ACID IN THE MULTIPLICATION OF INFLUENZA VIRUS

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THIS article is concerned with the effects of actinomycin *D*, an agent which blocks the function of cellular DNA, on the multiplication of two myxoviruses, influenza and Newcastle disease virus.

These viruses are structurally similar, but not identical, RNA viruses[1,2]. Fluorescent antibody investigations indicate that the RNA-containing component, the soluble antigen of influenza or of the closely related fowl plague virus, is produced in the nucleus of infected cells[3–5]. With cells infected with Newcastle disease virus, however, specific fluorescence can only be demonstrated in the cytoplasm; the nucleus does not appear to contain any virus specific antigen[6]. The importance of the nucleus in influenza multiplication suggests that nuclear DNA may play a part in the production of this virus.

The possible participation of cell DNA in the production of some RNA viruses has already been the subject of several papers. Simon[7] has reported that several agents which stop DNA synthesis in cells, namely, aminopterin (4-aminopterolyglutamic acid) and 5-fluorouracil, have no effect on the multiplication of either polio or Newcastle disease virus. Also, aminopterin does not affect the production of fowl plague virus[8]. Consequently, the synthesis of new cell DNA is apparently not required in the production of either Newcastle disease or influenza viruses. Reich *et al.*[9] have shown that the antibiotic actinomycin *D* will prevent the multiplication of the DNA virus, vaccinia, in *L*-cells, but not the multiplication of Mengo, an RNA virus. This substance is known to combine *in vitro* with DNA (ref. 10), and in mammalian cells its effect is to suppress selectively cell RNA synthesis. As it does not apparently interfere with the synthesis of DNA, its mode of action seems to be to prevent the functioning of cell DNA; "to block the expression of the genetic potentialities" of the cell[9]. This agent has been used as a means of blocking cellular DNA function in the work recorded here.

Experiments to test the effect of actinomycin *D* concentration on the yield of influenza or Newcastle disease virus were performed in strips of allantoic cells cut from eleven-day-old de-embryonated eggs[11]. Yields were determined by hæmagglutination 18 hr. after infection of cells with a saturating dose of virus (about 10 ID_{50} per cell). The strains of virus used were the *PR*8 and *MEL* strains of influenza *A*, and the California 11914 strain of Newcastle disease virus. Actinomycin *D* was kindly provided by Prof. E. F. Gale, Department of Biochemistry, Cambridge.

A series of experiments was performed in which a range of concentrations (1–10 μgm./ml.) of actinomycin *D* was added to cells at the same time as virus. Incubation of uninfected allantoic cells in these concentrations of actinomycin results in similar cytological changes to those found with mammalian cells[12]. Preparations of cells stained with acridine orange indicate that there is a loss of nucleoli and an absence of much of the cytoplasmic RNA. In experiments to test the effect of actinomycin concentration, 8–10 replicate strips of cells were used for each concentration. The results of typical experiments are shown in Figs. 1 and 2, for Newcastle disease virus and influenza, respectively. Concentrations as high as 10 μgm./ml. do not affect the production of Newcastle disease virus. With influenza, however, increasing concentrations produce a progressive decrease in yield. Pre-incubation of cell strips in actinomycin for up to 2 hr. before infection does not produce any alteration in the response obtained with either virus. From these results it appears that influenza is a virus which will only multiply in cells in which DNA function is unimpaired.

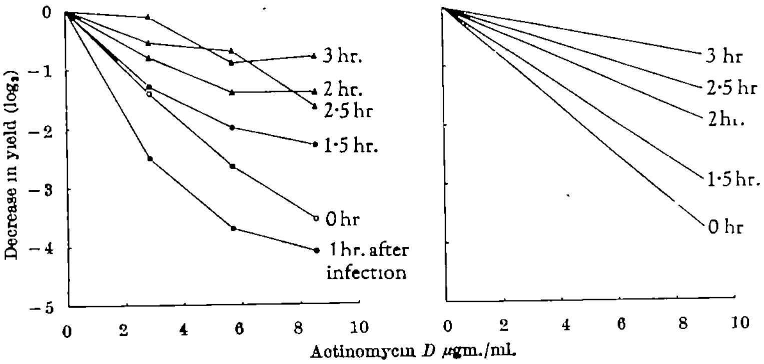

Fig. 3. Effect of time of addition of actinomycin *D* on the yield of influenza, strain *MEL*, from allantoic cells. The left-hand figure indicates the experimental values obtained. The right-hand figure is a diagrammatic representation of the effect of time of addition on virus yield

To investigate the nature of this dependence on DNA, experiments were performed in which actinomycin was added to cells at various times after infection with influenza. The results obtained are shown in Fig. 3. To ensure that virus production was synchronized in all cells, a multiplicity of about 100 ID_{50} per cell was used. Under these conditions, the degree of inhibition produced at 0 hr. was relatively less than found with smaller inputs of virus (Fig. 2). Addition of drug 1 hr. after infection produces rather more inhibition than if given earlier. From this time onwards, however, there is a gradual reduction in the extent of inhibition. This is first noticeable at 1·5 hr. and is virtually complete by 3 hr. The synthesis of virus specific RNA in fowl plague infected chick embryo cells has been shown by Scholtissek and Rott[8] to begin about 1 hr. after infection and to be near maximum at 3 hr. This is the same period of time over which the effects of actinomycin diminish in allantoic cells. Consequently, it appears that in influenza virus infected cells, actinomycin *D* is blocking production of virus specific RNA. This suggests that cell DNA is directly responsible for the production of influenza virus RNA. In the case of Newcastle disease virus, the evidence indicates that virus RNA synthesis is independent of cell DNA function.

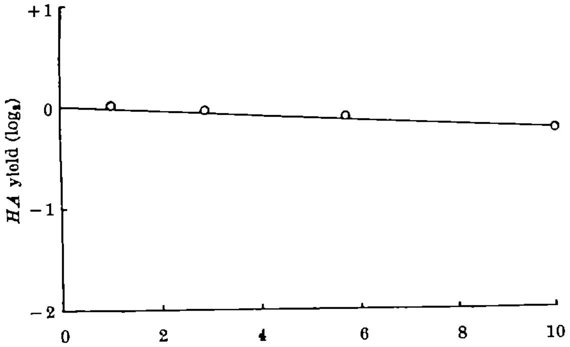

Fig. 1. Effect of increasing actinomycin *D* concentration on the yield of Newcastle disease virus from allantoic cells

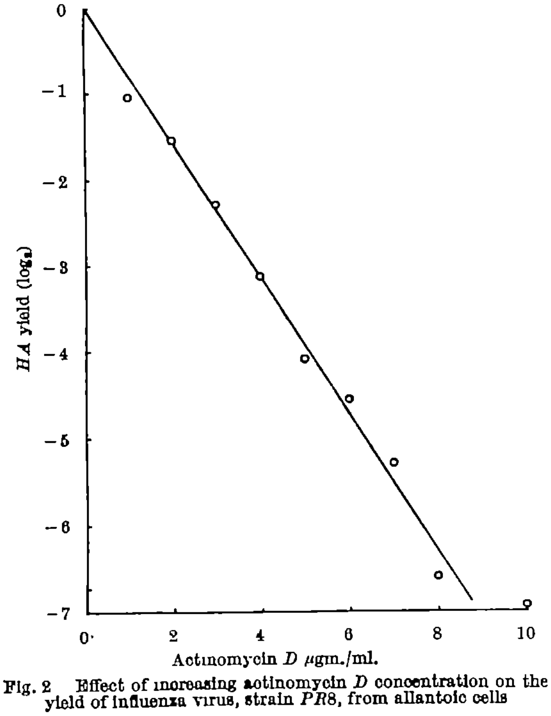

Fig. 2 Effect of increasing actinomycin *D* concentration on the yield of influenza virus, strain *PR*8, from allantoic cells

Concentrations of actinomycin as low as 0·005 μgm./ml. are capable of inhibiting the division of *L* cells, while 0·1 μgm./ml. is sufficient to prevent the production of vaccinia virus in these cells[9]. Compared with these amounts, the concentrations of actinomycin required to block influenza multiplication in allantoic cells are relatively high. On the other hand, the exponential nature of the response curve obtained (Fig. 2) suggests that a particular part only of the total cell DNA is essential in virus production. Taken together, these facts suggest that the effective amount of DNA required for influenza virus production in cells is relatively small. It is known that influenza virus, unlike other RNA viruses, undergoes a number of co-operative phenomena, such as multiplicity reactivation and incomplete virus formation[11,12]. The occurrence of these events suggests that one or a few topographical sites only are responsible for virus production. From the results presented here, it would appear that the site or sites involved are provided by a small part of the DNA of the host cell.

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PURIFICATION OF THE MOUSE MAMMARY TUMOUR VIRUS

By Dr. MICHAEL J. LYONS and Prof. DAN H. MOORE

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IN an earlier communication we discussed the purification of particles believed to represent the milk agent from mouse mammary tumours[1]. Although this procedure yielded pellets that contained areas rich in Bernhard's type *B* particles[2], they were not pure enough to permit chemical analysis nor were the bioassays very impressive. Three different preparations have been inoculated into 73 *C*57 mice. From two of the preparations no tumours have resulted, whereas from the third, where 40 mice were inoculated, only four tumours have developed. These bioassays are in agreement with viral inactivation of other viruses by repeated fluorocarbon extractions. Furthermore, when these fluorocarbon extracted pellets were re-suspended and examined in the electron microscope, either as shadowed or phosphotungstic acid treated dispersions, they revealed a high degree of heterodispersity, and definite identification of *B* particles in them has been difficult. Inasmuch as the negative contrast procedure of Brenner and Horne[3] greatly enhances the amount of detail which can be seen in viral structure, an effort has been made to secure preparations that could be examined by this method. This article describes procedures for obtaining purer and better preserved *B* particles and provides further details on their structure, although bioassay results will not be available for another year.

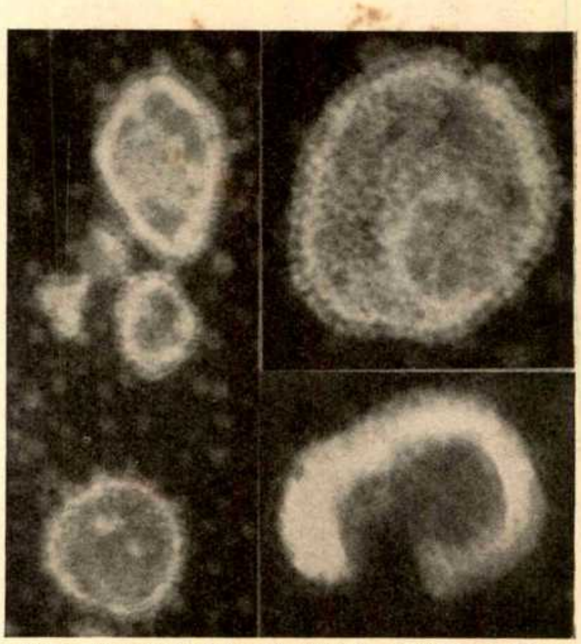

Fig. 1. Dispersions of type *B* particles separated from mouse milk (*R*III strain). The exterior surfaces of the particles are covered with radially extending spines characteristic of myxovirus. Particle at lower right has lost its internal contents by rupture. (Left, × 125,000; upper right, × 250,000; lower right, × 200,000)

Both milk and tumours from the *R*III strain of mice (mammary tumour incidence about 97 per cent) were used. In a typical experiment when milk was used about 4 ml. was centrifuged at 2,000 r.p.m. (1,000*g*) in an International refrigerated centrifuge for 1 hr. The milk, hereafter called skim milk, taken from between the cream and a small pellet was clarified by adding a few drops of 0·15 M ethylenediamine tetraacetic acid (EDTA or versene) which had been adjusted to *p*H 7·2 by the addition of sodium hydroxide. The clarified milk was then spun at 9,000 r.p.m. for 15 min. and the supernatant ultracentrifuged at 39,000 r.p.m. for 1 hr., whereupon the small pellet thus precipitated was re-suspended in a buffer 0·02 M with respect to sodium phosphate and 0·15 M with respect to sodium chloride (buffered saline). The 0·5 ml. of suspension was layered over 4½ ml. of a buffered rubidium chloride solution in the ultracentrifuge tube of a swinging bucket rotor (*SW*39). This solution contained 32 per cent w/v rubidium chloride, 8 per cent magnesium chloride and 0·05 per cent bovine plasma albumin in 0·02 M sodium citrate buffer, *p*H 6·7, and was similar to the solution used by Crawford and Crawford[4] in the isolation of infective Rous sarcoma virus. After centrifugation at 39,000 r.p.m. for 22 hr., the tube was found to contain 4 light-scattering zones. The zone containing the *B* particles lay at the middle of the tube. About 0·5 ml. containing this band was dialysed free of rubidium chloride against a 0·15 M sodium chloride solution containing 0·001 M magnesium chloride.

When tumour was needed a mouse was killed by severing the spine at the neck and the tumorous tissue immediately removed. A typical extraction was as follows: to 5 gm. of tumour was added 50 ml. of the buffered saline and 25 ml. of redistilled (b.p. 47·5°–48·5° C.) 'Freon 113' (Dupont). The mixture was homogenized at 0° C. for 1 min. in a Vertis blender running at full speed. The homogenate was quickly centrifuged in a trunnion cup rotor at 2,000 r.p.m. (1,000*g*) for 2 min. The supernatant was decanted and combined with 25 ml. of fluorocarbon and the process repeated. More *B* particles were obtained if the process was not carried to the point of having a clear supernatant. One or two fluorocarbon extractions are sufficient. The final supernatant was first centrifuged at 9,000 r.p.m. for 15 min. and then ultracentrifuged in 4 tubes of an angle head at 39,000 r.p.m. for 1 hr. The pellets were re-suspended in 1 ml. buffered saline. This opalescent solution was layered over 3 tubes of the buffered rubidium chloride solution and centrifuged at 39,000 r.p.m. in the swinging bucket rotor for 22 hr. Again the band lying at the middle of the tube was found to contain the *B* particles. In extracting *B* particles from tissue, it is important to work very fast at low tem-

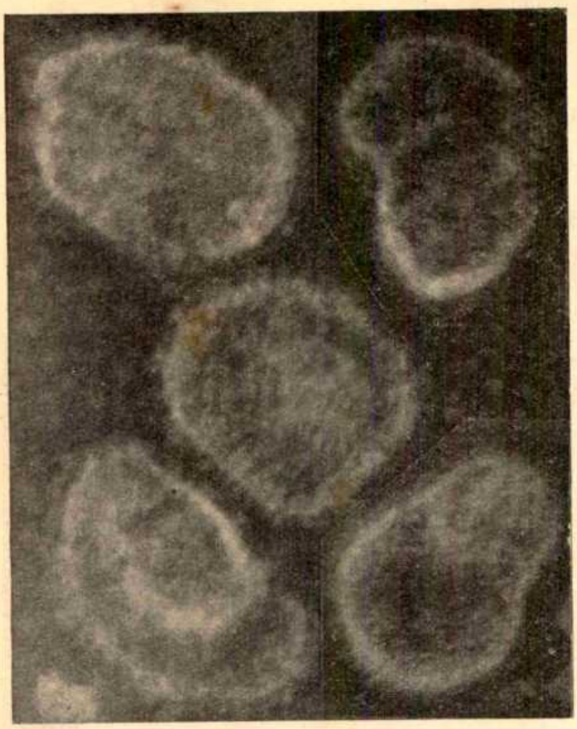

Fig. 2. Negative contrast dispersions of type *B* particles separated from tumours (*R*III strain). Eccentric 'nucleoid' areas are discernible in most of the particles. (× 200,000)

peratures because there is apparently rapid destruction of particles until they are freed of enzymes.

In the equilibrium ultracentrifugation, the density of the zone containing the *B* particles was 1·22. The density of such particles, however, may be influenced by the density of the medium if there is binding of rubidium chloride to the particles. Figs. 1 and 2 show particles, from milk and from tumours respectively, dispersed in buffered neutral phosphotungstate. They vary somewhat in size and shape but are predominantly spherical with an overall diameter of 130–160 mμ. They show two striking features: an outer region—very similar to the outer membrane of influenza virus—consisting of radially orientated spines about 10 mμ in length, somewhat shorter than those on influenza virus, and an internal structure which is quite different from influenza virus. The overall dimension is considerably greater than influenza, which ranges from 80 to 100 mμ. In osmium-fixed methacrylate embedded sections, the *B* particle measures 100–130 mμ (ref. 5), whereas influenza viral particles measure from 60 to 70 mμ (ref. 6). The outline of an eccentric nucleoid is clearly visible in many of the particles. The diameter of the nucleoid is usually a little less than half the diameter of the total virion. In other particles the nucleoid seems to consist of large granules. There is some similarity between the appearance of the nucleoid here and that of herpes simplex virus when studied by the same method[7]. There is little similarity between this *B* particle and the Rous sarcoma virus[8]. Rous sarcoma virions show a thin outer layer which is particulate but does not indicate the presence of distinct spines. Its overall diameter ranges from 70 to 100 mμ and the internal structure of the Rous virus is not as well defined.

The particles were found to disintegrate on treatment of suspensions with ether, and appeared to be resistant to degradation by trypsin, RNase and DNase.

Ultra-violet absorption analysis of dilute suspensions of *B* particles gave characteristic nucleoprotein absorption patterns. A preparation of influenza virus subjected to purification by the rubidium chloride density gradient gave a value of 1·14 for the ratio of optical densities at 260 and 280 mμ, while a *B* particle preparation gave the quotient 1·13.

Following an Ogur and Rosen[9] extraction procedure for nucleic acids, a positive orcinol test for ribose indicated the presence of RNA in the *B* particle preparation. Comparatively low quantities of DNA, representing possibly contaminants, were indicated by the diphenylamine test for deoxyribose. That the RNA was an integral part of the virus was indicated by positive ultra-violet absorption and orcinol tests on extracts obtained following incubation of suspensions for 30 min. at 27° with RNase and DNase (2 μgm. crystalline enzymes per ml.).

The presence of nucleic acid within the core of the particles was indicated by positive staining with uranyl acetate (0·5 per cent solution brought to *p*H 5·0 with 0·1 *N* sodium hydroxide.

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IMMUNOLOGICAL ENHANCEMENT OF A LEUKÆMIA

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THE growth of a tumour transplanted into a foreign host may sometimes be enhanced by treatment of the host with immune serum directed against the graft (see review by Kaliss[1]).

Much of the published work refers to sarcoma 1, a mouse ascites tumour which is remarkably susceptible to immunological enhancement. With other tumours it is not so easy to demonstrate this type of enhanced

Table 1. SUMMARY OF EXPERIMENTS ON THE GROWTH OF THE *C57BL* ASCITES LEUKÆMIA *EL4* IN FOREIGN HOSTS TREATED WITH IMMUNE SERUM

Exp.	Host strain	Serum	Titre	Dose-range tested (μl.)	No. of *EL4* cells inoculated ($\times 10^6$)	Result
A	*C3H/An*	$(C3H \times I)F_1$ anti *EL4*	1/512	20–0·02	0·6	See Fig. 1
B	$(C3H \times I)F_1$ and Swiss *Ha/ICR*	$(C3H \times I)F_1$ anti *EL4*	1/512	20–0·002	3	Inhibition at highest serum dose—otherwise no effect
C	*DBA/2*	*DBA/2* anti *EL4*	1/256	100–0·002	16	100 μl.—complete inhibition 20 μl. } 2 μl } moderate enhancement No effect with lower doses of serum
D	*C3H/An*	*C3H* anti *EL4*	1/64	20–0·002	11	No enhancement } No inhibition in this experiment
					2·8	Moderate enhancement with 20 and 2 μl. }
					0·29	No enhancement }
E	*DBA/1*	*DBA/1* anti *EL4*	1/512	100	29 70	See Fig. 2 See Fig. 2
F	*DBA/2*	*DBA/2* anti *EL4*	1/256	100 (single or repeated doses)	45	See Fig. 3

growth, and the opposite effect, suppression of tumour growth, may be seen, particularly if serum dosage is high[2]. In fact, suppression is the only recognized response of leukæmias inoculated into foreign passively immunized mice[3].

This has led to suggestions that transplanted tumours may be divided into two broad classes on the basis of their reactions to isoantisera[4-6]. Accordingly, leukæmias are regarded as highly sensitive to antibody, and for this reason, it has been supposed, they cannot be enhanced by immune sera[6]. In a separate category are the sarcomas and carcinomas which are less sensitive to antibody and are therefore susceptible as a class to the immunological enhancement effect.

This view has received support from the results of cytotoxic studies. These have shown that leukæmic cell populations are uniformly sensitive to the cytotoxic action of *H*-2 isoantibody and complement *in vitro*, whereas populations of sarcoma or carcinoma cells invariably contain a proportion of cells which are not affected under similar conditions[7-10]. Thus, some degree of resistance to antibody, both *in vivo* and *in vitro*, appeared to be a prerequisite of enhancement[3,6]. Naturally, these distinctions, if they can be maintained, favour the acceptance of a specific immune mechanism distinct from antibody formation in order to account adequately for the rejection of homografted carcinomas and sarcomas, and perhaps, by analogy, of their normal tissues of origin also.

Recently, however, we have reported that *BP*8, an ascites sarcoma which has been examined in detail in Gorer's laboratory and which can be enhanced by antibody with relative ease[2], is in fact completely sensitive to cytotoxic antibody *in vitro* provided that the test is carried out with sufficiently dilute cell suspensions[11]. Another sarcoma, a methylcholanthrene-induced fibrosarcoma recently converted to the ascites form, was also found to be completely sensitive under these conditions. Thus, *BP*8 furnishes a clear example of a tumour which can be enhanced by antibody *in vivo* despite the fact that it is completely sensitive to antibody *in vitro* under the proper conditions.

These findings led us to reconsider the possibility of obtaining immunological enhancement of highly sensitive cell populations like leukæmias. To achieve complete destruction of all sarcoma cells *in vitro*, the ratio of total antibody to the number of cells exposed must be higher, perhaps at least ten times higher in the case of *BP*8, than with a typical leukæmia. It seemed possible, therefore, that one might see immunological enhancement of a leukæmia if the ratio of total antibody administered to the number of cells inoculated were greatly reduced.

For this purpose, the ascites leukæmia *EL4* is an appropriate choice since, as a result of repeated tests in several laboratories, there is no doubt whatever as to its complete sensitivity to isoantibody *in vitro*[7,12,13]. Nor is there any doubt concerning its sensitivity to antibody *in vivo* as shown by the ease with which passive immunity can be obtained under the usual conditions of experiment without concomitant active immunization of the host[14]. It is strictly strain-specific, and has never killed an untreated mouse of any foreign strain in our laboratories.

In each of our experiments, with the exception shown in Table 1, the immune serum against *EL4* was prepared in the same strain as that to be used for the particular test. We used the conventional immunization schedule of three or four tumour inoculations over a period of six to eight weeks. Each pool of serum was titrated by the cytotoxic method[7] (against *EL4* in a cell concentration of 1–3 $\times 10^6$/ml.) before use. The titres recorded in Table 1 are 50 per cent end-points: all sera gave 100 per cent 'kill' at higher concentrations of serum.

Male hosts were used because they have been found to show enhancement more readily than females[2]. In every instance there was *H*-2 incompatibility between the host and *EL4*. The hosts were young adults of common inbred stocks from our own breeding nuclei, or, in a few instances, from commercial sources. Immune serum was diluted appropriately in saline to give a standard intraperitoneal injection volume of 0·5 ml. administered from 3 to 16 hr. before inoculation. *EL4* ascites cells were obtained from routine passage in *C57BL/6* mice. They were washed once by slow centrifugation and re-suspended in 'Medium 199'. No suspension contained more than 10 per cent dead cells according to trypan blue staining. After preliminary counting and appropriate dilution, the suspensions were inoculated subcutaneously into the shaved skin of the flank in volumes of 0·1 or 0·2 ml. by means of a tuberculin syringe and a No. 27 needle. The size attained by the grafts has been expressed as the average of two caliper measurements (mm.) at right angles to each other.

The ratio of antibody to the number of cells in the inoculum can be lowered either by reducing the amount of antiserum given or by increasing the size of the cell inoculum. As the summary of our results shows (Table 1) both methods can produce enhancement; but the latter gives more striking effects and serum treatment may then cause such massive growths that the hosts die (Exps. *E* and *F*, Figs. 2 and 3). With a small inoculum of *EL4*, enhancement can be obtained only with minute doses of serum. In Exp. *A* (Fig. 1), with a small inoculum of 6 × 10^5 cells, enhancement was seen with a serum dose of 0·02 µl.; higher doses gave increasing inhibition and apparently complete suppression with 20 µl.

Exp. *F* (Fig. 3) shows that continuous administration of serum over an extended period may be more effective than the usual single injection given before inoculation. It will be interesting to determine whether or not this manœuvre will enable us to secure enhancement of tumours in strains which normally do not show the effect easily. In this respect, our experience emphasizes once again the importance of the host strain in enhancement experiments[15]. Perhaps sufficient attention has not been paid in this connexion to the matter of genetically determined differences in the immune capacity of various strains. Since rejection, or failure to reject, must ultimately depend on this, it seems quite probable that the root of strains differences in the matter of enhancement may lie here. It is worth noting here that in general females respond better than males to certain immune stimuli[16-18] and that they are less favourable hosts for enhancement experiments[2]. *DBA*/2 and *C3H*, which in our hands gave good enhancement, respond poorly to immunization with heterologous red cells[19]. *DBA*/1 also gives good enhancement and is said to be peculiarly receptive to certain foreign tumours[20].

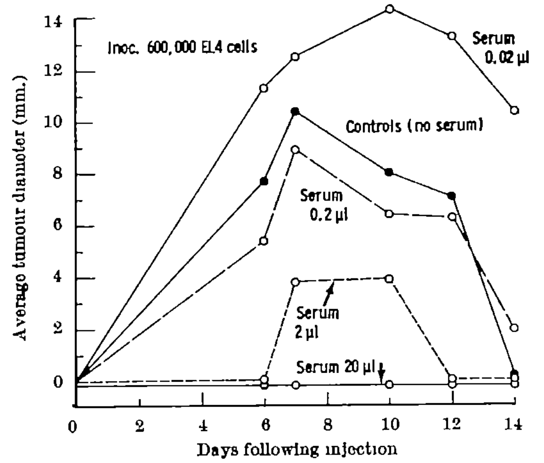

Fig. 1 Growth patterns of *EL4* inoculated subcutaneously into *C3H/An* hosts treated with various doses of immune serum (see Exp. *A*, Table 1): average readings for groups of 4 mice

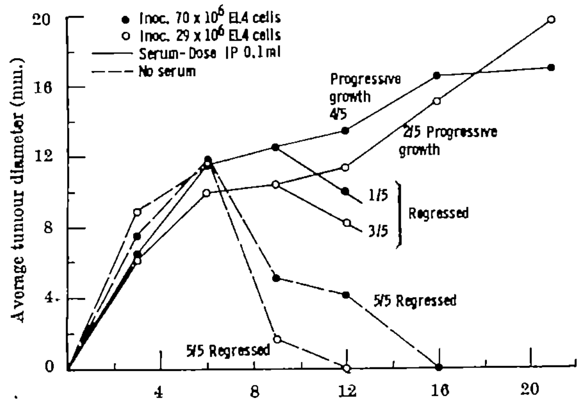

Fig. 2. Growth patterns of large subcutaneous inocula of *EL4* in *DBA*/1 hosts treated with immune serum 'Progressive growth' means that these hosts died with massive enlarging tumours (see Exp. *E*, Table 1)

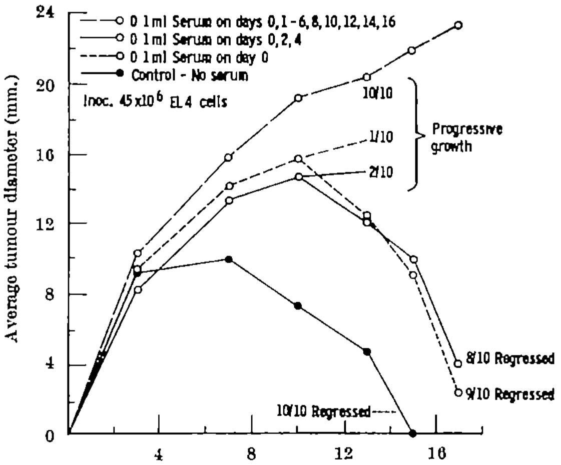

Fig. 3 Growth patterns of *EL4* inoculated subcutaneously into *DBA*/2 hosts treated either once or repeatedly with immune serum

In conclusion, it is clear that at least some sarcomas and leukæmias may not differ significantly in their reactions to isoantibody either *in vitro* or *in vivo* when a sufficiently large range of cell and serum doses is tested. The phenomenon of immunological enhancement therefore cannot be attributed to any inherent quality of resistance to antibody possessed by particular cell populations since one of the most sensitive types, *EL4*, can be enhanced by immune serum, given the proper conditions. Consequently, there is perhaps less need now than formerly to resort to theories of rejection which demand widely divergent immune mechanisms for tumour homografts of various types.

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DOSIMETRY OF CÆSIUM-137 IN SHEEP

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CÆSIUM-137 is considered one of the most significant constituents of world-wide fall-out because of a long half-life, energetic β- and γ-components and entry into the metabolic processes of plants and animals. Since it resembles potassium chemically, it is distributed quite uniformly in the animal body[1]. For the radionuclides which are rather uniformly distributed in body tissues, the National Committee on Radiation Protection and Measurements[2] has recommended the same occupational exposure limit that applies for external X-ray and γ-sources of radiation, that is, 5 rems per year. In such cases, the whole body is designated as the critical organ.

The estimation of radiation dose to an individual tissue or organ from cæsium-137 is complicated. This isotope emits 0·52 or 1·18 MeV. β-rays. Its daughter, metastable barium-137, emits an 0·66 MeV. γ-ray. For the most part, the β-rays are absorbed within the tissue in which they were emitted. The γ-rays, however, penetrate considerable distances so the dose they produce depends both on the concentration in and the position of other organs around the point in question. The relationship between radiation dose to whole body and gonads from internally deposited cæsium-137 was determined by dose measurements on young male sheep which were fed cæsium-137 (orally) for up to 20 days.

Three male sheep were confined in metabolism cages and fed a normal ration of alfalfa hay. With the morning meal each day for, 5, 10 or 20 days, the rams received a small feed pellet spiked with 1·25, 0·75 or 0·5 mc. of cæsium-137, respectively.

24 hr. after administration of the last dose, the animals were anæsthetized with a sodium pentobarbital solution. The body burdens were then measured by using either model 208 'Victoreen' stray radiation chambers or a scintillation counter with a 2 in. × 3 in. sodium iodide crystal. To compensate for differences in the distribution of cæsium-137 within the animals, measurements were taken with the detectors at various locations relative to the animals, and at least 1 m. from their bodies. Calibration for both methods was done by counting a 'standard' animal of similar size. The 'standard' had been prepared by intravenous injection of 5·5 mc. of cæsium-137 into an anæsthetized animal, which was killed approximately 2 hr. later. This delay before death allowed adequate time for the cæsium-137 to distribute throughout the body, yet not be excreted.

Immediately after whole-body burdens were determined, a number of 'Victoreen' pocket dosimeters were surgically inserted in and/or adjacent to the major organs *in situ*. Measurements were made in the testes after removal from the body and in the scrotum after removal of the testes. The dosimeters were encased in a condom to exclude moisture. The measurements were taken using a special pocket dosimeter reader described by Roesch *et al.*[3]. All readings were within ± 5 per cent and corrected for background radiation. These measurements were considered to represent only γ-radiation since work by Hubbell *et al.*[4] has shown this type of ion chamber to be relatively ineffective in measuring β-radiation in the range of energy emitted by cæsium-137. These measurements are given in Table 1.

Table 1. GAMMA- AND BETA-DOSIMETRY OF DAILY ADMINISTERED CÆSIUM-137 IN THREE RAMS

(Expressed as mrads/hr./mc. in body tissues and gastrointestinal tract at death)

	^{137}Cs regimen					
	1·25 mc./day (5 days)		0·75 mc./day (10 days)		0·50 mc./day (20 days)	
	Radiation					
	γ	$\beta+\gamma$	γ	$\beta+\gamma$	γ	$\beta+\gamma$
Total body	10	17	10	17	10	17
Testes (intact)	9	18	6	19	8	24
Testes (excised)	5	14	3	16	3	19
Scrotum (testes removed)	3		4		5	
Kidney	13	34	12	32	15	34
Kidney surface*	8		5		3	
Liver	18	29	16	27	10	21
Thigh muscle	7	14	8	19	11	26
Brain	6	8	9	12	11	17
Spleen surface	8	21	7	20		
Inside rectum	7	60	5	32	7	38
Inside rumen	6	14	6	9	12	
Body burdens as per cent of daily dose		240		330		280
Per cent of body burden in gastrointestinal tract	40–50		10–20		0–10	

* Approximate location of ovary in female animal.

The animals were killed with an overdose of anæsthetic and the tissue samples were obtained. Samples for radioanalysis were wet-ashed in nitric acid and duplicate aliquots were measured for cæsium-137 concentration using a well-type γ-scintillation counter with a 3-in. sodium iodide crystal. Values obtained are given in Table 2. β-Contribution to total dose was calculated from these values using a factor of 13 mrads/day per μc./kgm. of tissue. This value was estimated from a monogram for β- and γ-dosimetry of internally deposited radioisotopes[5].

Table 2. CONCENTRATION OF CÆSIUM-137 IN TISSUES OF MALE SHEEP

(Expressed as μc./gm. $\times \frac{\text{body-weight (gm.)}}{\text{daily dose } (\mu\text{c.})} \times 100$)

	^{137}Cs regimen		
	1·25 mc./day (5 days)	0·75 mc./day (10 days)	0·50 mc./day (20 days)
Muscle	200	490	550
Heart	470	600	560
Femur and rib shaft	38	44	110
Femur head	40	62	50
Rib end	120	170	100
Liver	500	520	420
Spleen	390	560	360
Kidney	640	900	720
Testis	280	610	610
Blood cells	31	56	130
Plasma	24	32	32
Body-weight of animals (kgm.)	67·4	72·4	70·2

Radiation dose-rates obtained for rams fed cæsium-137 for 5, 10, or 20 days are given in Table 1. All dose-rates are expressed as mrads/hr./mc. in the body tissues and gastrointestinal tract at the time of death. This expression allows direct comparisons between the different animals irrespective of body burdens. Thus, differences in dose-rates reflect distribution of cæsium-137 in the body to the number of days fed. Data for the γ-radiation dose-rates are those measured at the time the animals were killed. The combined γ- and β-radiation dose-rates were obtained by adding the γ-radiation dose-rates to the calculated β-dose-rates. A small correction was made for γ-activity of tissue displaced by the dosimeters.

The highest dose-rates were found in the rectum; however, these values only applied to the surface layers of the mucosa and were probably twice that received by most tissues in the rectal region. The highest tissue dose-rates were in the kidneys, where a rate twice that of the total body was measured.

The combined β- and γ-dose-rate to the intact testes were only slightly higher than that received by the total body. A comparison of values for the intact and excised testes reveals that approximately 20 per cent of the radiation dose to the testes originates from surrounding structures, such as the thigh muscle and gastrointestinal tract.

The present policy for establishing the maximum permissible body burdens of radionuclides considers the organ that receives the highest radiation dose relative to its radiosensitivity to be the critical organ. The maximum permissible limits for occupational exposure recommended by the National Committee on Radiation Protection and Measurements[2] were selected for most radionuclides on the basis of an average annual dose to the critical organ of 5 rems for the gonads or total body, 15 rems for most individual organs, and 30 rems for the thyroid or skin. Use of total body as critical organ for γ-emitting nuclides such as zinc-65 or cæsium-137 compensates for the potentially large contribution of the radiation dose to the gonads from concentrations of the radionuclide in surrounding body structures. Although the testis dose was always slightly greater than that of total body, the difference was such that use of total body as critical organ adequately accounted for the radiation dose to testes that originated from contiguous structures. γ-Dose measurements taken at the anatomical location comparable with that of the ovary were quite low. Considering the small size of the ovaries and assuming that they would not have high concentration, it would be expected that the total radiation dose to the ovaries of the ewe would be less than that to the testes of the ram.

Prolonged feeding of cæsium-137 may be expected to increase the gonad dose relative to the total body dose, providing the concentration of cæsium-137 increases in voluntary muscle relative to other tissues. From this brief 20-day study, it was not possible to determine accurately the extent to which this might occur. However, considering that during the period 5–20 days the radiation dose to the testes increased by only one-third, while the concentration of cæsium-137 in voluntary muscle increased nearly threefold, it appears that no great alteration in the relationship between total-body and gonad dose would take place even over extended periods of exposure to cæsium-137.

Obviously the anatomical relationships and metabolic characteristics of the male sheep are not identical to those of man and therefore preclude direct extrapolation to man. However, these results, obtained in a large species comparable to man in size, do help affirm the basic policy of using total body as an estimator of cæsium-137 radiation dose to the gonads.

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IMMUNIZING EFFECT OF IRRADIATED CERCARIÆ OF *SCHISTOSOMA MANSONI* IN RHESUS MONKEYS

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It has recently been reported[1,2] that previous exposure of mice to irradiated cercariæ of *Schistosoma mansoni*, or exposure of monkeys to irradiated cercariæ of *S. japonicum*, afforded protection against a homologous challenge with the unattenuated parasite. Concurrent work has been undertaken in this laboratory on the effect of irradiation on the behaviour of cercariæ of *S. mansoni* in hamsters, and the protective effect of such cercariæ against a homologous challenge in rhesus monkeys.

An Egyptian strain of *S. mansoni* was used and the methods of percutaneous infection, challenge, and worm recovery follow those described previously[3]. Irradiation of cercariæ was effected by X-rays; the cercariæ were collected in 10 mm. depth of filtered rain-water in a glass crystallizing dish 100 mm. in diameter. X-irradiation was delivered by a Newton–Victor *G* × 10 unit, and the radiation factors were 120 kV., 5·5 m.amp. with 1-mm. aluminium filter. The distance from the X-ray tube to the surface of the cercarial suspensions was 190 mm. and the exposure dose-rates ranged from 140 to 160 r./min.

Hamsters were used for estimating the dose of X-irradiation which was necessary to prevent cercariæ from developing to maturity, but which still allowed them to migrate through the lungs and reach the liver. It was found that an X-ray dose of 3,000 r. prevented any cercariæ from surviving for more than six weeks in hamsters; 2,000 r. prevented

Table 1 EXPOSURE OF RHESUS MONKEYS TO X-IRRADIATED CERCARIÆ OF *S. mansoni* AND RESULTS OF HOMOLOGOUS CHALLENGE

Monkey No.	Immunizing cercariæ: X-ray dose r.	No. in 1st exposure	Interval	No. in 2nd exposure	Interval	No. of cerc. in challenge	P.m. results 8 wks. after challenge: Worm recovery	Sterile females (per cent)	Lesions
39	—	—	—	—	—	1,000	328	—	Severe
44	—	—	—	—	—	1,000	586	1 8	Severe
46	—	—	—	—	—	1,000	420	0	Severe
37	2,000	3,000	6 wks.	10,000	8 wks.	1,000	166	59 2	Mild
38	2,000	3,000	6 wks.	10,000	8 wks.	1,000	341	56 0	Mild
45	2,000	3,000	6 wks.	10,000	8 wks.	1,000	456	60 9	Mild
40	3,000	3,000	7 wks.	7,000	6 wks.	1,000	73	3 5	Mild
47	3,000	3,000	7 wks.	10,000	6 wks	1,000	207	10 9	Moderate
48	3,000	3,000	—	—	13 wks.	1,000	440	1 8	Severe

all but about 1 per cent of the worms from surviving beyond this time. Most of the flukes surviving after exposure to 2,000 r. were stunted and the females were sterile. On one occasion, however, two females, each containing an egg *in utero*, were recovered from hamsters exposed to cercariæ irradiated at 2,000 r. although one of these females showed poorly developed vitellaria. On another occasion a few living eggs were found in the liver of a hamster exposed to cercariæ treated at 2,000 r. and one pair of mature worms was recovered; all other worms recovered from the same hamster and from another hamster exposed to cercariæ from the same suspension were stunted and sterile. After exposure to 1,500 r., approximately 5 per cent of cercariæ survived beyond six weeks, and although some of the surviving worms were stunted, others were able to produce live eggs. An X-ray dose of 1,000 r. caused only a slight reduction in the number of cercariæ developing to maturity as compared with a control infection, and live eggs were produced (Fig. 1).

Histological examination of tissues from hamsters exposed to cercariæ irradiated at 2,000 or 3,000 r. revealed that most cercariæ had passed through the lungs uninhibited, but on reaching the liver they died during the second, third or fourth week after exposure. Dead flukes were found in the smaller vessels of the liver, and were surrounded by an intense cellular reaction consisting mainly of mononuclear cells, with a few polymorphs. The dead flukes were gradually absorbed by macrophages. In later stages, fibroblasts were laid down until finally the vessels became completely occluded by fibrotic scars. In contrast with the reaction which occurs around schistosome eggs, giant cells were absent around dead schistosomula and no calcification occurred.

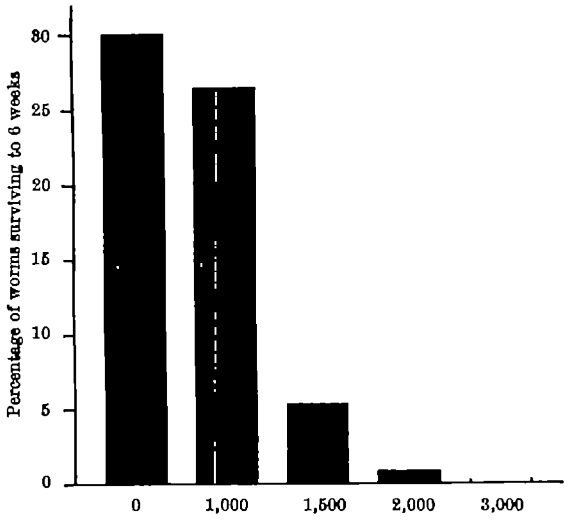

Fig. 1. Percentage of X-irradiated cercariæ surviving to 6 weeks in hamsters· 5 groups of 5 hamsters, each hamster exposed percutaneously to 380 cercariæ

Attempts to protect rhesus monkeys against subsequent challenge with *S. mansoni* were made by exposing them to cercariæ irradiated at 2,000 and 3,000 r. During the period of immunization, 6–9 fæcal examinations per monkey by the *AMS* III method[4] failed to reveal the presence of schistosome eggs as a result of the exposures to irradiated cercariæ. At varying periods after the exposures, each monkey was challenged with 1,000 normal cercariæ taken from a single suspension. The experiment is summarized in Table 1.

It is believed that the monkeys exposed to cercariæ irradiated at 2,000 r. developed a partial resistance to the challenge infection. Although the worm recovery figures from this group were not substantially lower than the worm recovery figures of the control group, the lesions in the liver and gut wall, due to egg deposition, were negligible compared with the severe lesions which occurred in the control monkeys. The reason for this became obvious on examination of stained preparations of the female worms, when it was found that more than half the females recovered from the 2,000 r. group were stunted, their vitellaria and usually their ovaries failed to stain, and their gut contents contained little or no pigment. It is believed that these sterile worms had survived from the immunizing exposures. In the 3,000 r. group, one monkey (No. 40) developed a marked protection against the challenge, but in a second monkey (No. 47) receiving almost identical exposures protection was less marked. The third monkey of this group, which received only one exposure of 3,000 irradiated cercariæ, failed to develop any recognizable signs of resistance against the challenge.

These results generally confirm the work of Villella *et al.*[1] on *S. mansoni* in mice, and Hsu *et al.*[2] on *S. japonicum* in monkeys. It appears, however, that under the conditions used by Hsu *et al.* a slightly lower X-ray dose than that needed to prevent egg formation in *S. mansoni* will sterilize the worms of *S. japonicum*. One fact is clear: large numbers of irradiated cercariæ are necessary to induce an incomplete protection against *Schistosoma* in rhesus monkeys.

I thank Dr. Janet Niven and Mr. M. J. Ashwood-Smith for their advice.

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TRANSPEPTIDATION MECHANISM IN BLOOD CLOTTING

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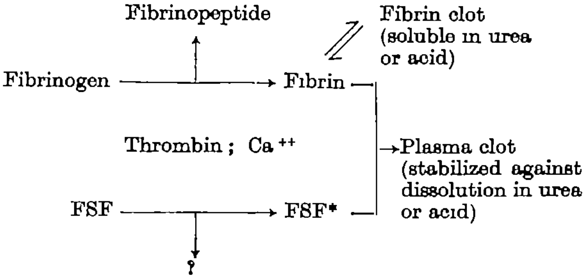
THE actual clotting phase in blood coagulation is initiated by two parallel sets of reactions catalysed by thrombin. Fibrinogen is converted to fibrin through the scission of fibrinopeptide from the parent molecule[1] and the more recently recognized plasma globulin, fibrin stabilizing factor (FSF), is also activated in some manner[2]. The thrombin-activated form of FSF (designated as FSF*) produces covalent cross-links in the clot polymer which is thus rendered insoluble in solvents such as 30 per cent urea or 1 per cent monochloroacetic acid[3]. Accordingly, physiological clotting may be outlined as follows:


Fibrinopeptide
Fibrin clot (soluble in urea or acid)
Fibrinogen
Fibrin
Thrombin; Ca ++
Plasma clot (stabilized against dissolution in urea or acid)
FSF
FSF*
?

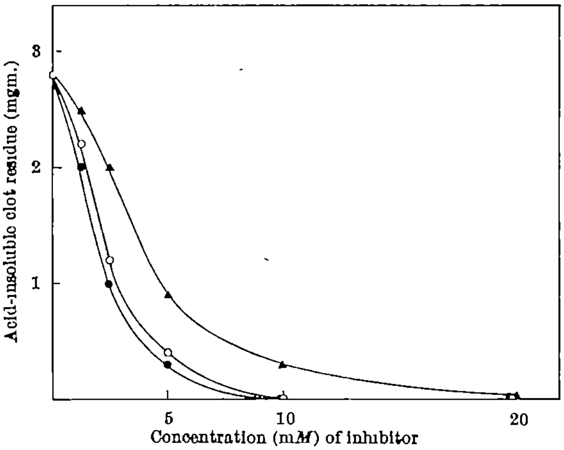
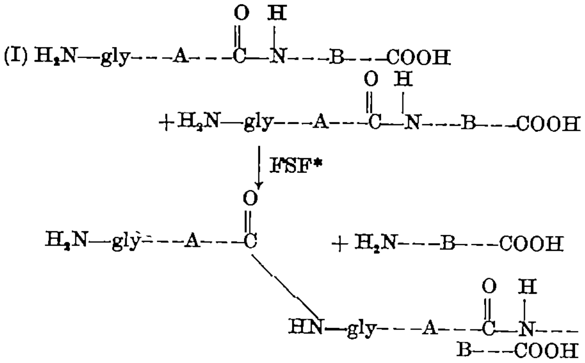


Fig. 1. Effect of inhibitors on the cross-linking of fibrin by the thrombin-activated fibrin-stabilizing factor. Buffer consisted of 0·05 *M* *tris*-HCl of *p*H 7·5 brought to an ionic strength of 0·18 by addition of sodium chloride. (*Tris* itself, probably because of its being an amine, is somewhat inhibitory on the cross-linking reaction studied, but the effect begins to be noticeable only at ten times the concentration used.) ***The activation mixture*** contained 0·4 ml. (65γ) of FSF, 0·1 ml. (2 units) of thrombin (ref. 8), 0·5 ml. each of 1 m*M* calcium chloride and 0·1 *M* neutralized cysteine, all being dissolved in the *tris* buffer. Following incubation for 10 min. at 20°, the action of thrombin was stopped by the addition of 0·2 ml. neutralized 0·25 *M* N$^{\alpha}$-tosylarginine methylester dissolved in *tris*. 2 min. later, 0·5 ml. of the neutralized inhibitor substance, dissolved in the buffer, was added in the concentrations shown on the abscissa. ***The polymerization mixture:*** Clotting was initiated 3 min. later by the addition of 0·8 ml. of purified fibrin, dissolved in 1 *M* sodium bromide containing 0·05 *M* acetate buffer at *p*H 5·3. Gelation occurred in all instances in less than 2 min. 30 min. after clotting, 2·5 ml. of 2 per cent monochloroacetic acid was added to the gel. After 24 hr. in the acid, the insoluble residue was washed and its protein content (shown on the ordinate) estimated. ●, Gly NH$_2$; ○, gly Me; ▲, gly gly Me

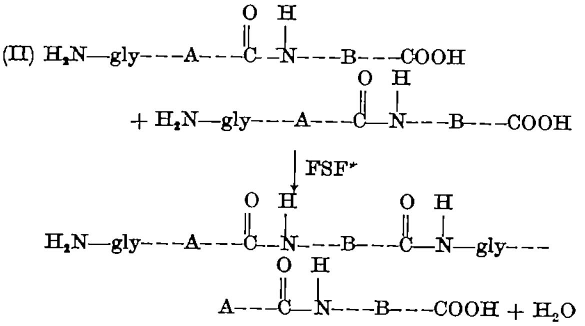
We have obtained some evidences which strongly suggest that the cross-linking of fibrin by FSF* belongs to the class of reactions generally referred to in protein chemistry as 'transpeptidation'[4].

(1) *Reduction in the number of amino end-groups in the cross-linked clot structure.* Bovine fibrin, purified[5] by repeated dissolution in urea and reprecipitation by dilution into a neutral buffer, contains *N*-terminal residues of glycine and tyrosine in amounts of about 1·1 and 0·5 moles per 100,000 gm. of protein respectively. When such purified fibrin is finally taken up in 1 *M* sodium bromide[6] and allowed to gel following an eight-fold dilution with a *p*H 7·5, μ : 0·18 *tris*(hydroxymethyl)aminomethane buffer in the presence of FSF* (prepared as described in the legend to Fig. 1), the clot becomes cross-linked so that it can no longer be dissolved in 30 per cent urea. Examination of the *N*-terminal residues of the urea-insoluble proteins shows only glycine and tyrosine end-groups, but in greatly diminished quantities (about 0·25 moles each per 100,000 gm. of protein) as compared with the starting fibrin material. The ε-NH$_2$ lysine contents of fibrin and stabilized fibrin are identical.

The observed reduction in the number of open peptide chains may be due to one of the following two possibilities, illustrated only for the dimerization of chains with glycine (gly) end-groups:



$$\text{(I) }\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}$$
$$+\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}$$
$$\Big\downarrow \mathrm{FSF^*}$$
$$\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\qquad+\mathrm{H_2N{-}{-}{-}B{-}{-}{-}COOH}$$
$$\diagdown$$
$$\mathrm{HN{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}$$



$$\text{(II) }\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}$$
$$+\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}$$
$$\Big\downarrow \mathrm{FSF^*}$$
$$\mathrm{H_2N{-}gly{-}{-}{-}A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}gly{-}{-}{-}}$$
$$\mathrm{A{-}{-}{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}{-}\overset{\mathrm{H}}{\overset{|}{\mathrm{N}}}\mathrm{{-}{-}{-}B{-}{-}{-}COOH}+\mathrm{H_2O}$$

Analysis of the clot liquor for the appearance of (H$_2$N---B---COOH) soluble fragments during stabilization of fibrin by FSF* will decide between the two mechanisms.

It should be mentioned that the tendency for reduction in the number of N-terminal glycines was also noticed by Middlebrook during the formation of a clot in whole plasma[7]. His results, concerning the release of end-groups into the supernatant of that system, seem to indicate to us that mechanism I is preferred. However, transamidation between a glutamine side-chain and a terminal amino group must also be considered[8].

(2) *Inhibition of the cross-linking of clot structure by certain amines.* Since the end-group measurements indicated mainly the involvement of α-NH_2 groups of glycine residues in the cross-linking of fibrin units, it was reasoned that the presence of a suitable glycine derivative during the polymerization of fibrin by FSF* might interfere with the cross-linking process. Indeed, as shown in Fig. 1, some simple amines, related to glycine in an N-terminal position, were found to inhibit the development of the acid-insoluble clot structure. At pH 7·5, minute concentrations of glycineamide, glycine methylester, glycylglycine methylester and glycylglycine were effective, whereas glycine, imidazole and aniline showed no appreciable inhibition. It must be emphasized that the inhibitors did not affect the onset of gelation; they appeared only to delay the cross-linking of the protein units of the clot as measured by solubility in 1 per cent monochloroacetic acid. The possibility that the inhibitors may become temporarily incorporated into the clot proteins and act as transient chain terminators in the polymerization processes is now being examined.

It is noteworthy that the inhibitors, when added to oxalated plasma, did not interfere with the onset of clotting after recalcification, but again inhibited the development of the acid-insoluble, stabilized clot structure. A wider survey of inhibitory amines is in progress.

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OCCURRENCE OF CITRULLINE IN PROTEINS

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REPORTS in the literature claiming that the amino-acid citrulline is present in many proteins have appeared from time to time[1–3]. These claims have not been substantiated. Furthermore, the application of recent developments in amino-acid analysis has not revealed citrulline in any of the well-characterized proteins.

More recently the presence of citrulline was reported in the red alga, *Chondrus crispus*[4]; but it was later shown to be combined in small peptides and not in protein[5].

In previous work we demonstrated the presence of significant amounts of citrulline in hydrolysates of the hardened inner root sheath cells of the hair follicle[6–8]. The evidence favoured the view that the citrulline was covalently bound by peptide linkage to the proteins of these cells and not simply adsorbed by secondary forces[6,8]. The present results support this view.

In these experiments citrulline-rich polypeptides have been released from the cell proteins by tryptic digestion. However, owing to the small size of the inner root sheath even in large hair follicles and the attendant difficulty of obtaining sufficient material for examination, attention was turned to the medullæ of hairs and related structures, namely, porcupine quills, where the medullary cells had been shown to have proteins which contain citrulline[8]. Both inner root sheath cells and medullary cells give a strong colour in the Fearon test for citrulline which is now shown to be incorporated in the peptide chains of the insoluble proteins in these cells.

Inner Root Sheath Cells

The inner root sheath layers were stripped from vibrissal hairs with 'roots' attached, plucked from rats (Wistar strain), and from the soft 'root' ends of quills plucked from the African porcupine (*Hystrix cristata*).

The sheath layers, which consist mostly of hardened protein-filled cells, were treated with 8 M urea to remove the unhardened cells containing trichohyalin[6], washed and dried, then 1–2 mgm. quantities of each were digested with 50 μgm. crystalline trypsin, pH 8, for 4 hr. The digests were centrifuged to remove cellular debris, lyophilized, and then examined by unidimensional paper electrophoresis. Bands of separated polypeptides were detected by spraying with ninhydrin, and on duplicate strips citrulline-containing peptides were visualized by the yellow colour produced when sprayed with Ehrlich's reagent under mild acid conditions. The patterns obtained are shown in Fig. 1*a* and *b*. Five separate ninhydrin-positive bands were usually distinguishable; one which moved the fastest towards the anode (1) reacted strongly for citrulline whereas another band (4) reacted only faintly. The others (2, 3 and 6), more basic in character than band (1), did not react for citrulline.

From this distribution it follows that in the inner root sheath protein the citrulline is concentrated into regions of the peptide chains where the concentration of dicarboxylic amino-acids is higher than that of the basic amino-acids. The large peptide fragments,

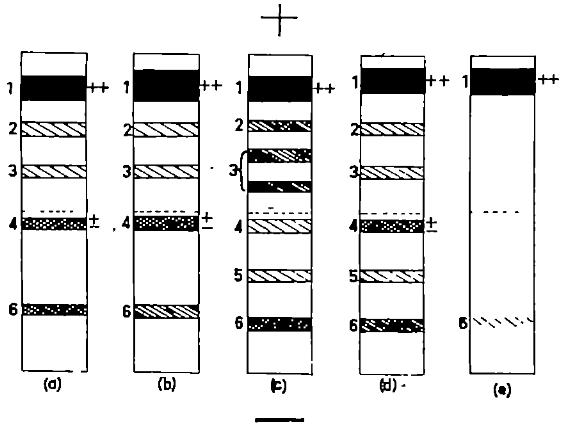

Fig. 1. Paper electrophoresis band patterns of polypeptides obtained from tryptic digests of (*a*), inner root sheath cells from rat hair follicles and from (*b*) African porcupine quill follicles, (*c*) medullary cells of medullated reindeer hair and Lincoln wool, (*d*) medulla from African porcupine quill, (*e*) medulla from African porcupine quill after fractionation according to the scheme in Fig. 2. Relative intensities of ninhydrin colour are indicated by shading. Degree of reaction with Ehrlich reagent for citrulline is signified by ++ (strong) and ± (weak). Run in 0·04 *M* phosphate *p*H 6·8, 7·5 V./cm. for 4 hr. in a Spinco model *R* paper-electrophoresis apparatus.

containing citrulline, glutamic acid (and aspartic acid) and smaller amounts of other amino-acids, are liberated when trypsin hydrolyses lysyl and arginyl linkages of the protein.

It could still be argued that these results do not exclude the possibility of strong adsorption of citrulline to the insoluble protein before trypsin digestion. However, this possibility seems most unlikely when the results obtained with medullary cells are taken into account.

Medullary Cells

Amino-acid composition. Paper chromatograms of acid hydrolysates of medullary cells isolated from rabbit fur by the alkali method[9] and of medullæ dissected from the quills of African, North and South American porcupines (*Hystrix* sp., *Erithizon* sp. and *Coendou* sp. respectively) revealed similarities to inner root sheath cells in their qualitative amino-acid composition, in particular in their high contents of citrulline and glutamic acid. Quantitative amino-acid analysis of rabbit fur by the Moore and Stein procedure[10] (Table 1, col. *A*) revealed the presence of citrulline originating from the medulla, but greatly diluted by the contribution of cortical keratin. Medullary cells isolated from the fur by alkali treatment[9] were richer in citrulline and also glutamic acid (Table 1, col. *B*). The medulla manually dissected from African porcupine quill had a comparable composition (Table 1, col. *C*). Other features of the amino-acid compositions of the medullæ are the relatively high leucine and aspartic acid figures, the low content of tyrosine and the virtual absence of cystine, the total pattern resembling that of the inner root sheath (Table 1, col. *E*).

Identity of citrulline. The identification of the citrulline in medulla proteins does not rest solely on the position of the amino-acid in Moore and Stein amino-acid analyses or paper chromatograms. Citrulline was isolated in quantity from medulla hydrolysates by ion-exchange chromatography and converted to the dinitrophenyl (DNP) derivative. The properties of this derivative, such as melting-point, elemental analysis, infra-red spectrum and R_F values in a variety of solvents, were indistinguishable from those of an authentic sample of DNP-citrulline. Both DNP derivatives were also remarkably stable to hydrolysis by 6 *N* hydrochloric acid at 110° C. for 16 hr. but a trace of α-DNP-ornithine was observed in both cases.

Isolation and composition of citrulline-rich peptides. Although resistant to solution by the usual protein solvents medullary cells are rapidly digested by trypsin[9,12] and unidimensional paper electrophoresis of tryptic digests of medulla material from hairs and porcupine quills gave band patterns (Fig. 1*c* and *d*) similar to those of the inner root sheaths. Usually six or seven ninhydrin-positive bands were obtained, the most prominent again being one which moved rapidly to the anode (1) and gave a strong reaction for citrulline. The citrulline-rich polypeptide material of this band was separated from the more basic polypeptides of the remaining bands according to the scheme shown in Fig. 2. Fraction *A* and fraction *B*, which were obtained in the ratio of about 2 : 1, moved almost identically on paper electrophoresis in a position similar to the fastest migrating anodic band 1, and were only slightly contaminated by the basic material of band 6 (Fig. 1*e*). Both polypeptide fractions did not dialyse through 'Cellophane' and therefore have molecular weights of about 10,000, which is in accord with preliminary ultracentrifuge data. Since they are produced by protein splitting with trypsin, each are mixtures of polypeptides, but their electrophoretic and chromatographic behaviour strongly suggests that although being mixtures they are closely related in molecular size and in composition. Fractionation of them by ion-exchange chromatography on DEAE-cellulose columns has not yet led to the separation of discrete components.

The amino-acid composition of fraction *B* only is given in Table 1 (col. *D*). A striking feature is that citrulline and glutamic acid together constitute

Table 1. QUANTITATIVE AMINO-ACID ANALYSES* OF CITRULLINE-RICH COMPONENTS

Proteins hydrolysed in 6 *N* hydrochloric acid at 110° C.; *A* and *B*, 16 hr.; *C*, *D* and *E*, 10 hr. The lysine figure in each case includes ornithine originating from citrulline breakdown and not separately resolved. Thus the citrulline figure is a minimal one.

	A Whole rabbit fur (medullated) N = 16 per cent	*B* Rabbit fur medullary cells (isolated) N = 14·4 per cent	*C* African porcupine quill medulla‡ N = 16·8 per cent	*D* Medulla protein fraction *B* N = 16·9 per cent	*E* Whole inner root sheath (ref. 7) N = 16 per cent
Amino-acid	µmoles/gm.	µmoles/gm.	µmoles/gm.	µmoles/gm.	µmoles/gm.
Lysine	266	815	580	511	192
Histidine	145	47	89	88	65
Arginine	503	302	365	90	294
(Ammonia)	(838)	(722)	(912)	(1,610)	(250)
Aspartic acid	375	249	568	242	534
Threonine	345	88	256	0	201
Serine	615	142	445	73	372
Glutamic acid	782	2,232	2,120	2,960	1,280
Proline	513	87	162	364	—
Glycine	542	146	651	46	387
Alanine	286	286	480	283	348
Cystine/2†	1,040	0	102	0	42
Valine	253	121	405	162	154
Methionine	0	0	35	0	10
Isoleucine	175	103	197	0	122
Leucine	482	818	760	600	542
Tyrosine	177	82	338	0	116
Phenylalanine	158	149	494	157	164
Citrulline	18	178	373	1,410	350

* Beckman–Spinco *MS* analyser was used for analyses in *C* and *D*. *A*, *B* and *E* were by a laboratory constructed machine (ref. 11) from which the nitrogen recoveries were low (70–85 per cent), especially in *E*, because of the minimal quantity of sample available for analysis and presence of non-protein contaminants.

† Originating from α-keratin.

‡ Dissected from the quills and contaminated with α-keratin.

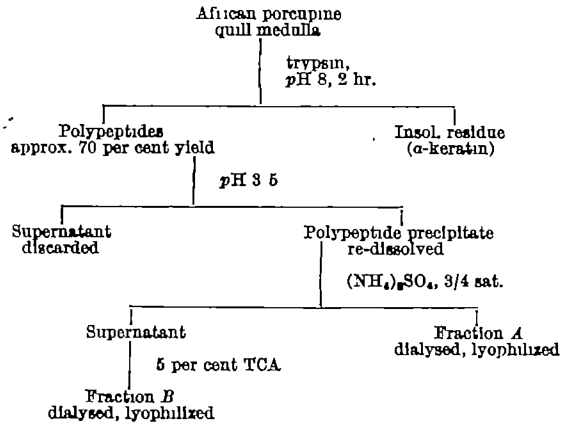
African porcupine quill medulla
trypsin, pH 8, 2 hr.
Polypeptides approx. 70 per cent yield
Insol. residue (α-keratin)
pH 3 5
Supernatant discarded
Polypeptide precipitate re-dissolved
(NH4)2SO4, 3/4 sat.
Supernatant
Fraction A dialysed, lyophilized
5 per cent TCA
Fraction B dialysed, lyophilized

FIG. 2. Isolation of citrulline-rich polypeptides from trypsin digest of quill medulla

58 per cent of the total nitrogen and on a weight basis the citrulline content of fraction *B* is 24·7 per cent, but since it was found that 16 per cent of the citrulline was converted to ornithine during hydrolysis of fraction *B* for 10 hr. in 6 *N* hydrochloric acid at 110° C. the corrected value for zero time is 28·6 per cent. An independent determination of citrulline by a colorimetric method[13] gave the same value. This means that 1 residue in 2·8 is glutamic acid and 1 in 5 is citrulline. The next most abundant amino-acids from Table 1 (col. *D*) are leucine and lysine, whereas tyrosine, isoleucine, threonine, cystine and methionine are absent. The composition of fraction *A* was similar, but the glutamic acid and citrulline contents were slightly lower than in *B*.

Peptide bonds involving citrulline. A most significant finding indicative of the citrulline being combined in peptide linkage in medullary proteins was the release of free citrulline from fractions *A* and *B* by both carboxypeptidase and leucine amino-peptidase. Using the 'molecular-sieve' technique[14,15] it was shown that in addition to citrulline these enzymes liberated four or five other amino-acids, including glutamic acid. When pepsin was allowed to act first, about 25 acidic peptides separable by the 'fingerprinting' technique[16] were produced and about 10 of these contained citrulline. Subsequent action of carboxypeptidase and leucine aminopeptidase on the pepsin digests released greatly increased quantities of amino-acids. About twelve liberated amino-acids were detected by paper chromatography, with citrulline and glutamic acid in concentrations relatively much higher than the others. Leucine aminopeptidase was more effective in liberating citrulline and glutamic acid than was carboxypeptidase and the evidence indicates that in the initial digestion of the medulla the trypsin hydrolysed citrullyl bonds only very slowly compared with arginyl and lysyl bonds.

General Conclusions

The present results show that the proteins of medullary cells (within the hair) and inner root sheath cells (external to the hair) are very similar in properties. This is not altogether unexpected on histological grounds for trichohyalin 'droplets' are found in both types of cell[17] during development in the follicle. Consequently some properties of the medulla are likely to apply to the inner root sheath.

The amino-acid compositions are distinctive and the evidence for the identity of citrulline and its occurrence in peptide linkage is now unequivocal for medulla, and almost certainly for the inner root sheath as well. Of particular interest is the release from both cell proteins by trypsin of closely related polypeptides which contain virtually all the citrulline and much glutamic acid. This observation and the action of pepsin and exopeptidases in liberating large amounts of both amino-acids not only rules out the possibility of adsorption of citrulline to the protein but also indicates the frequent occurrence of trypsin-resistant cit-cit, cit-glu and glu-glu sequences gathered together and forming citrulline-rich regions in the medulla proteins.

Earlier investigations of medulla pointed to features which distinguish it from α-keratin; for example, a β-type X-ray diagram[18], avidity for dyes and rapid digestion by trypsin[9,12,19], alkali resistance[9], and low cystine but high glutamic acid content[20]. The present work has demonstrated even more striking dissimilarities from α-keratin in amino-acid composition, including a low content of the aromatic amino-acids contrary to reported high values[9]. Moreover, the majority of the cystine present in the whole medulla analysed in the present work probably arose from the keratinous septæ present in quill of African porcupine[21] and was not detected in the citrulline-rich fraction of medulla.

At the present time it is not known whether during growth citrulline *per se* is incorporated into the medulla and inner root sheath proteins or whether citrullyl residues are derived from arginyl residues. The conversion of arginyl residues to citrullyl while in protein combination has been suggested for the inner root sheath[6-8] and supporting histochemical evidence has been obtained for this mechanism in both the medulla and the inner root sheath[22]. A mechanism of this kind, if it exists, would be unique and could constitute a specialized process in protein biosynthesis in which the imino-nitrogen of the guanidino group might be released (as ammonia or derivative) to nearby cells synthesizing keratin. In addition to introducing difficulties for the 'triple-letter code' of Crick[23] for 20 amino-acids, the direct incorporation of citrulline into proteins would require a specific activating enzyme and *s*-RNA. Nevertheless, a search for such a system in hair follicles is being undertaken as well as investigations into the alternative conversion mechanism.

I thank Mr. A. S. Inglis and Mr. R. J. Rowlands for the amino-acid analyses.

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SPORIDESMIN POISONING IN THE RAT

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SPORIDESMIN is a toxin of as yet unknown chemical structure (empirical formula $C_{18}H_{22}O_6N_3S_2Cl$ (ref. 1)) which is produced by the fungus *Pithomyces chartarum* (formerly *Sporidesmium bakerii*). The toxin is closely associated with the development of facial eczema, an affliction of sheep of great economic importance mainly in New Zealand. Done, Mortimer and Taylor[2] have reported the pathological disturbances in field cases of facial eczema in sheep; similar results have been found by the New Zealand workers to occur with rabbits, guinea pigs and sheep after dosing with sporidesmin (Taylor, A., personal communication). At the request of Dr. Taylor we commenced a study of the effect of sporidesmin on the rat and a comparison of its action with another liver poison, icterogenin[3].

Poisoning was induced in female rats (body-weight approx. 150 gm.) by a single intraperitoneal injection of 1–5 mgm./kgm. sporidesmin. Dosages of the highest level often produced ascites and/or pleural effusions; with such an insult approximately 1 rat in 4 died between the second and eighth days. The rat is apparently more resistant to the toxin than either rabbits or guinea pigs, in which serious liver damage occurs at a much lower dose[1]. The male rat appears more resistant than the female, apparently due to a more rapid detoxication of the toxin. Preliminary experiments in which *SKF*–525 *A* was given to male rats concurrently with sporidesmin indicated that a greater toxicity was obtained than with sporidesmin alone; *SKF*–525 *A* inhibits microsomal detoxication enzyme systems[4].

Animals injected with sporidesmin showed a variety of symptoms associated with tissue damage. Changes in the serum included variable increases in cholesterol, glutamic–pyruvic transaminase and malic dehydrogenase; there were no increases in serum alkaline phosphatase, bilirubin and β-glucuronidase. There were variable decreases in total serum protein; on occasions this was decreased by more than 30 per cent. Serum ribonuclease was also decreased. Serum mucopolysaccharide, as measured by the diphenylamine method[5], was slightly increased (+15 per cent); the increase was of the same order as that found to occur in serum from bronchitic patients[6]. Starch-gel electrophoresis of serum from rats poisoned with sporidesmin showed that in acutely ill animals a new protein band appeared in region *a* (Fig. 1). The position of the new band is similar to the band found to appear after poisoning by certain heavy metals[7] and also to appear in pregnant, immediately post-weaned, and tumour-bearing rats[8]. The electrophoretograms show other differences which will be discussed in more detail at a later date.

Rats treated with sporidesmin developed a proteinuria associated with marked oliguria; the total amount of protein excreted daily was not much affected. Total urinary carbohydrate was virtually unaffected. The urinary amino-acid pattern was normal. There was a moderate increase in the urinary excretion of 17-hydroxy corticosteroids and 11-deoxy-17-hydroxy corticosteroids; the increases were consistent with an ACTH–mimetic effect. Urinary glucuronic acid excretion increased rapidly during the first 24 hr. after injection but had returned to normal by the second day.

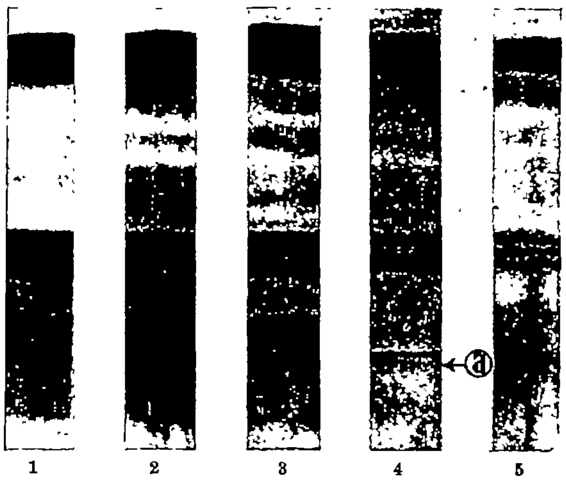

Fig. 1. Starch gel electrophoresis of serum samples from normal and sporidesmin-treated female rats. Electrophoresis was carried out by Poulik's method (ref. 10). Strip 1, normal serum, strips 2–4, sporidesmin-treated animals. The rat corresponding to serum 4 had extensive ascites and the electrophoretic pattern of this fluid is shown in strip 5

Many sporidesmin-treated rats showed decrease in the liver weight although total liver RNAP and DNAP remained in the normal range. Even in animals which were acutely ill there were no noticeable differences in the acid-soluble ribonucleotide pattern; the level of adenosine triphosphate (ATP) was slightly higher in the treated animals than in normal rats. This is possibly a result of the animals entering a state of shock in which the level of liver glycogen is known to fall and that of ATP to rise. In agreement with previous work on the sheep, it was found that treated rats showed adrenal cortical hyperplasia[2]; in the limited number of determinations made, adrenal ascorbic acid and dehydro-ascorbic acid were in the normal range.

Sporidesmin was tested for its effect on several enzyme systems *in vitro*. At concentrations equivalent to 10^{-3}–10^{-5} *M* it had no effect on DPNH–oxidase (with malate or α-ketoglutarate as substrates); on glucose-6-phosphate dehydrogenase; on cytochrome oxidase; on succinate–neotetrazolium reductase; and on the ratio of free/bound ribonuclease in a lysosomal preparation.

Dr. C. H. Gallagher has since informed us that sporidesmin *in vitro* leads to an increased mitochondrial permeability to pyridine nucleotides and, as a consequence, produces a decreased activity in pyridine nucleotide dependent systems. Gallagher has also found that sporidesmin has no effect *in vitro* on succinic oxidase. Although we could find no effect of sporidesmin on DPNH oxidase *in vitro* we did find that the level of DPN in the livers of sporidesmin-poisoned rats was slightly decreased.

In vitro, sporidesmin caused mitochondrial swelling to a limited extent as measured by Tapley's procedure[9]. In addition to this effect on mitochondrial permeability, sporidesmin caused a delayed increase in capillary permeability as measured by the method of Spector[10]. Large amounts of sporidesmin, when injected subcutaneously, produced intense inflammation and abscess formation. It was very active in releasing from rat plasma slow-reacting substances that stimulated the guinea pig ileum and rat uterus preparations. The intense irritant effect of sporidesmin after subcutaneous injection into rabbits had been reported to us previously (Mortimer, P. H., personal communication). Since this manuscript was prepared and sent to Ruakura, New Zealand, for preview, we have learnt that Mortimer, working in Ruakura, has found effects in rats similar to some of those reported here, that is, a delayed increase in capillary permeability and also the production of necrotic zones in the liver.

Pathological lesions produced by sporidesmin in the rat were different from those previously described in the sheep. In almost every instance there was lung damage which took the form of a sustained protein-rich pulmonary œdema with a variable inflammatory or parenchymal reaction. Liver damage was not marked but when it did occur it was in the form of focal necrosis and periportal abscess formation. Biliary hyperplasia was sometimes present but never extensive. The kidney usually showed no abnormalities but in one case there was a necrotizing renal papillitis. Organizing peritonitis and necrotizing inflammation of the subcutaneous and muscular tissues of the injection site were sometimes seen. Tissues routinely examined were liver, lung, kidney, adrenal, ovary, uterus, spleen, heart; and, on occasion, intestine, peritoneal membrane, testis and brain. These results will be described in full elsewhere.

Supplies of sporidesmin were kindly made available to us, through Dr. A. Taylor, by the team working on facial eczema at the Ruakura Animal Research Station, Hamilton, New Zealand. They also gave us much information concerning their own work. We also thank Drs. M. Bulbrook, E. Huehns and D. Y. Wang and Mrs. B. Sawyer for assistance.

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FACTORS INFLUENCING SYNTHESIS OF URIDINE-5′-MONOPHOSPHATE IN RAT LIVER

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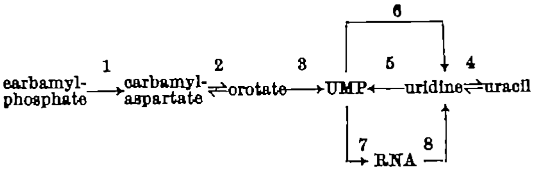
THE pyrimidine nucleotide moieties of nucleic acids are derived, directly or indirectly, from acid-soluble uridine nucleotides, of which the simplest is uridine-5′-monophosphate (UMP). The latter nucleotide arises from orotate ('*de novo* pathway') or, in some circumstances, from uracil or uridine produced when ribonucleic acid (RNA) or UMP itself is catabolized ('salvage pathway')[1]:



Steps 2, 3, 7 and 8 are actually multi-stage, but can be considered as single steps for the present purpose —which is to survey the overall behaviour of processes for synthesis of UMP in liver, both normally and in certain experimental situations (adrenalectomy, hyperthyroidism and carcinogenesis).

The finding of a change in enzymatic activity *in vitro* does not prove that the rate of the process is correspondingly altered in the animal. In multi-step reaction sequences only one step—perhaps the first step[2]—is likely to be rate-limiting. The limiting step would be expected to have low activity *in vitro* even when assayed under optimal conditions, for example, with respect to *p*H and to substrate concentration. *In vivo*, the conditions may, of course, be less favourable, and there may be other adverse factors: for example, Pardee[2] has shown with a bacterial system that step 1 in the synthesis of UMP is subject to 'feed-back inhibition' by one of the ultimate products (cytidine-5′-monophosphate). With the tissue present at a high concentration in the assay system, as in the present measurements of steps 1, 2, 3 and 5, the observed activity may reflect to some extent any influence of endogenous inhibitors or activators[3].

Steps 1–6 *in normal liver*. In assaying these steps the *p*H was within the range 7·2–8·0, with magnesium ions present in the case of steps 3, 5 and 6, and adenosine triphosphate (ATP) in the case of steps 3 and 5.

Table 1. CHANGES IN ENZYMATIC ACTIVITIES

Changes are expressed on a percentage basis (see text), and are followed by the standard error of the mean (except where there were fewer than 4 observations) and, in parentheses, by the No. of degrees of freedom and by the P value if less than 0·1, where P is the probability that the change could be due to chance. For changes given in bold figures, the P value was $<$0·05

Reaction: Reference No. in diagram, trivial name of enzyme, and International Union of Biochemistry Code No.		1: Aspartate transcarbamylase, 2.1.3.2	2 : Dihydro-orotase (carbamylaspartate dehydrase), 3.5.2.3, and dihydro-orotate dehydrogenase, 1.3.3.1		3: Orotidylate pyrophosphorylase, 2.4.2.10, and orotidylate decarboxylase, 4.1.1.23	4: Uridine phosphorylase, 2.4.2.3	5: Uridine kinase, ? 2.7.1.21	6: Uridine 5′-phosphatase, ? 3.1.3.5
			'Free'	'Total'				
Mean activity in normal liver, µmoles/gm./min.		0·48	0·0021	0·0054	0·0074	0·42*	0·022	1·6
Change in liver after adrenalectomy	At 3 days	+4 ±9·6% (11)	**+38** ±12·4% (9; $P <$ 0·025)	**−29** ±11·5% (7; $P <$ 0·05)	Unchanged (ref. 17)	Unchanged (ref. 17)	+25 ±24·5% (6)	Unchanged (ref. 19)
	Beyond 9 days	**−37** ±8·7% (5; $P <$ 0·01)	−24 ±14·9% (5)	**−20** ±6·7% (6; $P <$ 0·025)			**+68** ±13·8% (7, $P <$ 0·005)	
Change in liver after injection of L-thyroxine	2 × 0·2 mgm., 44 and 20 hr. previously	−42	−34	+7	Unchanged (ref. 16)	Unchanged (ref. 16)	+1	Unchanged (ref. 17)
	1 × 0·1 mgm., 20 hr. previously	−9	−56	+3			+30	
		−27 ±9·7% (10; $P <$ 0·025)	**−50** ±11·9% (5; $P <$ 0·01)	+5 ±30% (4)			+24 ±12·2% (9; $P <$ 0·1)	
Change in liver after azo-dye feeding ('precancerous liver')	5–20 days	+70 ±51 (3)	+30 ±23% (3)	+40	+20	−8	+62	−20% (2)
	21–41 days	**−27** ±7·9% (7; $P <$ 0·025)	−31% (2)	+28	+53	−9	+36	+67 ±47% (6)
				+28 ±21·5% (11)	+33 ±17·6% (12; $P <$ 0·1)	−8 ±7·1% (13)	**+53** ±14·7% (15; $P <$ 0·005)	
Change in primary hepatoma		**+91** ±17% (3; $P <$ 0·025)	+74 ±33·6% (7; $P <$ 0·1)	**+89** ±38·0% (10; $P <$ 0·05)	**−67** ±7·8% (5; $P <$ 0·001)	**+280** ±59% (6; $P <$ 0·025)	**+140** ±19% (5; $P <$ 0·001)	**+30** ±13·4% (15; $P <$ 0·05)

* Excluding old rats (controls for the hepatoma-bearing rats), for which the value was 0·13.

For steps 1, 2, 3 and 5, use was made of substrates labelled with carbon-14, and the reaction product was finally separated by chromatography on 'Dowex-1' resin. The choice of substrate concentration, pH, incubation time, and other variables, was guided by information in the literature[3–10]. Most of the assays were done with whole homogenates (0·25 M sucrose medium) or homogenates freed from nuclei; but for step 3 mitochondria were also removed, to eliminate interference from step 6 (ref. 6). For steps 4 and 5 particle-free supernatant fractions were used.

The enzyme concerned in step 1 is probably located in mitochondria[7] but easily detached[3,4,10]. The observation[8] that nuclear or microsomal fractions can effect step 2 has now been confirmed, but the mitochondrial fraction is more active and may be the true location of the enzymes concerned[7]. It has now been found that two-thirds of this activity is in a latent form which, as for the latent activity of lysosomal enzymes[11], becomes manifest if the tissue preparation is repeatedly frozen and thawed. Assays previously reported[4,5] may well have revealed only the 'free' activity and not the latent activity. Step 6 resembles step 2 in its intracellular location[6] and in its partial latency.

The rate of formation of carbamylphosphate with normal liver *in vitro* is so fast[12] that this process could scarcely be rate-limiting in the *de novo* pathway for the synthesis of UMP. Moreover, it is questionable whether step 1 (formation of carbamylaspartate) is rate-limiting in liver as distinct from bacteria. Step 1 has indeed been reported to have low activity in liver[4,7], of the same order as that of step 2, and the overall conversion of aspartate to UMP was found to be slow in Potter's laboratory (citation in ref. 13). However, in agreement with findings in Cohen's laboratory[3], we have observed the normal activity of step 1 to be as high as 0·5 µmole/gm./min. We have found the normal 'free' activity of step 2 to be only 0·002 µmole/gm./min. (Table 1)—a value of the same order as may be calculated from other reports[4,5]. Although step 3 likewise has low activity (Table 1) and, moreover, is irreversible[1,13], this step is unlikely to be rate-limiting since orotate is undetectable in normal liver[1,13].

Accordingly, the evidence from normal liver argues that in the *de novo* synthesis of UMP it is step 2 (and, in particular, dihydro-orotase[14]) that is rate-limiting —a conclusion which is supported by data discussed below. In the formation of UMP by the salvage pathway, step 5 is presumably limiting; to show even the low activity stated in Table 1, it is necessary to have uridine present in high concentration[9].

Since liver has a high capacity for dephosphorylating nucleotides such as UMP (Table 1, step 6), and a low capacity for the synthesis of UMP, it appears paradoxical that uridine nucleotides are normally present in quite high amount[15,16]. However, the dephosphorylating enzymes are only partly in 'free' form in fresh homogenates and, moreover, can scarcely be saturated with substrate *in vivo*. The concentration of UMP in liver (0·1 µmole/gm. (ref. 16)) is only one-twentieth of that required to achieve saturation when assaying the dephosphorylation of UMP *in vitro*, as in the experiments of Table 1 (step 6).

Enzymatic activities following adrenalectomy, injection of thyroxine, or feeding with a carcinogenic azo-dye. The animals and treatments, and the techniques for homogenization and centrifugation, were as described elsewhere[16–19]. The azo-dye 3′-methyl-4-dimethylaminoazobenzene was fed for 3 months to obtain primary hepatomas (which arose 3–5 months after cessation of dye feeding), and for periods less than the 'critical period'[20] to obtain 'pre-cancerous liver'. Experiments not presented here, with carcinogenic and non-carcinogenic analogues of 3′-methyl-4-dimethylaminoazobenzene[20], have suggested that the biochemical changes observed are specifically associated with carcinogenesis. In the work on adrenalectomized rats, no differences were seen between sham-operated controls and intact controls.

In the work on hepatomas and pre-cancerous liver, enzymatic activities have been calculated per gram of fresh tissue, this basis being considered preferable to a 'per cell' basis[20]. In the hormonal work, activities have been calculated per 100 gm. of body-weight; but the conclusions would be similar if based on tissue weight uncorrected for changes in liver size.

In each group of experimental rats there were certain clear-cut changes in enzymatic activities, as indicated in bold type in Table 1. These changes are best discussed in conjunction with results previously reported from this laboratory, concerning changes in the levels of uridine nucleotides.

Pattern of changes in the hormonal studies. Adrenalectomy produces a biphasic change in the level of UMP: there is an increase at 3 days after the operation, followed by a decline[17]. (An analogous biphasic change has been observed for incorporation of amino-acid into microsomal protein *in vitro*[21].) As predicted[17], it appears that altered availability of orotate accounts for this change in the level of UMP. The 'free' capacity for forming orotate is transiently increased at 3 days, although the 'total' capacity revealed by freezing and thawing the tissue sample is impaired (Table 1, step 2). With the eventual decrease in UMP, the situation becomes more complex. There is an increase in the capacity for the synthesis of UMP by the salvage pathway (step 5); the capacity for synthesis of carbamylphosphate is little altered[22], but there is evidence of impairment of the *de novo* pathway at steps 1 and 2 (Table 1), and also of increased utilization of UMP for the synthesis of RNA (ref. 17)—a process which may be quite fast *in vivo* even with normal liver (say, 0·01 μmole of UMP incorporated/gm./min.).

Thyroxine, even in a single dose of 0·1 mgm., decreases the levels of uridine nucleotides such as uridine triphosphate (UTP), although not of UMP itself[17]. Since ATP is also decreased[17], there may well be impairment both of the synthesis of UMP and of phosphorylation of the UMP to UTP. A single dose of thyroxine does in fact reduce the 'free' capacity for the synthesis of orotate (Table 1, step 2). The activity of step 1, by which carbamylaspartate is formed, can likewise be reduced by thyroxine, but only slightly with a single dose of 0·1 mgm.—which supports the argument that this step is less important than step 2 in governing the *de novo* synthesis of UMP.

It is unlikely that the effects of adrenalectomy and of thyroxine on uridine nucleotide levels are due to altered rates of nucleotide catabolism. There are no changes in the 'free' capacity for dephosphorylation of UMP, in the 'total' capacity as tabulated (Table 1, step 6), or in the capacity for dephosphorylation of UTP.

Pattern of changes in the azo-dye studies. The levels of uridine nucleotides, and particularly of UMP, show a marked rise within 10 days of commencing azo-dye feeding[18,23]. The data in Table 1 for step 5 suggest that this is due to increased activity of the salvage pathway. The rise in uridine kinase is evidently not a sequel to an enzyme 'deletion' in the *de novo* pathway. Step 1 of the latter pathway initially shows variable behaviour and later a decline which, however, is evident only after 3 weeks of dye feeding (Table 1); moreover, as already argued, it is unlikely that this step is rate-limiting. Steps 2 and 3 show no change in activity. It is conceivable that with the rise in UMP there is a depression of some step of the *de novo* pathway *in vivo*, not evident *in vitro*; but such a 'feed-back inhibition' of step 1[10], 2[10] or 3[18] by UMP is demonstrable *in vitro* only with UMP at a concentration much above that found in pre-cancerous liver.

The rise in the level of UMP is, then, not due to increased activity of the *de novo* pathway; nor is it attributable to decreased dephosphorylation of UMP (Table 1, step 6), or to decreased utilization of UMP for the synthesis of RNA (ref. 18). It is, however, not proved that the increased activity of uridine kinase (step 5) found *in vitro* connotes acceleration of the salvage pathway in the animal; one reason for hesitation is that ATP, which is requisite for this step, is diminished in amount in pre-cancerous liver[18,23].

In primary hepatomas the levels of certain uridine nucleotides, including UMP and UTP, tend to rise, but less strikingly than in pre-cancerous liver[18,23]. As is shown in Table 1, the capacity for the *in vitro* synthesis of UMP by the salvage pathway (steps 4 and 5) is greatly increased, in accordance with observations by Rutman *et al.*[24], who injected labelled uracil into hepatoma-bearing rats and found a high uptake by the hepatomas. (Since, however, with the high dose of uracil used in their work the tissue was probably 'flooded' with uracil, it is not proved that there was an increase in the actual, as distinct from potential, rate of synthesis of UMP from endogenous uracil or uridine).

Study of the *de novo* pathway in primary hepatomas (Table 1) has shown that step 3 is impaired, step 1 is enhanced (in agreement with a previous report[3]), and step 2 is usually enhanced at least with respect to 'total' activity. (In transplanted hepatomas, step 1 is undiminished[25]; the capacity for supply of carbamylphosphate is lowered[25], but it is doubtful if this supply is rendered limiting.) If the conversion of orotate to UMP (step 3) were to become rate-limiting in primary hepatomas, orotate would accumulate and, by a mass-action effect[1], damp down its own synthesis. However, we have so far found no evidence of such an accumulation, and conclude that the *de novo* pathway is unimpaired or even enhanced.

If, then, there is a high capacity for the synthesis of UMP in hepatomas, at least by the salvage pathway, why do hepatomas show only a moderate rise in nucleotides such as UMP and UTP? Possibly there is faster incorporation of uridine nucleotides into RNA (step 7)[16]. Another factor may be a higher capacity for the catabolism of uridine nucleotides, as now shown with UMP (Table 1, step 6) and with UTP (not tabulated).

The catabolism of RNA to uridine (step 8) may likewise be accelerated in hepatomas, since there is evidence of increased 'free' activity of acid ribonuclease[20] and of a higher capacity for dephosphorylation of the uridylic acid (3′-isomer of UMP) thus liberated. Evidently primary hepatomas do not show a general suppression of catabolic processes[9], although there may indeed be a slowing of late stages of catabolism such as the oxidation of hypoxanthine[20]. The evidence concerning UMP and RNA points to faster turnover in hepatomas, rather than to a serious imbalance between anabolism and catabolism.

Primary hepatomas as now obtained admittedly show some histological and biochemical variability[20], but they often show little necrosis (except in the 'core', which is routinely rejected) and, moreover, they appear to have arisen from parenchymal cells. It is therefore felt that the present results are a valid contribution to the understanding of neoplasia. In regenerating liver, there is an early rise in uridine kinase[8,9] and in aspartate transcarbamylase[3], and an eventual rise in uridine phosphorylase and (in contrast with hepatomas) in the enzyme system which converts orotate to UMP (ref. 8). Further study is warranted concerning analogies between liver regeneration and azo-dye carcinogenesis.

Conclusions. In the *de novo* pathway for the biosynthesis of uridine nucleotides in rat liver, the rate-limiting step is probably the conversion of carbamylaspartate to orotate. Even with tissue homogenates treated so as to liberate latent activity, this conversion is notably slow. Changes in the rate of this conversion underlie the changes in uridine nucleotide levels observed 3 days after adrenalectomy or in hyperthyroidism.

In pre-cancerous liver, the observed rise in the level of UMP is attributable to enhanced activity of uridine kinase. In primary hepatomas, uridine kinase is likewise increased, and the activity of the *de novo* pathway appears to be increased or at least undiminished. The lack of a marked rise in the level of UMP in hepatomas is attributable to faster turnover; the belief that catabolic processes are suppressed in tumours is an over-simplification.

Controlling factors not disclosed by the present assays may be operating in the animal. Moreover, increased activity of uridine kinase cannot be taken to prove that the salvage pathway is accelerated *in vivo*. Experiments in this laboratory with labelled uracil, injected in low dosage, have shown no increase in uptake when uridine kinase activity is increased[23]; to prove that the salvage pathway is nevertheless accelerated, it would be necessary to show that the endogenous 'pool' of uridine or uracil is enlarged.

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INHIBITION OF GLUTAMIC-ASPARTIC TRANSAMINASE BY ANTIBODIES

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CINADER[1] has reviewed a good deal of the work on the neutralization of enzymes by antibodies but very few of the numerous investigations concerning glutamic-oxaloacetic transaminase (GOT) enzymes are of this nature. The reasons for this may be: transamination involves a shift of amino-groups from one compound to the other rather than the formation of a totally new product. Size of substrate, which as pointed out by Cinader[2] is so important in these reactions, is here comparatively small. The apoenzyme, against which one would like to direct the antibodies, has only recently been described by Banks[3] as purified and, finally, the apparent fact, discovered by Fleisher[4], of a family of 'isoenzymes' in a given tissue does not favour this method of approach.

Córdoba *et al.*[5] have been able to obtain enzymatic inhibition by using sera from GOT-injected rabbits and hens. This article describes some of the serological characteristics of this neutralization.

Human hearts and livers, monkey (*Macacca cynomolgus* hearts and livers and pig hearts were cleaned, minced and homogenized with three volumes of cold water. Fibrous material was removed by gauze filtration and 30 gm. ammonium sulphate added to the supernatant and the precipitate discarded.

Table 1. PERCENTAGE INHIBITION OF CRUDE MONKEY HEART GOT WITH HEN ANTI-MONKEY HEART PLASMA GLOBULINS

Plasma inhibitor (mgm./protein)	Enzyme activity tested 10 units	27 units	30 units	40 units
0·118	10	2	0	0
0 237	21	7	3	0
0 475	43	22	14	2
0·950	73	58	50	38
1 900	94	80	71	60

Table 2. EFFECT OF TIME OF INCUBATION AND DILUTION ON THE INHIBITION OF MONKEY HEART GOT WITH HEN ANTIBODIES

Incubation time (hr.)*	Per cent inhibition of 15 units	Incubation vol.† (ml.)	Per cent inhibition of 9 units
2	75, 73	0·4	83, 86
24	78, 78	0·8	84, 87
48	73, 73	1·3	86, 83
—	—	1·7	86, 83

* All tubes incubated 2 hr. at room temperature and additional time in the refrigerator at 4° C.

† Final volumes of 3 7 ml. Experiment conducted for 2 hr. incubation at room temperature.

Table 3. INHIBITION AND HÆMAGGLUTINATION OF MAMMALIAN TISSUES GOT WITH HEN ANTI-MONKEY HEART GLOBULINS

Bleedings of hen No. 2	Enzymes tissue sources: Monkey heart Inhib. per cent	Monkey heart Hæmgl. titre	Pig heart Inhib. per cent	Pig heart Hæmgl. titre	Monkey liver Inhib. per cent	Monkey liver Hæmgl. titre	Human liver Inhib. per cent	Human liver Hæmgl. titre
Pre-immuniz.	0	0	0	12	0	0	0	12
First week	93	1,536	95	1,536	88	192	50	384
Second week	85	384	58	96	50	192	50	96
Third week	10	48	45	24	16	12	—	12

Inhib., percentage inhibition tested with 10±3 units of enzyme. Hæmgl., inverse of titre in the tannic acid hæmagglutination test.

According to Green *et al.*[6], 40 gm. of the same salt precipitates the bulk of the enzymes. This precipitate was dissolved in 0·9 per cent sodium chloride dialysed extensively and stored in small vials in a freezer.

Immunization was carried out by injecting into the wing veins of adult hens (local strain 500) 1 ml. of the monkey heart preparation containing 18·7 mgm./protein/ml. analysed by the Folin reagent of Kabat and Mayer[7]. Monkey liver preparation, at a concentration of 24 mgm./protein/ml., was similarly injected three times a week. At the end of each week, blood was collected into centrifuge tubes, filled with 0·25 vol. 1·1 per cent sodium citrate, and the plasma fractionated according to Gregory[8], by addition of one-third (v/v) saturated solution of ammonium sulphate at room temperature. The γ-globulin precipitate was dissolved in saline, dialysed and stored in the cold at − 30° C.

The measurement of enzymatic activity and the serological inhibition was obtained by spectrophotometric determinations of the oxalacetic acid produced by the action of the enzymes in a set-up as follows: 0·1 ml. of enzyme dilution, pyridoxal phosphate (20 μgm.), 2 ml. substrate in *tris* buffer at *p*H 8·6 with 200 μmoles aspartic and 2 μmoles α-ketoglutaric acids, 100 μmoles sodium tetraborate and 1 μmole magnesium sulphate. The controls were completed to volume with 0·8 ml. saline and the experimental ones with 0·1–0·3 ml. of the γ-globulin solutions plus the required saline. The oxalacetic acid formed was measured at 1-min. intervals by readings of the optical density at 290 mμ (1 unit: 0·001 *O.D.*/min : 0·0011 μmole oxalacetic acid) in the Beckman *DU* spectrometer. For the evaluation of the antibody in the neutralizing sera Boyden's tannic acid hæmagglutination test, as modified by Stavitsky[9], was used. 0·25 mgm./protein of each tissue enzyme preparation was used to sensitize 1 ml. of a 1 : 40 suspension of absorbed tannic acid-treated normal red cells. The agglutination patterns were examined 18 hr. later.

The inhibition plots using monkey heart enzymes with hen anti-monkey heart γ-globulins presented in Table 1 show a correlation between neutralization and amount of neutralizer on one hand, and degree of inhibition and enzyme concentration on the other. These results are comparable with recent findings of Gregory and Wróblewski[8] in the lactic acid dehydrogenase anti-L.A.D. systems.

Results in Table 2 show that the inhibition is not altered by changing the time of interaction of enzyme (antigen) with antibodies from the usual 2-hr. period up to 48 hr. It is also shown how the neutralization effect is not sensitive to certain changes in volume in that length of time. Variations of volume up to four times did not modify appreciably the observed neutralization. In this respect, the transaminase–anti-transaminase system behaves like most antigen–antibody reactions[7].

In Table 3 are given the serological results obtained using monkey heart anti-transaminase against different tissue enzyme preparations. The activity of the enzyme sources was pre-set at 10 ± 3 units and were tested for antibody neutralization by the usual procedure. The same enzyme extracts were then used for sensitizing 'tanned' erythrocytes and tested against the anti-monkey heart globulins in the hæmagglutination test. The first week's blood shows a titre (inverse of the dilution) of 1,536 and an inhibiting power of 93 per cent when tested with the homologous antigen. The titre in the second week's blood is only 384 and the inhibiting power 85 per cent. By the third week, the titre has dropped to 48 with only 10 per cent of neutralization capacity. The pig heart preparation, when tested against the same antiserum, behaved strikingly similar. Both human and monkey liver enzymes, on the contrary, were agglutinated and inhibited to a much lesser degree.

Absorption on the anti-monkey heart globulins was carried out with the three heterologous extracts during one week in the refrigerator. The supernatant was tested again for neutralization to the monkey heart preparation; but this time no inhibition was demonstrable. This result suggests a combination of the anti-enzyme(s) with heterologous tissue extract components with a consecutive removal of inhibitors from the antibody solution.

The enzyme–anti-enzyme combination produced discrete precipitates. Attempts to locate the missing activities by re-suspending the precipitates in saline with co-factor and substrate failed. The addition of an excess of soluble enzymes, in an attempt to bring the precipitates to antigen excess and liberate the supposedly trapped activity, was also unsuccessful.

The neutralization observed seems compatible with an immunological effect. The plasma globulins from the immunized animals showed inhibitory power subsequent to the injections, and this was reflected in an agglutinating ability roughly parallel to the neutralization of the enzyme(s). The fact that the precipitate was inactive, favours the hypothesis of a close combination of the enzyme(s) with the antibodies. Although this interpretation is attractive, confirmation awaits the purification of the GOT enzymes in these extracts by methods like those used by Córdoba and Ortega[10]. The absorption of the inhibiting activity by heterologous tissue extracts also runs in favour of the immunological nature of the phenomenon, and here again only quantitative immunochemical analyses with homogeneous antigens can prove the validity of this conclusion.

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LIPID METABOLISM IN HIGHER PLANTS*

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INFORMATION concerning the anabolic and catabolic fate of lipids in higher plants merits the attention of not only the plant biochemist and the plant physiologist but also the comparative biochemist who is interested in the metabolic mechanisms of a broad spectrum of organisms. As summarized in Fig. 1, during the past ten years we have been examining some of these mechanisms in higher plants; here I shall limit myself to a discussion concerning the degradation and synthesis of straight chain fatty acids. I shall not cover areas involving phospholipids or the glyoxylate bypass.

α-Oxidation. In 1952 Newcomb and Stumpf[1] observed that carbon dioxide labelled with carbon-14 was rapidly released when palmitic-1-^{14}C was added to a homogenate of cotyledons of germinating peanut seeds. Differential centrifugation of the homogenate yielded several fractions of which both the microsomal fraction (obtained by centrifugation at 100,000*g* for 1 hr.) and the supernatant fraction were active. However, while the supernatant fraction only decarboxylated palmitic-1-^{14}C, the microsomal fraction could readily release $^{14}CO_2$ from both internally labelled and carboxyl labelled palmitic acid, providing NAD$^+$ had been added to the reaction mixture. When the supernatant soluble proteins were submitted to ammonium sulphate fractionation, all decarboxylation activity was lost, but this could be restored on addition of boiled crude supernate. Castelfranco *et al.*[2] isolated and identified the unknown co-factor as glycolic acid. Further experimentation readily proved that an α-hydroxy acid in the L-series could substitute for glycolic acid in the decarboxylation of palmitic acid. In 1956 Stumpf[3] demonstrated that glycolic acid functioned as a substrate for glycolic oxidase for the purpose of forming hydrogen peroxide. In addition to carbon dioxide, long-chain aldehydes were also formed. Finally, in 1959, Martin and Stumpf[4] described the interrelationship of two discrete enzymes: (*a*) a specific fatty acid peroxidase which catalysed the decarboxylation of long-chain fatty acids from C_{13} to C_{18} to yield carbon dioxide and a long-chain aldehyde containing one less carbon atom ; (*b*) a long-chain aldehyde dehydrogenase which in the presence of NAD$^+$ oxidized the aldehydic group to the carboxylic group.

This reaction sequence has been named α-oxidation to distinguish it from the more widespread β-oxidation sequence which occurs in plant cells. α-Oxidation is a limited type of degradation but has been found in several plant tissues. The commonly occurring plant peroxidases are totally inactive in this reaction. The role of α-oxidation in plant cells is somewhat puzzling although one can speculate possible functions: (*a*) as a source of long-chain aldehydes and their reduction products, long-chain alcohols, which may then be used as substrates for the formation of waxes; (*b*) for the formation of odd-chain fatty acids which may then enter the β-oxidation sequence and be converted to propionic acid.

β-Oxidation. Although considerable difficulty has been experienced in the past in attempts to demonstrate the occurrence of β-oxidation, in 1956 Stumpf and Barber[5] realized that the success of its demonstration depended on using only trace amounts of fatty acid substrates since amounts greater than 0·5 μmole proved to be toxic and markedly inhibited mitochondrial respiration. Barber was able to prepare mitochondrial particles which, when supplemented with ATP, CoA, Mn^{++}, NAD$^+$, NADP$^+$, and a TCA cycle acid, could readily oxidize a long series of fatty acids to carbon dioxide and water. Mitochondrial preparations from peanut cotyledons, avocado mesocarp, lupine and pine seedlings have been found to contain the system. Of interest is the observation that propionic acid is also readily oxidized.

Modified β-oxidation of propionic acid. The comparative biochemistry of propionic acid is of interest. In 1957, Flavin and Ochoa[6] had shown that the following sequence holds as the major pathway of oxidation in animal tissues:

$$CH_3CH_2COOH + CoA + ATP \rightarrow CH_3CH_2CO - CoA \quad (1)$$

$$CH_3CH.CO - CoA + CO_2 \rightarrow CH_3CH(COOH)CO - CoA \rightarrow \text{succinyl} - CoA \quad (2)$$

Recently it has been shown that biotin is the co-factor for the carboxylation reaction and a cobamide derivative is the co-factor for the subsequent isomerization reaction. Of some interest is the repeated observation made by many workers that vitamin B_{12} or its derivatives do not occur as a functioning unit in the normal plant cell. Plants can passively accumulate vitamin B_{12} from the soil environment; but the vitamin does not appear to participate in any reactions in the plant cell. A notable exception is the observation made recently by Kliewer and Evans[7] that cobamides occur in nodules of roots of legumes and the alder tree and may indeed play an important part in the process of symbiotic nitrogen fixation. Because of the absence of vitamin B_{12} or its derivatives in the normal plant cell, the Flavin–Ochoa mechanism can be dismissed as occurring in plants. Moreover, Hatch and Stumpf[8] have recently shown that methylmalonic acid is inert in plant slice experiments although it can be formed by the carboxylation of propionyl CoA.

On the basis of the kinetics of formation of $^{14}CO_2$ from differently labelled propionic acid, the isolation of β-hydroxypropionic acid from reaction mixtures, and the location of radioactive carbon in succinic acid (formed the reaction mixture) depending on the position of the label in the initial substrate, pro-

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* Substance of two special lectures given at Bedford College, University of London, on February 19 and 21. A more detailed account of these lectures will appear in *Biological Reviews*, 1963. Since this article is not intended as a review of the subject, only references pertinent to the discussion are cited.

† Senior Postdoctoral Fellow of the U.S. National Science Foundation, 1961; John Simon Guggenheim Fellow, 1962.

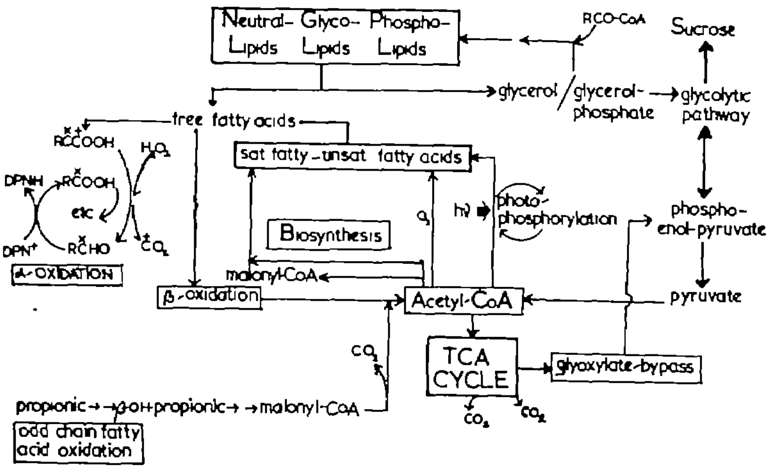

Fig. 1. General summary of lipid metabolic activities in higher plants

pionic acid, Giovanelli and Stumpf[9] have proposed the pathway outlined as follows:

$$\begin{aligned} &CH_3CH_2COOH + CoA + ATP \rightarrow CH_3CH_2CO - CoA \rightarrow \\ &CH_2 = CHCO - CoA \rightarrow CH_2OHCH_2CO - CoA \rightarrow \\ &CH_2OHCH_2COOH \rightarrow CHOCH_2COOH \rightarrow \rightarrow \\ &\quad CoA - COCH_3 + CO_2 \rightarrow \text{TCA cycle acids} \qquad (3) \end{aligned}$$

It should be noted that Vagelos and Stadtman[10] have proposed a slightly different pathway for propionate degradation in several bacteria. Their pathway differs from that which occurs in plants in that β-hydroxypropionyl CoA, formed from propionic acid by similar reactions, is oxidized as a CoA derivative and therefore the carbon dioxide is derived from C-3 of propionic acid rather than from C-1 (as in plants), and acetate is formed from carbons 1 and 2 rather than from carbons 2 and 3, as in plants.

Propionic acid is a rare acid in plant tissues. It is quite possible that by the participation of an effective oxidative pathway, the plant cell can readily remove any newly formed propionic acid and thereby propionic acid-levels never build up to a significant concentration. Moreover, propionic acid may in part act as a precursor of the pyridine ring in the alkaloids nicotine and ricinine. Byerrum *et al.*[11] have recently suggested that carbons 1 and 2 of the pyridine ring may be derived from carbons 3 and 2 of propionic acid. Similar results have been obtained by Waller and Henderson[12] in their studies on the pyridine ring derivative of ricinine. In addition, Hatch *et al.*[8] have shown that β-alanine is formed from propionic acid in a variety of plant tissues. It is also of interest that glycerol, another product of lipid metabolism, contributes its carbon skeleton to carbons 4, 5 and 6 of the pyridine ring[13]. Thus we can implicate two substrates in the area of lipid metabolism, propionic acid and glycerol, as important precursors of pyridine biogenesis and CoA biogenesis (β-alanine).

Table 1. COMPOSITION OF FATTY ACIDS IN AVOCADO MESOCARP AND NEWLY SYNTHESIZED FATTY ACIDS BY PREPARATIONS OF AVOCADO MESOCARP

Type of tissue	Percentage Palmitic	Stearic	Oleic	Linoleic
Avocado mesocarp	20	1	60	18
Mitochondrial lipid	15	1	70	15
Per cent incorporation of acetate-^{14}C by various preparations:				
Slice	48	10	31	3
Mitochondrial prep.	50	30	20	1
Acetone powder extract	25–40	60–75	0	0
Sonicate prep.	14	59	9(+13)*	—

* 13 per cent includes polar long-chain fatty acid.

Biosynthesis. In 1952 Newcomb and Stumpf[14] fed radioactive substrates to slices of cotyledons of maturing (synthesizing) and germinating (degradative) peanut seeds. In both systems, acetate was the most effective of several tested for incorporation into long-chain fatty acids. In 1957, Stumpf and Barber[15] initiated a series of investigations of the incorporation of acetate into long-chain fatty acids by various preparations obtained from avocado mesocarp. Table 1 summarizes some of the results obtained with various preparations. Note the alteration of the composition of newly synthesized fatty acids from acetate by the different preparations. It would appear as if the unsaturation process is rather unstable and tends to disappear in the various cell-free systems. A more detailed analysis of these preparations will now be undertaken:

Particulate system. Stumpf and Barber[15] prepared a particulate system from ripe avocado mesocarp. This preparation possessed TCA activity, oxidative phosphorylation, and β-oxidation, three typical systems always found associated with mitochondrial particles. The soluble proteins freed from mitochondrial particles had no capacity to synthesize fatty acids, while the particulate system readily incorporates acetate into palmitic, stearic and oleic acids. ATP, CoA, Mn++ and carbon dioxide were essential co-factors. When either stearic or palmitic acid is added as substrate both are incorporated unchanged into triglycerides and phospholipids, providing both ATP and CoA are present. Thus while these acids are readily activated to acyl CoA derivatives, there is no evidence that they are elongated or serve as precursors of oleic acid. Mudd and Stumpf[16] have considerably extended the original observations, particularly with regard to the factors involved in oleate biosynthesis from acetate. The critical factor proved to be the degree of aerobiosis in a given reaction mixture. Thus under strictly anaerobic conditions acetate is incorporated only into palmitic acid (61 per cent) and stearic acid (39 per cent) whereas under aerobic conditions 53 per cent palmitic and 1 per cent stearic are formed as well as 46 per cent oleic. Oleic acid synthesis is not inhibited by cyanide or azide. At first glance it would appear as if stearic acid had a precursor relationship to oleic acid; however, under strongly aerobic conditions, while oleic acid is formed from acetate readily, added palmitic or stearic acid does not affect oleic synthesis. Either stearic acid is not a precursor or if it is it must be enzyme bound and therefore not in equilibrium with exogenous stearic acid.

Acetone-powder extracts of particulate fraction of avocado mesocarp. Barron, Squires and Stumpf[17] have prepared extracts of acetone powders of avocado mesocarp particles which readily incorporate either acetyl CoA or malonyl CoA into long-chain fatty acids with little, if any, formation of oleic acid. These acetone powders are extremely stable in that they can be kept for two or more years at − 10° with little if any loss in activity. The saturated fatty acid synthesizing system is extremely sensitive to avidin with 20γ giving a 78 per cent inhibition when acetyl CoA is the initial substrate; with malonyl CoA as the substrate avidin is ineffective. ATP and carbon dioxide are required with acetyl CoA as substrate whereas with malonyl CoA these co-factors are not required. The properties of this system seem to be very similar if not identical to those described by Lynen[18] for yeast system and by Wakil[19] for chicken liver system. However, in contrast to these two systems, the avocado system is always associated with particles.

Sonicated avocado particles. Since acetone treatment of the particulate system completely destroys the capacity of the particles to synthesize oleic acid, several attempts were made to solubilize the unstable enzymes associated with these particles[20]. An active preparation could be obtained by exposing the particles to a brief sonication under hydrogen with a subsequent high-speed centrifugation. The clear supernatant had the capacity to synthesize long-chain fatty acids. Surprisingly, when malonyl CoA was the initial substrate only palmitic and stearic acids were formed whereas with acetyl CoA 59 per cent of the total radioactivity was associated with stearic acid, 14 per cent with palmitic, 9 per cent with oleic, and the remaining 13 per cent with a long-chain polar acid having properties of a hydroxy acid. Addition of octanoic, decanoic, palmitic and stearic acids or palmityl CoA and stearoyl CoA to the enzyme preparation did not lead to oleic acid synthesis. It would appear as if there are two separate pathways for the synthesis of fatty acids, one for unsaturated and the other for saturated fatty acids. What the nature of these pathways is remains for future exploration.

Light-stimulated chloroplast incorporation of acetate. Chloroplasts[21] are usually considered to be the site of photosynthesis and consequently the site of sugar biogenesis. They are unusually rich in lipids, which presumably maintain the lipo-protein insulating layers in the grana where the photo-excitation process occurs. Smirnov[22] has already reported a biosynthetic system in spinach chloroplasts which requires light, ADP, Mg^{++}, CoA and acetate to form long-chain fatty acids. We have recently examined lettuce chloroplasts[23] for their capacity to synthesize fatty acids from acetate and have described a system which requires ATP or ADP, inorganic phosphate, CO_2, NADPH, and oxygen to synthesize palmitic and oleic acids from acetate. In the dark the system incorporates only small amounts of acetate; but in the light a very marked increase is consistently observed. Light undoubtedly participates by activating a non-cyclic photophosphorylation mechanism which thereby supplies to the lipid-synthesizing system ATP, NADPH, and oxygen, all important components in the incorporation of acetate into long-chain fatty acids. However, there is also evidence to suggest that light has an additional function in the synthesis of fatty acids. Apparently both photophosphorylation and lipid synthesis are tightly coupled and any disturbance in this coupling will disrupt lipid synthesis. Thus, ammonium ions and CMU, both effective inhibitors of photophosphorylation, will greatly decrease acetate incorporation into long-chain fatty acids. Moreover, ATP, NADPH and oxygen, the primary products of non-cyclic photophosphorylation, will not replace the requirement for light.

In summary, an examination into some of the mechanisms in higher plants for the synthesis and degradation of fatty acids has revealed several new and interesting systems as well as systems which are very similar to, if not identical with, those found in other living organisms. The incorporation of this knowledge into such diverse systems as carbohydrate and alkaloid biogenesis and photosynthesis once more demonstrates the interdependence and intermeshing of seemingly unrelated biochemical areas.

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METAL DEFICIENCIES IN BANANAS

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Manganese

THE only record known to me of a minor element deficiency in the banana is that for zinc described by Moity[1] on Dwarf Cavendish bananas in the French Guinea. Recently, a peculiar disorder has been observed on the Lacatan, Robusta and Gros Michel varieties in a few areas; the results of foliar spray treatments and leaf analysis suggest a manganese deficiency.

The earliest visible symptom is a marginal, interveinal chlorosis on the younger leaves. This chlorosis advances towards the mid-rib, and pinhead-sized dark brown and black spots appear on the upper surface of the leaf, mainly within the chlorotic area; spots in the green area of the leaf usually have a pale yellow margin. The spots, mainly confined between the veins, increase in diameter to 1–3 mm. and begin to coalesce until oblong necrotic patches with orange margins are formed. Necrotic areas eventually fuse to form a brown margin often around the entire leaf. The margin appears crinkled, drawn together and gives the leaf a boat-shaped appearance when untorn. Examination of the necrotic band reveals many brown or black, circular and oval spots on the dead tissue. The inner part of the leaf-blade remains green with varying numbers of necrotic areas with orange-yellow margins. Marginal necrosis advances with age of leaf until eventually the whole leaf turns brown and dies (Fig. 1).

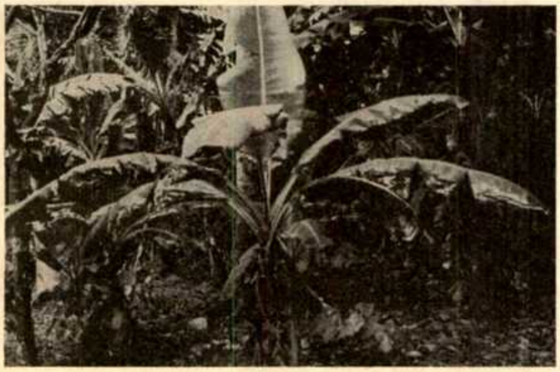

Fig. 1. Banana plant suffering from a deficiency of manganese

Affected plants usually produce bunches of fruit with widely distributed raised spots, dark brown to black in colour. Meredith[2,3] has described this unusual form of fruit spot and has discovered that the fungus *Deightoniella torulosa*, which causes 'speckle' (fruit-spot or 'swamp-spot') of Jamaican bananas also causes the scab-like spots on the fruit. In fact, on affected fingers there is usually a range of spots from the tiniest fruit spot with water-soaked halo to the large scabs up to 6 mm. in diameter (Fig. 2). The fact that the scab-like spots on the fruit were confined to certain areas, and had not spread, strongly suggested that some other factor, possibly a soil or nutritional condition, was associated with the disorder.

Preliminary examination revealed that there were two soil types on which the condition occurred. One known as 'Wait-a-bit clay', a normally acid, highly leached soil, showed up the disorder on any alkaline spot such as roadside ditches with limestone washings from the road. The other soil type, known as 'Killancholly clay', was a rendzina and the condition occurred on areas with very high lime contents.

Foliar spray treatments were carried out on two severely affected plants using the following treatments: 2 per cent magnesium sulphate, 1 per cent manganese sulphate, 1 per cent ferrous sulphate, 1 per cent borax, 1 per cent ammonium molybdate, 1 per cent zinc sulphate, 1 per cent ammonium sulphate. There was also a soil treatment of 16 lb. sulphur/root given once a year. The foliar spray treatments were given once every two months at first, but after four months this was changed to once every month. The manganese sulphate treatment improved the condition of the affected plants. At the time of shooting one of the plants so treated had the four youngest leaves unaffected, the next three leaves slightly affected and the last two leaves severely affected. The other plant had all eight leaves unaffected. The treatment has also considerably reduced the degree of spotting or 'scabbing' on the fruit.

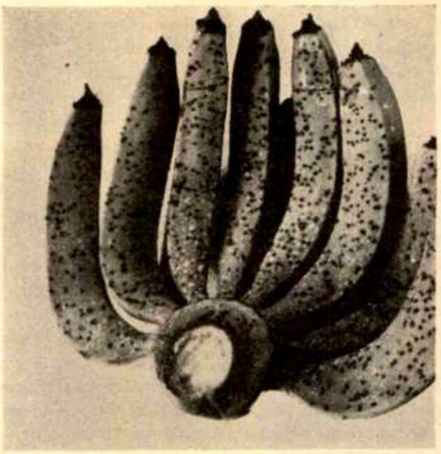

Fig. 2. Hand of fruit from manganese-deficient plant. Note wide distribution of spots

A weekly foliar spraying treatment of a similar nature as the former was carried out and plants sprayed with manganese sulphate gave response within two weeks. Leaves produced after this interval were all unaffected and after fourteen weeks the condition was non-existent. Leaf samples were taken from affected and also unaffected plants in one such area at Hopewell, St. Mary. The method of sampling is that of Boland[4]. The results showed that manganese was very significantly lower ($P < 0{\cdot}001$) in affected leaves.

The relation of soil *p*H on the 'Wait-a-bit clay' and the disorder described are in agreement with the existence of a manganese deficiency, as the main factor which controls the available manganese in the soil is *p*H. The more acid a soil, the greater is the availability, the more alkaline a soil the less is it.

Usually where the black scab occurs on banana fruit there is every gradation in size of spots from the typical 'speckle' to the scabs of varying diameters. Both are caused by the same fungus, but the black spots or scabs only exist in certain areas where, as a result of the lime content of soils, the plants suffer from a manganese deficiency. Stahel[5] describes *D. torulosa* as a powerful parasite, and thus it may be that 'fruit-spot' or 'speckle' may be reduced, though perhaps not prevented, by optimum levels of manganese in the banana plant. It may also be that apart from the factors detailed by Meredith[2] as affecting the incidence and degree of speckling the available manganese in the soil might be operative. Experiments are to be set up to test this.

Zinc

A peculiar chlorosis and narrowing of the leaves of Lacatan bananas has been observed on the St. Catherine plains, Jamaica, for a number of years. Preliminary work[6] showed that the soils and the leaves of plants in such areas were very high in available phosphate ('Truog') and percentage phosphate, respectively. The results of recent foliar spray treatments and the similarity of the foliar symptoms to those described by Moity[1] on Dwarf Cavendish bananas in the French Guinea suggest a zinc deficiency.

Symptoms may appear in 1-month-old plants, but they are more commonly observed in plants 2–5 months old. The foliage may appear bunched and the growth habit stunted; leaves are uniformly narrow, or narrower towards the tip end than the petiolar end, or less often, narrower towards the petiolar end than the tip-end. Length–breadth ratios of leaves of up to 8·6 : 1 have been observed. Affected leaves may be (*a*) apparently wholly green, (*b*) green, but with transverse pale stripes bleached of much of the chlorophyll alternating with transverse greener bands, or (*c*) bleached of the normal green colour thus being creamy white (sometimes the leaves are almost completely bleached at the petiolar end but green with transverse chlorotic bands towards the tip end). In the pale green, bleached areas of the leaves, numerous lighter coloured blemishes, oblong in shape, 1–5 mm. in length, and parallel to the leaf venation develop, and many of these 'dashes' become necrotic on ageing. A number of the chlorotic or whitish areas on affected leaves may develop into necrotic spots, 2 or more inches wide, and with concentric rings. Older leaves sometimes show a general bronzing or chlorophyll deficiency with a brown scorching and rotting along the margin or over the entire leaf-blade.

Leaves described under (*b*) and (*c*) soon become yellowed, particularly in the pale green and bleached areas; this yellowing is usually more pronounced on the side facing the Sun. Leaves described under (*a*) may also show a yellowing, but there are plants which do not have the chlorotic striping or any noticeable narrowing of the leaves but have yellow patches particularly near the midrib on the side of the leaf facing the Sun. These yellow patches are sometimes 'burnt out', leaving dead areas. Affected plants may produce a few normal leaves which, in turn, may be followed by affected ones. Many plants grow out of the condition but it may recur when the plants have fruited. Plants showing symptoms of the deficiency at shooting develop more marked symptoms with yellowing and bronzing during fruit development. On some plants without discernible symptoms before fruiting, as the fruit develops the leaves become bronzed and yellow, particularly near the petiolar ends.

On severely affected plants fruit development is slow, the young bunch remaining horizontal or near horizontal for an unusually prolonged period. The fingers seem twisted and are shorter, smaller, thinner and usually lighter green in colour than those of normal fruits. The distal ends of the fingers of affected fruit are nipple-shaped (Fig. 3) in appearance whereas normal Lacatan fingers possess a blunt distal end. A comparison of fruit from affected plants with that from unaffected plants with a similar number of hands showed a considerable reduction in weight of fruit from affected plants.

The soils in St. Catherine where the condition exists are known as Caymanas sandy loam and Caymanas clay loam. Symptoms are usually more severe on the sandier areas. These soils are alkaline in reaction, usually high in available phosphate and potash, well drained and were originally some of the highest yielding banana soils in the island.

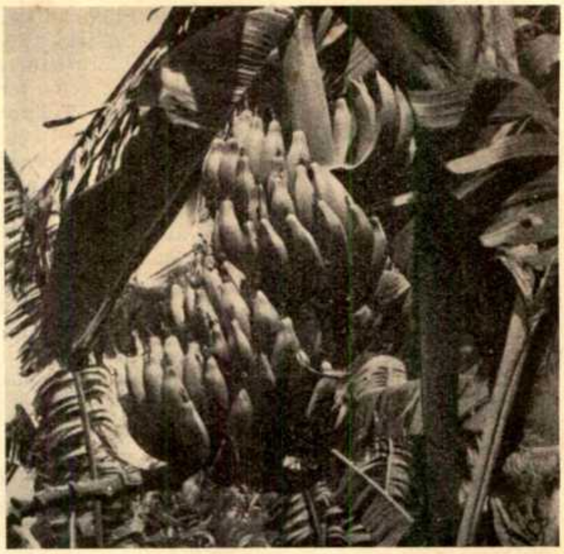

Fig. 3. Fruit from zinc-deficient plant

A weekly foliar spray treatment was carried out on three each severely affected young banana plants using the following treatments: 1 per cent zinc sulphate, 1 per cent manganese sulphate, control (water); 'Triton *X*-114' was added to all solutions. Despite some scorching of the foliage, plants sprayed with zinc sulphate produced greener leaves of normal shape and size. Later, another foliar spray treatment was carried out using weaker concentrations of the salt: 0·5 per cent zinc sulphate, 0·5 zinc sulphate neutralized with calcium hydroxide, control (water), all with 'Triton *X*-114'. Within two weeks there was a response to zinc sulphate; leaves produced after this interval were unaffected and in three months the condition had disappeared.

The foliar symptoms of the disorder, with the exception of the yellowing, closely match the symptoms of zinc deficiency as described by Moity[1]. Sunlight and/or temperature may be partly responsible for yellowing but, since there were plants just as exposed but not showing yellowing, it is possible that soil or nutritional factors predispose plants to become yellow. This may be wholly or partly the zinc deficiency. Zinc-sulphate sprayed plants did not show up marked yellowing but there was still a discernible difference in colour between the sides of some leaves which faced the Sun and the shaded side. It is not known whether this difference is due to sunlight only or to some other factor in combination with it. Many workers have shown that high light-intensities are conducive to the development of zinc-deficiency symptoms.

The soil factors responsible are likely to be alkalinity, sandy texture and high phosphate content. Numerous investigators have observed that zinc deficiency usually occurs on soils of *p*H 6 or higher. Camp[7] concluded ". . . it seems reasonably well established that the availability of zinc declines as the *p*H of the soil rises, the critical point being between the *p*H range of 5·5 and 6·5". Many workers, for example Chandler[8], have noted that the deficiency is most apt to show up on sandy and gravelly soils. Zinc deficiency has frequently been reported in soils containing abnormally high contents of soluble or total phosphates. Some investigators have reported reduced zinc availability when phosphatic fertilizers are added to the soil.

Mention must be made of the similarity of this disorder with many of the symptoms of alkali chlorosis described by Wardlaw[9] as occurring in certain alkaline areas around Delugé in Haiti. It seems that this alkali chlorosis may in fact have been partly if not wholly a zinc deficiency. There is also the possibility of mistaking zinc deficiency for nitrogen deficiency, and virus diseases such as described by Wardlaw and McGuire[10] as occurring in Brazil, Wardlaw[11] as occurring in Trinidad, or infectious chlorosis as described by Magee[12] in Australia as some of the foliar symptoms are close to those of zinc deficiency. Moity[1] has also directed attention to this.

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PLANT ANALYSIS BY X-RAY FLUORESCENCE SPECTROGRAPHY: DETERMINATION OF CALCIUM AND POTASSIUM

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X-RAY fluorescence spectrography[1,2] is a powerful non-destructive analytical technique capable of determining elements above atomic number 12 (magnesium) with speed and accuracy over a wide concentration-range. There is a rapidly developing application of the method in the geological[3–5] and biological[6–10] sciences. Vacuum or helium path instruments now permit quantitative analysis of elements lighter than titanium, among which are many of the major rock-forming and biologically significant elements.

Work of the Nature Conservancy at Bangor[11] is concerned with the ecology of mountain grasslands, and in this work there is a need for a wide range of rock, soil and plant analyses. Many of the analyses of macro- and micro-elements involved appeared likely to be amenable to rapid and accurate determination by the X-ray spectrograph. Accordingly, a Hilger and Watts vacuum spectrograph, the Flurovac, has been installed and the results reported here were obtained with this instrument. Some of the practical details of sample preparation and instrumental technique in X-ray fluorescence spectrography of plant material are discussed and the X-ray data correlated with potassium and calcium determinations by flame photometry.

As an illustration of the preparation of calibration curves we report here results for analyses of mixed herbage grasses, selected from a range of material kindly provided by the Soil Chemistry Department, N.A.A.S., Ministry of Agriculture, Bryn Adda, Bangor. Twenty-five samples covered a range in potassium content of 5·7–25·3 mgm./gm. dry matter and twelve samples a range of calcium content of 4·6–9·6 mgm./gm. dry matter. The selected samples were afterwards analysed by flame photometry after ashing at 520° C., potassium being analysed directly on the diluted ash solution and calcium after an oxalate precipitation procedure.

We have previously reported[12] that potassium, calcium, manganese, iron and zinc are elements present in adequate amount for quantitative work by direct X-ray fluorescence spectrographic analysis of vegetation dried at 80° C. and milled to pass a 0·5-mm. sieve. It is possible to get a rapid and moderately reproducible result for these elements when milled vegetation is loosely tapped down in a sample-holder having a Melinex (Mylar) window through which the primary X-ray beam and the secondary emission both pass. Radiation from light elements such as potassium and calcium is reduced in intensity by the presence of even the thinnest practicable Melinex film (about 0·006 mm.). Additionally, packing and particle size have an important effect on the amount of secondary radiation emitted. Both finer particle size and greater degree of close packing increase the obtained count. Loose packing in a Melinex window holder is not generally precise enough for quantitative work, but can be useful for rapid comparative examination of samples, particularly when the material is required for further destructive analyses.

The simplest and quickest sample preparation should be used for quantitative analysis which is commensurate with adequate radiation emission and with reproducibility of count from replicate samples. To improve the reproducibility over that obtained by packing loosely in a holder, a 2·0-gm. sample is compressed into a disk, of 32 mm. diameter, large enough to cover completely the sample-window of the spectrograph. The disks are made in a tool-steel mould using a hydraulic press (Research and Industrial Instruments, Ltd., London, Type *H* 30–1 *Mk*. II) at an indicated operating pressure of 5,000 lb./in.^{2}, moderate deviations from which do not significantly affect count-rate. Aliquots of a sample milled to pass from 0·5 to 2·5 mm. sieves did not give significantly different counts when compressed into disks, but finer particles bond together more effectively. Disks made in this way are reasonably rigid but can be greatly improved by being given a cellulose backing. A layer of powdered cellulose (Whatman's standard grade) is added above the plant material in the mould and the sample with cellulose backing is then compressed into a disk. The cellulose bonds well with the plant material, increasing the rigidity of the sample.

Table 1

Weight of plant material (gm.)	Mean disk thickness (mm.)	Count (Se $K\alpha$) without Se backing	Count with Se backing
2·0	2·8	528	12,089
2·5	3·4	521	7,891
3·0	4·1	533	4,767
3·5	4·7	536	2,552
4·0	5·5	528	1,634
4·5	6·2	532	1,133
5·0	7·0	520	882
5·5	7·6	536	705
6·0	8·2	523	609
7·0	9·6	525	524

The question of a suitable thickness of disk required some investigation. Mechanical strength of the disks is too low under vacuum when less than 1 gm. of material is compressed into a 32-mm. diameter disk. Tungsten radiation, which is the primary radiation used, has a deep penetration in a very light matrix such as is provided by plant material. The thickness of material through which secondary fluorescent radiation travels to contribute to the analytical count (the effective sampling depth of the material), depends on the nature of the sample matrix and on the element being determined. The lighter the matrix, the deeper the effective thickness for any element; the lighter the element, the less the effective thickness in a similar matrix. The magnitude of this effect is negligible for practical thicknesses of sample of heavy matrix, but is appreciable for heavy elements in a light matrix, such as plant material. The magnitude of this effect was tested by counting radiation emitted by a pure element, used as a backing on increasing thicknesses of sample disk. When emitted secondary radiation from the backing element failed to penetrate the grass, the count-rate for the element remained constant with increasing thickness of the sample disk. In this way curves could be drawn for each element, plotting thickness of sample disk against count-rate.

If the thickness of sample is less than that at which counts with and without backing are identical, then variations in count-rate may be caused by variation in thickness of disk. Thickness of disk can be carefully controlled but preparation is then tedious, and too low a sample-thickness means that the count-rate is not the optimum obtainable from the specimen. Table 1 shows the general form of the relationship of disk thickness to count, when a backing sample of pure selenium is used behind varying thickness of disk (without backing cellulose). Since it is easier to prepare disks from a weighted aliquot, corresponding weights are included in Table 1.

From data of this type, it is possible to say for any particular element in a plant material matrix what is the thickness of sample disk required to ensure that minor thickness variations have no effect on count-rate. Table 2 shows minimum weight of disk required to ensure complete secondary emission within the specimen for a given element, against atomic number of the element.

Tables 1 and 2 show that pure selenium as a backing contributes to the selenium count up to 6·5 gm. (8·9 mm.) of sample disks. However, if a grass matrix containing 5 per cent added selenium is made up in disks of different thicknesses, an increase in counts, of practical significance, is only obtained up to a 3·0 gm. (4·1 mm.) disk. The effect is negligible for the elements we find readily detectable in non-ashed material (up to zinc) at the concentrations in which they are present, if a standard sample of 2 gm. (2·8 mm.) is taken. When ashed material is used for determination of other elements, the depth of penetration in this matrix is much less and a smaller weight in a thinner disk is acceptable on these grounds.

Table 2

Element	Atomic number	Weight of disk (gm.)
Manganese	25	1·5
Nickel	28	1·8
Copper	29	3·3
Zinc	30	3·8
Selenium	34	6·5
Zirconium	40	10·0

The Hilger and Watts instrument uses a reference monitoring system, whereby counts of secondary radiation are accumulated on the analytical channel until a pre-set count has been accumulated on the reference channel. The reference channel monitors a constant, but adjustable, proportion of the primary tungsten radiation. The analytical count is determined against a constant reference of a standard quantity of primary radiation reaching the sample. Counts of different samples for the same element are therefore strictly comparable regardless of slight fluctuations in primary radiation. The X-ray data are recorded as total counts accumulated for a

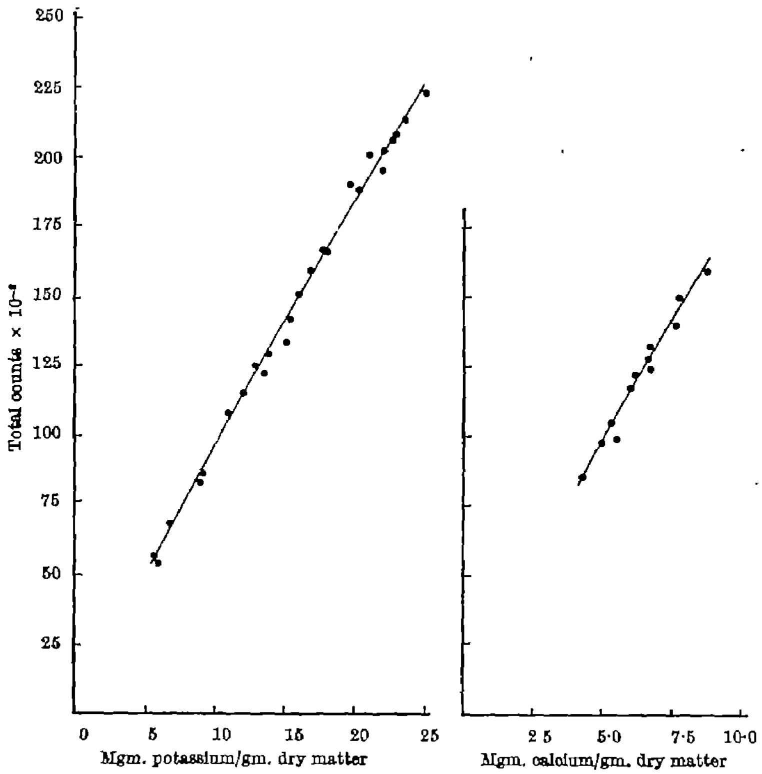

Fig. 1. X-ray fluorescence spectroscopy calibration curves for potassium and calcium determinations in samples of mixed herbage grasses

given reference count, rather than as counts per unit time.

Normally the X-ray generator is operated at 40 kV., 20 m.amp., and the reference monitor adjusted to approximately 4,000 c.p.s. For potassium and calcium determinations, 10^5 reference counts are accumulated, with an $N/4$ scaler in operation. Thus a counting time of approximately 100 sec. is used for each determination. Potassium and calcium are determined under vacuum using an ethylenediamine ditartrate analysing crystal and gas-flow proportional counter. Pulse-height analysis is used, with settings of 15 V. low-level and 33 V. window-width for potassium; 16 V. low-level and 44 V. window-width for calcium.

Fig. 1 gives calibration graphs for potassium and calcium in a series of mixed herbage grass samples. The X-ray counts given on these curves are from the $K\alpha$ lines of the elements and are of single determinations against 10^5 reference counts. The correlation thus shows what is attainable under rapid routine operating procedure for large numbers of samples. For practical purposes the calibrations can be considered to be linear, but they are in fact slight curves of the type to be expected for a moderately light element in a lighter matrix[2]. The speed of manufacture of disks from dried material is approximately 24 per hr. Analytical time, including application and release of vacuum for each sample and change-over of samples, is also 24 per hr. In a normal 8-hr. day, allowing time for the instrument to stabilize, approximately 150 single-element analyses can reasonably be made, which include determinations to check the calibration curve and the stability of the instrument. It is usual to determine one element in a large batch of samples as a continuous run.

Analyses of the same sample disk inserted and removed in the standard fashion throughout a day showed variation of ± 1–2 per cent in counts over the standard length of count of approximately 100 sec. Several disks made from the same bulk material showed errors no greater than this statistical counting error. It was found that the X-ray count on the same disk left under vacuum increased by about 1 per cent over a 1-hr. period. Our standard practice is to count each sample immediately the vacuum gauge indicates < 1 mm. mercury.

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PURE MUTANT CLONES INDUCED BY ULTRA-VIOLET LIGHT IN THE GREEN ALGA, *CHLAMYDOMONAS REINHARDI*

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AUXOTROPHIC mutants have been obtained in *Chlamydomonas reinhardi*[1] and other species of *Chlamydomonas*[2,3] by irradiating vegetative cells in liquid medium and plating them on complete agar medium, or else plating the cells to be treated on complete agar medium and then irradiating them. After the irradiated cells have formed distinct colonies they are replica-plated to minimal medium and to complete medium. The colonies which are unable to grow on minimal medium, but which do grow on complete medium, are presumed to be auxotrophs and are then tested for their biochemical requirement. Eversole[4] has varied this method by allowing the irradiated cells to undergo two divisions in liquid complete medium prior to plating them on complete agar medium for growth and replica-plating.

The difference between the method used by Eversole, which allows cellular division prior to the plating of irradiated cells, and the first method which does not, is important for the following reason. If cells are plated to an agar medium immediately following irradiation, two classes of mutants might be expected. The first class would consist of cells which only give rise to mutant progeny and thus to a pure clone of mutant cells. The second class of mutants would be composed of cells which give rise to colonies containing both prototrophic and auxotrophic progeny. Mixed clones of this type would not be evident on replica-plating to minimal medium since the prototrophic cells in the clone would overgrow the auxotrophic cells and the clone would appear to be wild-type. Such clones would be detected only if they were broken up into their component cells at some time prior to replica-plating, and if the clones resulting from these progeny cells were those replica-plated to minimal medium.

In order to determine whether or not mutants of the second class were induced, cells were irradiated and plated immediately following irradiation. After several cell divisions had taken place, the colonies on a portion of the plates were broken up ('spread' series) while on the remaining plates they were left undisturbed ('unspread' series)[5]. On replica-plating the resulting colonies in the two series of plates, one of two results was to be expected. First, if mutants occurred only as pure clones then the ratio of plates bearing mutants to plates lacking mutants would be the same in both 'spread' and 'unspread' series. Second, if mutants occurred in mixed clones as well as in pure clones, the ratio of plates bearing mutants to plates lacking mutants would not be the same in the two series. A larger proportion of the plates in the 'spread' series would have mutant colonies. The possibility that mutants occurred only in mixed clones is eliminated because the first method described in this communication has been used with success in obtaining auxotrophic mutants. The detailed experimental procedure and results of experiments carried out to test for the two classes of mutants are described in the succeeding paragraphs.

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The mutational system which was adopted in these experiments is one using forward mutations to acetate dependence. When mutations to acetate dependence are sought by the first method mentioned in this article, they are found to occur at a frequency of 0·1 per cent at an ultra-violet dose of 97 ergs/mm.^{2}/sec. given for 30 sec. This dose permits a survival of about 50 per cent. In each of the three experiments discussed here wild-type cells were removed from yeast extract agar medium[6], incubated for two days on minimal agar medium, and then placed in liquid minimal medium and shaken for several hours on a Gyrotary shaker. At the end of this time, an aliquot of cells was fixed and stained by the method of Levine and Folsome[7] to determine the proportion of multinucleate cells in the population. A minimum of 200 cells was counted in determining this proportion. The suspension was adjusted to a density of 5×10^6 cells/ml. by hæmacytometer count. A 4-ml. aliquot was then placed in a small Petri dish and irradiated for 30 sec. with two 15-W. General Electric germicidal lamps at a dosage of 97 ergs/mm.^{2}/sec. The suspension was mechanically agitated during irradiation. At the end of the treatment, the suspension was diluted and aliquots (0·1 or 0·2 ml.) were plated on acetate agar medium. The size of the aliquot plated in both the spread and unspread series was the same in any given experiment and contained between 50 and 100 viable cells. To control photoreactivation all post-irradiation procedures were carried out under the light of a General Electric 'Bugaway' yellow lamp, and the plates were incubated in the dark for 12 hr. prior to returning them to the light.

The colonies on the plates comprising the 'spread' series were broken up with a glass rod after the irradiated cells had been allowed to go through about four divisions (the number of divisions was determined by breaking up at least 30 microcolonies with a glass needle and counting the number of cells per colony under a dissecting microscope). The efficiency of spreading was determined by counting the number of colonies seen on a set of plates before and immediately following spreading. The colonies on both the 'spread' and 'unspread' series of plates were replica-plated to minimal medium and to minimal medium plus acetate after sufficient growth had occurred.

The results of the three experiments are given in Table 1. It can be seen that the number of mutants recovered in the 'spread' and 'unspread' series is about the same in each experiment. The data from all three experiments were combined, and a two-by-two contingency test was performed. In this test the number of plates with and without mutants in the 'spread' series was compared with the number with and without mutants in the 'unspread' series. The test shows that the data ($\chi^2 = 0{\cdot}49$; $P = 0{\cdot}48$) allows the conclusion that the number of plates bearing mutants in both series is not significantly different. Table 1 also shows that multinucleate cells and ineffective spreading have little effect on the results. In addition, the results are not affected by spontaneous mutations since none was observed among 60,450 untreated cells.

Table 1. FREQUENCY OF ULTRA-VIOLET-INDUCED ACETATE MUTANTS AMONG SPREAD AND UNSPREAD PLATES IN THREE EXPERIMENTS

	Experiment					
	1		2		3	
Per cent survival	70		41		47·5	
No. of surviving cells tested	16,800		10,270		8,670	
Per cent multinucleate cells	1·6		0·0		2·7	
Average No. of divisions at time of spreading	4·2		4·4		3·9	
Spreading efficiency (per cent)	98·5		98·5		75·0	
	U*	S	U	S	U	S
Plates bearing mutant colonies†	6	7	4	6	5	6
Total No. of plates	198	198	162	155	188	162
Per cent plates with mutants	3·0	3·5	2·5	3·9	2·6	3·7

*U, unspread; S, spread.

† Plates with mutants in the unspread series bore only a single mutant colony. Since spreading disrupts colonies, plates of the spread series bearing mutants had several mutant colonies.

Although it has been known for some years[1–3] that ultra-violet-induced mutations arise in pure clones in *Chlamydomonas*, it was not known until these experiments were performed whether ultra-violet-induced mutations occurred in clones mixed with prototrophic cells as well. The results reported here are in accord with the hypothesis that the majority of ultra-violet-induced mutations to acetate dependence in *C. reinhardi* arise only in pure clones. This result is of interest because Doudney and Haas[8–10], from their investigations of ultra-violet-induced mutations in *Escherichia coli*, have suggested a mechanism of induced mutation in which ribonucleic acid (RNA) is modified by the incorporation of radiation-altered precursors. The altered RNA causes a corresponding modification in newly synthesized deoxyribonucleic acid (DNA). In brief, their hypothesis predicts that an ultra-violet-induced mutation should appear initially in the first DNA synthesized after irradiation. The results reported here for *Chlamydomonas* are in disagreement with this prediction for the following reason. In *C. reinhardi* the DNA of mitotically dividing cells is replicated semiconservatively[11]. Therefore, if an ultra-violet-induced mutation arose initially in the first DNA synthesized after irradiation, a clone would arise consisting of both mutant and wild-type cells. The absence of a significant number of mutants which occur in mixed clones in the experiments reported here suggests that in *C. reinhardi* ultra-violet light induces mutations by a mechanism that directly affects the parental DNA.

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LETTERS TO THE EDITOR

PHYSICS

Production of Continuous High-Current Discharges in Gases

The properties of high-current arcs under stationary conditions have been studied, in general, using low-pressure mercury vapour. Thonemann and Cowhig[1], for example, have made probe measurements in a mercury arc at currents up to 100 amp. Much higher currents are used in technical devices such as rectifiers and inverters.

The reason for the use of mercury is that a cathode spot on a mercury pool can supply virtually unlimited currents. Furthermore, the cathode is indestructible since the mercury which is blasted away from the spot afterwards returns to the pool.

It is possible, however, to utilize the properties of a mercury pool to obtain a high electron current which is then sent into a gas other than mercury. This can be done by introducing the gas into a mercury vapour discharge tube in which a cold trap prevents the mercury from entering the part of the tube which contains the anode.

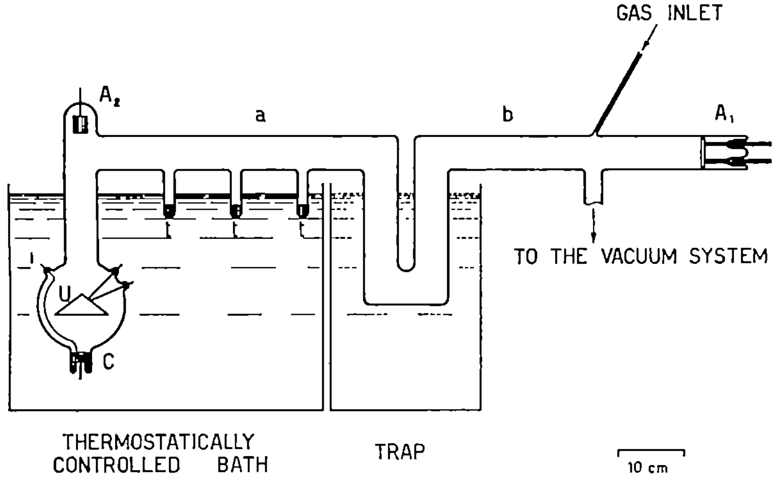

Fig. 1

The experimental tube (Fig. 1) is of 'Pyrex' and has a water-cooled principal anode A_1, a mercury pool cathode C and an auxiliary anode A_2. U is an umbrella which shields the main discharge space from jets of mercury emanating from the cathode spot and i is an electrode which is used to start the discharge. The bulb containing the cathode pool and the three tubes t containing further mercury are submerged in a thermostatically controlled bath. The large U-bend, which is surrounded by a vessel containing dry ice, constitutes the trap. The discharge tube is connected to a two-stage mercury diffusion pump through a liquid nitrogen trap.

Using a suitable optical system, light from regions a and b of the discharge (Fig. 1) illuminates respectively the lower and the upper parts of the entrance slit of a Hilger–Barfit constant deviation spectrometer. Thus the two spectra can be viewed simultaneously through an eyepiece. The measurements presented here were obtained using a photomultiplier which views individual lines through a slit.

Table 1. Intensity of Spectral Lines in Arbitrary Units

Wave-length (Å.)	Light from *a*	Light from *b*	Light from both *a* and *b*
5876 (He)	0·0	2·0	2·0
5770/91 (Hg)	3·5	0·0	3·5
5461 (Hg)	16·0	0·0	16·0
5016 (He)	0·0	8·0	8·0
4922 (He)	1·0	2·0	3·0
4713 (He)	0·0	1·5	1·5
4471 (He)	0·5	0·5	1·0
4358/39 (Hg)	28·0	0·0	28·0

Experiments have been carried out using helium. The pressure of the latter was 10μ mercury, and the mercury vapour pressure was 2·6μ mercury, corresponding to a bath temperature of 30° C. The arc current was 1 amp. and the arc voltage 190 V. Measurements were made of the intensity of the light arriving from region a, from region b and from both regions together. The first two measurements were made by blocking the light beam from part b and part a respectively. Typical results are given in Table 1. These measurements have not been corrected to allow for the variation of the sensitivity of the photomultiplier with the wave-length of the incident light. The figures given are galvanometer readings, which are to the nearest 0·5 division. Successive series of measurements, however, indicate that the error is somewhat greater than this.

It is seen from Table 1 that the light emitted from part a is due almost entirely to excitation of mercury atoms. The essential result, however, is that there is no mercury in part b of the tube, within the limits of experimental error. By analogy with normal vacuum practice it is clear that a discharge tube with a more sophisticated trap system would be even more efficient.

In the present experiment a current of only 1 amp. was used but the method can be extended to much higher currents.

We thank Prof. B. Brunelli for his interest.

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Current Distribution in Thin Superconducting Films

The problem of the current distribution in a thin superconducting film has recently been discussed by several authors[1,2]. Although the solution of the London equations for a thin rectangular strip carrying a current cannot be given in a simple analytical form, W. A. Bowers (unpublished, quoted by Glover[3]) has given an approximate solution for the case in which the film thickness d is comparable with the superconducting penetration depth λ, and the width w is greater than λ so that $wd \gg \lambda^2$. Assuming the current density to be constant through the thickness of the film, Bowers finds that the current density at a distance x from the centre of the strip is given by $J(x) = J(0)\{1 - (2x/w)^2\}^{-\frac{1}{2}}$ provided x is not too near $w/2$, while near the edge the solution has the form.

$$J(x) = J\left(\frac{w}{2}\right) \exp - \left\{\mathrm{d}\left(\frac{w}{2} - x\right)\Big/ a\lambda^2\right\}$$

where a is a constant of the order of unity. The two solutions are joined at a point distant $a\lambda^2/2d$ from the edge of the film, so that:

$$J\left(\frac{w}{2}\right) = \exp\left(\frac{1}{2}\right)\sqrt{wd}\, J(0)/\lambda\sqrt{2a}$$

The solution for the central region is the same as the charge distribution on the surface of a thin conducting strip in the analogous electrostatic problem.

A simple test of the validity of this solution can be made by comparing the external magnetic field which would be produced by such a current distribution with that measured experimentally. An experimental study of the perpendicular component of the magnetic field produced by a current in a superconducting lead film 2000 Å. thick × 1·5 mm. wide has already been made by Broom and Rhoderick[4], and we give here a comparison of their results with the magnetic field distribution to be expected from Bowers's expression for the current density. In calculating this field we shall assume that the current density is given by $J(x) = J(0)\{1 - (2x/w)^2\}^{-\frac{1}{2}}$ right up to the edge of the strip; although this gives an infinite density at $x = w/2$ it does not give an infinite magnetic field outside the strip, and the error caused by ignoring the edge solution is negligible for any practicable separation between the film and the magnetic field detector. Let the y-axis be perpendicular to the plane of the strip and introduce new variables $\xi = 2x/w$, $\eta = 2y/w$; then the component in the y-direction of the magnetic field set up by the current distribution will, at an external point (ξ, η) relative to the centre of the film, be proportional to:

$$\int_{-1}^{1} \frac{(\xi - t)\mathrm{d}t}{(1 - t^2)^{\frac{1}{2}}[(\xi - t)^2 + \eta^2]}$$

that is, to the real part of

$$\int_{-1}^{1} \frac{\mathrm{d}t}{(1 - t^2)^{\frac{1}{2}}(\zeta - t)}$$

where $\zeta = \xi + i\eta$. The complex integral is equal to $\pi(\zeta^2 - 1)^{-1/2}$. The real part of this integral is plotted in Fig. 1 for the case $\eta = 0{\cdot}08$, which corresponds to the separation between detector and film used in the experiments, together with measurements of the perpendicular component of the magnetic field. To make comparison possible, the horizontal co-ordinates of the experimental points have been scaled to make the positions of the theoretical and experimental maxima coincide, while the vertical scale has been adjusted to give agreement for $\xi > 2$. This procedure was adopted because neither the calibration of the mechanical drive for the detector nor the sensitivity of the latter was known to better than 10 per cent. The significance of the comparison therefore lies in the shape of the curve, and in this respect the agreement with the experimental results is remarkably good. As an indication of the extent to which the shape of the curve is sensitive to the current distribution, we show in Fig. 1 the magnetic field variation which would be obtained if the current were distributed uniformly across the strip. There is a marked difference between this curve and the experimental results.

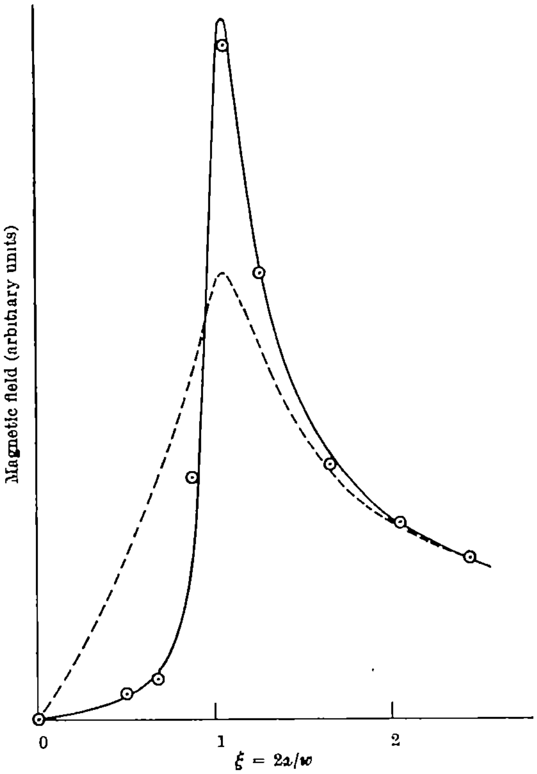

Fig. 1. Comparison of the perpendicular component of the magnetic field due to a current through a superconducting lead strip 2000 Å. thick × 1·5 mm. wide with that to be expected theoretically. ○, Experimental points; —, theoretical for current distribution of the form $J(\xi) = J(0)\ \{1-\xi^2\}^{-1/2}$; ‐ ‐ ‐, theoretical for uniform current distribution

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Packing of Liquids for Irradiation in Nuclear Reactors

The 'packing' of liquids for irradiation in nuclear reactors is still in many cases a problem. In our case we had to make use of the self-service channel of the *BR*1 reactor at Mol (Belgium), with a flux of 10^9 neutrons/cm.^{2} sec.—a channel in which irradiation is usually carried out with the samples packed in a 'Perspex' ball, the dimensions of which are shown in Fig. 1.

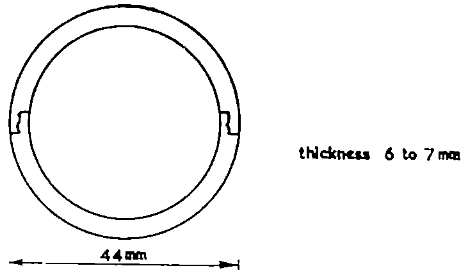

Fig. 1

In this ball the samples are present in the solid state, usually pressed to disks. Because our experiments had to be performed with liquids, we tried to find another way. Two possibilities were at hand.

At first we used a 'Perspex' ball as shown in Fig. 2. The advantage of this method is the irradiation of sample and standard at the same time. However, the disadvantages are: (*a*) the difficulty of cleaning the two spaces; and (*b*) the costly construction of the apparatus.

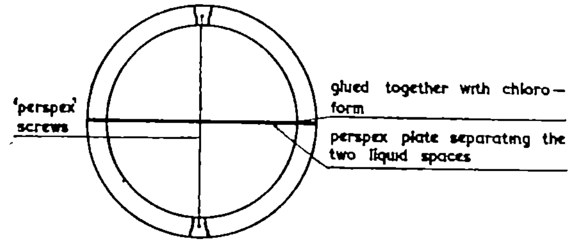
'perspex' screws
glued together with chloroform
perspex plate separating the two liquid spaces

Fig. 2

Therefore, a simple and cheap method was tried out which makes use of table-tennis balls. For this purpose two tiny holes are drilled in the balls at a short distance from each other, and the ball is filled with liquid by means of a funnel with an injection needle, connected with the stem by a piece of plastic tube (Fig. 3). The holes are closed with a plastic glue, and, in order to avoid contamination of the reactor in the event of leakage, the ball is packed in closely fitting polythene shells, tied together with adhesive tape (Fig. 4).

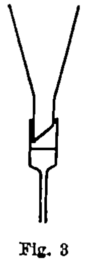

Fig. 3

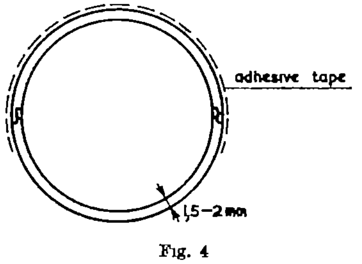

Fig. 4

The standard material is packed in a separate table-tennis ball and irradiated simultaneously with the sample. If it is not possible to bring them together on a spot in the reactor with sufficiently constant neutron flux, sample and standard must contain an internal standard in order to compare the flux that each receives. Afterwards the balls are cut open and 10 ml. of the liquid is pipetted into a bottle to be counted.

The advantages of the use of table-tennis balls are: (1) They are remarkably strong, even after being filled with water, and can stand falls of 8 ft., a temperature of 80° C. and dilute mineral acids for a long time. (The first mentioned was necessary because the ball had to be dropped into the reactor from the height of 8 ft. on to a steel plate.)

(2) They do not contaminate the sample; for example, dilute nitric acid, irradiated in a ball for half an hour and counted 15 min. in a thallium-activated NaI-well crystal detector connected with a multichannel energy spectrometer, showed no trace of activity, either γ-peaks or bremsstrahlung, caused by dissolved impurities of the ball.

(3) They are cheap and easy to obtain in quantity. For these experiments 'Halex' two and three star balls were chosen.

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GEOPHYSICS

Effect of Magnetic Disturbances on the Equatorial Ionospheric Jet Current

The existence of an anomalously large diurnal variation of the horizontal components of the Earth's magnetic field near the equator was first noticed at Huancayo[1]. Later, to explain this, Egedal[2,3] and Chapman[4] postulated the existence of an equatorial jet current flowing around the Earth. Several studies of this jet current have been made[5], and naturally the first attempts have concentrated on the days which were magnetically quiet. I have been interested in ionospheric effects near the equator on magnetically quiet days and on magnetically disturbed days, and have found that many of the anomalous phenomena which occur near the equator seem to be markedly reduced under magnetically disturbed conditions. These phenomena include: (*a*) intensity of day-time sporadic *E* of equatorial type; (*b*) amount of night-time spread-*F*; (*c*) the large values of the semi-

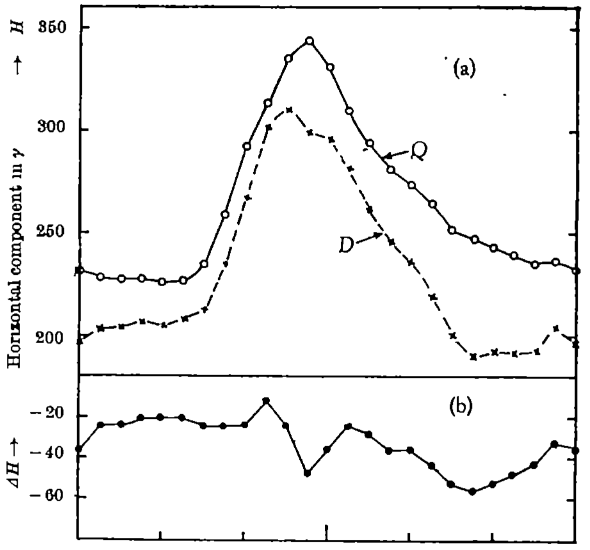

Fig. 1. *a*, Variation of horizontal component of Earth's magnetic field *H* on magnetically quiet days *Q* and magnetically disturbed days *D*; *b*, difference between variation of *H* on quiet and disturbed days $H_D - H_Q = \Delta H$

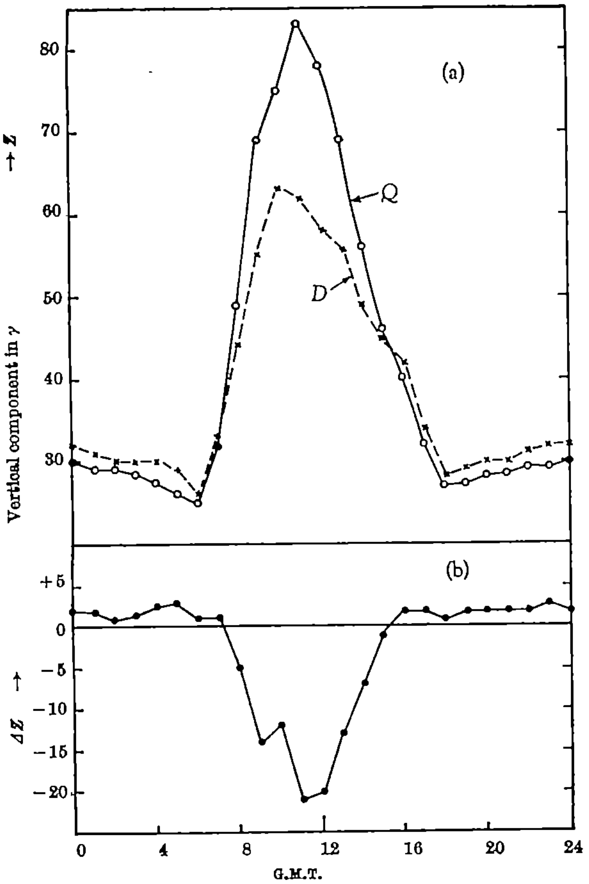

Fig. 2. *a*, Variation of vertical component of Earth's magnetic field Z on magnetically quiet days Q and magnetically disturbed days D; *b*, difference between variation of Z on quiet and disturbed days $Z_D - Z_Q = \Delta Z$

thickness, Ym, of the F-layer in the day-time; (*d*) the mid-day bite-out of the F_2-layer, and (*e*) the separation of the F_2-layer from the F_1-layer.

I have therefore examined the magnetic data obtained during the International Geophysical Year at Ibadan, Nigeria, 3° south of the magnetic equator, to see what effect magnetic disturbance has on the ionospheric jet current. Ibadan is just at the edge of this equatorial jet current, and has therefore a large diurnal range of both H, the horizontal magnetic component, and Z, the vertical magnetic component.

The hourly values of H and Z components of the Earth's magnetic field for the five magnetically quiet days from each month were taken and the mean diurnal variation calculated. The data for the five disturbed days in each month were treated in the same way. The results averaged over the whole 18 months of the International Geophysical Year are shown in Fig. 1 for H and in Fig. 2 for Z. In the case of H for disturbed days, a non-cyclic variation of about 36γ has been removed in the usual way.

There are two points of interest to be noted from the graphs: (1) The reduction in range of diurnal variation of Z of about 40 per cent on magnetically disturbed days. The diurnal range of H is almost unaffected. (2) The overall depression of H by about 30γ with no corresponding change in Z.

The latter effect is well known and indicates currents flowing in regions where the conductivity is independent of time of day, and either a current sheet or a current at a great distance from the Earth. This is explained in a general way by the ring-current theory which is developing from the suggestions of Chapman and Ferraro[6].

The former effect does not appear to have been noted before. The only previous similar investigation is that of Johnston and Bartels[7], who examined the data from Huancayo. The marked change of Z observed here at Ibadan was not observed, but the change in the diurnal variation of H was similar to that found here. This is probably due to the fact that Huancayo is right under the equatorial jet current, and thus the current produces negligible diurnal variation of Z.

The jet current is thought to be about 4° of latitude wide, at about 100 km. height, and in Nigeria centred on 10° N. geographic latitude (see Onwumechilli[5]). One explanation of the behaviour shown in Figs. 1 and 2 would seem to be that on magnetically disturbed days the anomalous behaviour leading to the belt of high conductivity is reduced, so that the current is much less channelled into a jet. The overall diurnal range of H would then be about the same, but the diurnal range of Z at Ibadan would be much reduced. However, the jet current has been ascribed by Martin[8] to polarization inhibiting the Hall currents in the ionosphere. It would suggest, then, that this polarization is reduced on magnetically disturbed days. This would, however, lead to a reduction of the conductivity and hence of the current, and this should be evident from a reduction in the diurnal range of H which is not observed.

An alternative explanation is based on the fact that Ibadan is very close to the region where the diurnal range of Z is a maximum[5]. Therefore, if the exact position of the jet current moves erratically and is most affected on D-days, then the effect will be to give rise to a reduction in the average diurnal variation of Z. There is some evidence for considerable day-to-day changes in the position of the jet current (Rivers, D., private communication). More work is required to examine the suitability of such an explanation.

The results of this analysis will be published in more detail elsewhere. I am grateful to Prof. N. S. Alexander and Dr. C. A. Onwumechilli for making data available.

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METEOROLOGY

Some Amino-Acids as Ice Nucleators

THE occurrence of organic nitrogen in snow has been known for many years[1]. More recently amino-acids have been identified in rain[2], and in free air and snow[3].

We have examined amino-acids for possible activity as ice nucleators. At first sight, it did not seem likely that they would be active as a class, since most of them are soluble in water, and water insolubility has generally been conceded to be a pre-requisite for ice-forming nuclei. However, some amino-acids are soluble only with difficulty in cold water.

The following substances were tested: glycine, *l*-leucine, *dl*-leucine, *dl*-isoleucine, *dl*-alanine, *dl*-serine, *dl*-aspartic acid, *l*-glutamic acid, *dl*-phenylalanine, *l*-tyrosine, *l*-tryptophan, *dl*-tryptophan, *dl*-valine.

Nucleating ability was tested in a 4·5 cu. ft. cold box using both soap films and supercooled sugar solutions as detectors of ice nuclei. When using the sugar solution detector the relevant temperature was taken as that of the solution, care being taken that the ambient temperature was maintained at that same value, and that contamination from colder parts of the chamber did not occur. Those substances which cannot be finely powdered and so do not form a stable aerosol when blown into the chamber were tested by dusting a small quantity directly on to the detecting surfaces and watching for the appearance of small ice crystals in the usual fashion.

The threshold temperatures for ice nucleation of those amino-acids found to be active above −10° C. are shown in Table 1.

Table 1

Substance	Threshold temperature (deg. C.)
dl-Alanine	−7
l-Leucine	−4·5
l-Tryptophan	−5·5
dl-Aspartic acid	−6

It has recently been found[4] that nucleating ability with some other organic substances occurs with those possessing —OH, =O, or—NH_2 groups, so that the activity of amino-acids fits into this pattern. The recent suggestion[5] that good ice nucleators such as silver iodide act because of hydrophilic sites on the crystals would also be compatible with the hydrogen bonding properties of the —NH_2 group. It is also interesting that proteins (that is, complex groupings of amino-acids) are stated by V. J. Schaefer to be good ice nucleators[6].

The amino-acids found in the atmosphere have been identified[3] as glycine, alanine, valine, leucine, and (less certainly) glutamic acid, aspartic acid, and proline. Thus, among the naturally occurring amino-acids in the atmosphere are two with excellent nucleating power.

The origin of the amino-acids in the atmosphere and in precipitation is not known with certainty. It has been suggested that they may be synthesized in the atmosphere from simpler compounds[3,6], that they may be formed during combustion processes[2], or that they may arise during decomposition of organic matter[3]. It has been suggested that the surface of the ocean is the source of the organic nitrogen in precipitation[7,8], the organic material being released by the bursting of foam bubbles in surf and in storm waves.

It appears to us that a world-wide class of atmospheric ice nuclei may therefore be certain amino-acids. If this is so, their origin is a matter of some importance.

Amino-acid nuclei if released from the sea or the soil would almost certainly be associated with other substances. We have mixed 1 per cent leucine with a 30 per cent saline solution. When this was sprayed into the cold chamber, it was inactive in producing ice crystals at temperatures above − 15° C. When the same solution was evaporated to dryness, the crystalline powder gave ice nuclei active at − 4 to − 5° C. Natural ocean water[9] and sea salt[10] both give ice nuclei active at about − 15° C.

Some mineral dusts are good ice nucleators; kaolinite is the most active of these with a threshold temperature of − 9° C.[11]. When 1 per cent leucine was added to a slurry of kaolin, the powder resulting from evaporation to dryness was active as an ice nucleator at − 6·5° C. Neither diatomaceous earth nor sand (mainly quartz) showed any noticeable increase in nucleating power under similar treatment.

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CHEMISTRY

Iodine as a 'Non-Destructive' Colour Reagent in Paper- and Thin-layer Chromatography

IODINE has been used as a colour reagent for paper chromatography of amino-acids and their derivatives[1,2], imidazoles[3], and other nitrogen-containing compounds[1,4]. Steroids are also revealed by this technique, which is sufficiently sensitive to enable location of 0·5 µgm. oestrone[5]. The method has not gained wide acceptance possibly only because it cannot be relied on to yield a colour diagnostic of a class of compounds—some nitrogen-containing compounds are not revealed—but its application is simple, and if unsuccessful, other colour reagents can be applied to the same chromatogram. In the most convenient procedure, the dried paper is exposed to iodine vapour (supplied by a few iodine crystals in a closed battery jar) for 10–30 sec.; or the paper may be sprayed with a solution of iodine in carbon tetrachloride[3], or may be dipped into a saturated solution of iodine in petroleum ether (b.p. 60°–80°)[5]. Compounds are revealed usually as brown spots, which fade rapidly.

The method is applicable also to 'thin-layer' chromatography[6], in which the stationary phase is a water-moist layer of a powdered silica gel–calcium sulphate mixture ('Kieselgel *G*') supported on a glass plate. Larger quantities of material can be eluted on this phase, in comparison with paper chromatography, and more compact spots are obtained, and this has enabled me to demonstrate that compounds are revealed by the iodine vapour procedure as loose addition compounds, as suggested by Brante[1]. DL-Serine methyl ester hydrochloride was eluted on 'Kieselgel *G*' with butanol/acetic acid/water (6 : 2 : 2) and was located by exposure of the dried plate to iodine vapour; the powder supporting the spot was collected, and was extracted with ethanol. The concentrated extract was shown by comparative paper chromatography in several solvent systems to contain only unchanged DL-serine methyl ester. γ-Benzyl-DL-glutamic acid and benzyloxycarbonyl-DL-methionine were similarly recovered unchanged.

The only alternative 'non-destructive' method for revealing positions of components of a mixture separated by chromatography depends on possible fluorescence in ultra-violet light; in the special case of bacteriostatic compounds a further alternative is available[7]. The iodine technique applied to 'thin-layer' chromatographic separations of mixtures may make possible the isolation of valuable components for further examination.

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Heat of Reaction of Polyriboadenylic Acid and Polyribouridylic Acid

POLYRIBOADENYLIC acid (poly A) and polyribouridylic acid (poly U) have been shown by sedimentation[1], light-scattering[2], X-ray[3] and ultra-violet absorption[1,4] investigations to interact in solution to form, depending on external conditions and the mole ratio of reactants, either a two-stranded (A + U) or a three-stranded (A + 2U) helical complex[3,4]. At 25° C., *p*H's between 6 and 9, and ionic strengths of 0·1 or greater, analysis of ultra-violet spectral mixing curves has shown that the formation of the complex species attains completion[5]. At U : A ratios greater than 1·0 the A + U species can, depending on external conditions and the molecular weights of the reactants, add a second poly U strand to form the A + 2 U triple-stranded helix[1,4]. It is not possible to ascertain by spectral analysis the extent to which formation of the A + 2 U species can occur at U : A ratios less than 1·0 under these conditions, as the absorbancy changes for the two steps appear to be equal[1,5].

The A + U complex has been shown to have a conformation which is remarkably similar to that of natural deoxyribonucleic acid (DNA)[3]. It has, moreover, been postulated to be stabilized by inter-base hydrogen bonding similar to that believed to exist between adenine and thymine in DNA[3]. The degree of molecular organization is very high, the evidence from ultra-violet hypochromism indicating that virtually all possible adenine-uracil linkages are formed[5].

The molecular conformations of the two reactants are not entirely equivalent. While both are believed to be single-stranded, poly A, but not poly U, appears to possess some degree of internal hydrogen bonding which results in a fractional helical content[6].

The current concept of the conformation of the (A + U) species represents it as stabilized by hydrogen bonds between the N_{10} nitrogen of adenine and the C_6 carbonyl of uracil and between the N_1 of adenine and the N_1 of uracil[3]. In addition, van der Waals interactions between adjacent pairs of planar purine and pyrimidine rings, which are stacked in a parallel array in the (A + U) complex, are probably of major, although imperfectly understood, importance.

The purpose of the present communication is to report the enthalpy of reaction for this important process in the region of poly A excess.

All the poly A and poly U preparations which were used in the course of this investigation were prepared by the action of the polynucleotide phosphorylase of *M. lysodeikticus* on the corresponding nucleoside diphosphate[7]. The sedimentation coefficients ($S_{0,20}$) of the poly U samples ranged from 4·5 to 10·0 svedbergs; those of poly A (unless otherwise specified) ranged from 8·0 to 12·0. No systematic variation with molecular size was observed and the results for different preparations were averaged without regard for this parameter (Table 1). The A : U ratio was always > 1·0. No variation of the heat with ratio was observed in this range. The question of the distribution of the complex species between the A + U and A + 2U forms in this region of poly A excess must be left open at present. The number of adenine : uracil linkages is, of course, independent of this.

Table 1. HEATS EVOLVED PER MOLE URACIL FOR THE INTERACTION OF POLY A AND POLY U

Effect of ionic strength (average of determinations for 14 preparations)

Molarity potassium chloride	$-\Delta H$ (calories)
0·10	5,900 ± 200
0 50	5,250 ± 200
1·00	4,750 ± 300

Effect of varying size of poly A* (in 0 1 *M* potassium chloride)

$S_{0,20}$ (svedbergs)	$-\Delta H$ (calories)
12·2	5,870 ± 100
4·3	6,050 ± 100
3 2	6,020 ± 100
2·1	5,850 ± 100

In each case 0 01 *M* sodium cacodylate, *p*H 6·6, was present. The temperature was close to 25° C. Poly A was in excess.

* These samples were obtained by stepwise alkaline hydrolysis of a single poly A preparation.

Measurements of evolved heat were made with the heatburst calorimeter developed by Benzinger and Kitzinger[8]. This has been amply described in other publications[8,9]. A 'Teflon' reaction vessel, containing two compartments of 7 ml. each, was used. Reaction was initiated by rolling the vessel and thereby mixing the reactants which were initially separated by an air bubble.

The molarity in nucleotide of the reactants varied from 2 × 10^{-4} to 5 × 10^{-5}. Concentration was determined by removing an aliquot, hydrolysing it quantitatively to mononucleotide (1 hr. at 100° in 0·1 *M* potassium hydroxide), and measuring the absorbancy at 260 mμ after suitable dilution.

Prior to measurement the poly A and poly U solutions were dialysed versus several changes of buffer in the same container. This served to eliminate any gradient of *p*H or electrolyte concentration.

Table 1 cites the average values of the heats obtained for 14 different preparations at three ionic strengths. A slight decrease in heat occurs with increasing ionic strength between 0·1 *M* and 1·0 *M* potassium chloride.

Table 1 includes the heats evolved with a single poly U preparation with a series of poly A preparations obtained by stepwise alkaline hydrolysis of a single poly A sample. No significant variation in heat was obtained for preparations of $S_{0,20}$ ranging from 12·3 to 2·1, corresponding to molecular weights ranging from 10^6 to 10^4 (ref. 10).

It is clear that the enthalpy of reaction of poly A and poly U to form a helical species is a well-defined quantity which is independent of the molecular weight of the interacting species for a considerable span of values of the latter However, it is not yet possible to equate the observed heat of reaction with the intrinsic heat of formation of the adenine–uracil linkage, in view of the uncertain contribution of any internal hydrogen bonding of poly A itself[6] This would necessarily be broken up by formation of the helix and might contribute to the observed heat.

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Sedimentation of β-Lactoglobulin A under Aggregating Conditions

IN a previous paper[1] we compared the experimental results of Townend, Weinberger and Timasheff[2] for the sedimentation of the genetic species β-lactoglobulin *A* and *B* (ref 3) under dissociating conditions with theoretical estimates based on the corresponding light scattering data by the same authors. There appeared to be a good correlation between the two types of data.

Perhaps more important than dissociation in acid solution of the two species, *A* and *B*, is the association[4] which occurs maximally at *p*H 4·40–4·65 for β-lactoglobulin *A* but not for *B*, since this allows the species to be distinguished readily by physico-chemical means.

Timasheff and Townend[5] have shown by light-scattering experiments that in the range of *p*H 3·7–5·2 at low temperatures and ionic strength 0·1, β-lactoglobulin *A*, which exists at room temperature in the form of particles corresponding to dimers of molecular weight 36,000, aggregates reversibly to octomers of molecular weight 144,000. In this *p*H-range the dimer is sufficiently stable for its dissociation to be discounted[2] and for it to be taken as the basic aggregating species. For the present purpose, therefore, the system will be likened to a model in which monomer aggregates to tetramer, the monomer and the tetramer in the model being equivalent to the dimer and octomer of the actual system.

It is now proposed to repeat the procedure adopted in the previous paper[1] and to compare the dependence on concentration of the sedimentation velocity of β-lactoglobulin *A* with that calculated using the light-scattering data[5] of Timasheff and Townend for the extent of association of the molecule The method of calculation is the same as described earlier[6,7], and eliminates the need for the empirical correction for hydrodynamic effects used by Timasheff and Townend[4,8]. The main weakness of this type of calculation lies in the neglect of diffusion, but this does not usually seem to be as serious a defect as might be expected.

The following assumptions are made: (1) A rapidly reversible equilibrium exists between monomer, dimer, trimer and tetramer species formed by polymerization through identical bonds. Equilibrium is governed by the mass-action equations $a_2 = ka^2$, $a_3 = k^2 a^3$, and $a_4 = \gamma k^3 a^4$, where concentrations are in moles of the species per litre. The equilibrium constant for monomer–tetramer equilibrium has been represented by the compound term γk^3 to indicate that two bonds instead of one are formed during the addition of the fourth molecule to the trimer according to the model of Timasheff and Townend[5], and that this accentuates the stability of the tetramer γ times. From light-scattering data[2] $\gamma k^3 = 5 \times 10^{11}$ l^3/mole^3 at 2° C., *p*H 4·65, ionic strength 0·1. The value of k is not known, but has been fixed by *arbitrarily* choosing $\gamma = 100$, whence k becomes 1,710 l/mole. With a value of γ as high as this, k is sufficiently small for the amounts of dimer and trimer to be relatively insignificant, in conformity with the light-scattering data[2], and for the precise value of k to be therefore rather unimportant. (2) The limiting sedimentation coefficient $(s_1)_0$ of the monomeric species (molecular weight 36,000) is taken as in the previous paper[1] to be 2·87 svedbergs. Polymers of degree n are assumed to have sedimentation coefficients equal to $(s_1)_0 n^{2/3}$, that is, they are assumed to sediment as if they all had the same frictional coefficient and had volumes proportional to n. Thus the tetramer, for example, has a limiting sedimentation coefficient of 7·23 svedbergs. (3) The sedimentation coefficient of each species obeys the usual relation applicable to proteins near their isoelectric point, that is, $s_n = (s_n)_0\,(1 - gc)$, where g is a constant and c is the concentration of protein. The value of g is assessed from the experimental results for the dependence of s on c at high concentration. In the present instance a value of $g = 0{\cdot}058$ (gm./100 ml.)$^{-1}$ has been adopted, and this lies within the range to be expected for a protein near its isoelectric point.

These assumptions are sufficient for the evaluation of s as a function of c (refs. 6 and 7). The schlieren pattern breaks into two peaks[6], in agreement with the experimental patterns[4,5]. Two sedimentation coefficients can therefore be calculated, one for the trailing and one for the leading peak, and each of these sedimentation coefficients can be found by taking either the weight average or the median of the peak as the representative velocity. Weight average values

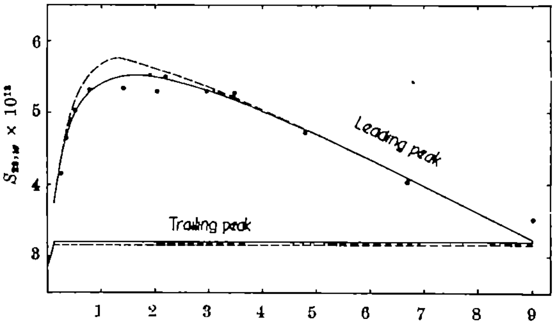

Fig. 1. Sedimentation of β-lactoglobulin *A* under aggregating conditions, *p*H 4 65, 2° C. ——, Weight average (theory); – – –, median (theory), ○, experiment (Timasheff and Townend, ref. 5)

have the advantage that they are less sensitive to the effect of neglecting diffusion, and are therefore likely to correspond more closely to experimental values.

In Fig. 1 theoretical weight average and median velocities for the trailing and leading peaks are shown and experimental points for the leading peak taken from the paper of Timasheff and Townend (ref. 5, Fig. 7) are added for comparison. It will be seen that there is a reasonable degree of correlation between the theoretical sedimentation curves based on data from light scattering, and the sedimentation velocities actually observed.

It is desirable to prepare families of curves in order to determine the sensitivity of the curves to adjustment in the parameters γ and g. This is now being undertaken with the aid of a digital computer.

A comparison of the theoretical and experimental velocity of the slow peak is not possible in the present instance because some 10 per cent of non-aggregating impurity in the lactoglobulin *A* masks the slow peak in the experimental schlieren patterns[5].

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Determination of Parathion

IN chemical determination of parathion (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate) and closely related compounds according to Averell and Norris[1], the nitro-group is reduced by zinc and hydrochloric acid, followed by diazotization and coupling of the primary aromatic amine thus produced. The method has become extensively used[2], but, to our knowledge, the descriptions available comprise only reduction with zinc and hydrochloric acid or similar heterogeneous systems.

p-Nitrophenol can be determined by an indophenol test after reduction to *p*-aminophenol with titan-III-chloride[3]. As a determination, according to the method of Averell and Norris, could be carried through with titan-III-chloride as reducing agent in a homogeneous system, the following procedure was elaborated.

The test solution is presupposed to be a neutral alcohol-containing solution, to 5 ml. of which are added: 50 µl. of titan reagent (2 min.); 200 µl. of about 8 *N* hydrochloric acid (2 min.); 100 µl. of about 3·2 per cent sodium nitrite (4 min.); 500 µl. of about 2 per cent sulphamic acid (3 min.); 500 µl. of about 1 per cent *N*-1-naphthyl ethylenediamine dihydrochloride; alcohol and water to make 10 ml.

The details of the procedure are such that semi-automatic Lang–Levy constriction pipettes could be used. These permit addition of reagents at intervals of 10 sec., so that a series of twelve analyses can be dealt with in 2 min. The reagents and reaction times (stated above in brackets) followed the methods of Averell and Norris[1,2] as well as Terp[4] (determination of procaine). The procedure has been tested to such an extent as to allow a convenient and reproducible technique to be established; but the reagents and reaction times make no claim to be optimal.

The titan reagent consists of 1 vol. of a 15 per cent solution of titan-III-chloride, 1 vol. of 2 *M* acetic acid and 2 vol. of 2 *M* sodium acetate; the amount mixed is such as to correspond with only one or two sets of analyses. In the circumstances, the reduction is performed in a weak acid solution, which is intensely greyish so long as the reducing titan is present in excess. (An old titan solution can be regenerated by adding a piece of zinc a few minutes before the dilution with acetate buffer; deposits of titanic compounds can be removed with hydrochloric acid and hydrogen peroxide.)

The amount of nitrite is somewhat larger than usual as some of it serves to remove the excess of titan. The hydrochloric acid should be added before the nitrite solution in order to avoid precipitation of titan compounds.

If the analysis is performed in a purely aqueous system, the colour develops fairly slowly, with absorption maximum at 555 mµ. Alcohol accelerates the colour development, but at alcohol concentrations of about 80 per cent and higher in the test solution, a different red colour is produced (a single observation implies the hydrochloric acid concentration to be too low in such cases). Usually the test solution should contain 20–70 per cent alcohol, and the final dye solution 30–50 per cent. The absorption maximum is now at 560 mµ. The colour seems to have become fully developed in the course of 10–20 min. and remains constant at least until the next day. The molar absorptivity is of the order of 46,000 M^{-1}cm.$^{-1}$. This is higher than the value found by Blinn *et al.*[5], possibly due to the presence of alcohol. Highly coloured solutions can be diluted with 50 per cent alcohol containing hydrochloric acid to prevent the dye from changing to yellow[5]. The stated quantities of reagents allow total amounts of up to 300 µgm. parathion being determined. In these cases the dye solution was made up to 100 ml. with the alcohol–acid–water mixture.

Using the method described here, the reduction time has been reduced from 10–20 min. (on boiling water bath) to 2 min. (at room temperature). Furthermore, the reduction with titan is performed in a homogeneous system, so that removal of the reducing agent by filtration or otherwise is avoided. Both processes contribute towards a considerable shortening of the process of analysis. Moreover, the somewhat simpler titan procedure opens up possibilities of performing the analysis on very small volumes.

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BIOCHEMISTRY

Partial Synthesis of Vitamin B_{12} Coenzyme and Analogues

The structure of the vitamin B_{12} coenzyme[1] has been determined recently by X-ray crystallography[2]. It seemed possible (cf. ref. 3) that a synthesis of this compound, which contains a 5′-deoxyadenosyl group attached direct to the cobalt atom by a cobalt–carbon bond, could be achieved from reduced hydroxocobalamin and a suitably 5′-substituted adenosine derivative. 5′-Tosyladenosine is known[4] to cyclize easily to the tosylate of *cyclo*adenosine but the 5′-tosyl-2′,3′-isopropylidene derivative of adenosine can be prepared without difficulty[4]. Hydroxocobalamin was reduced in aqueous or ethanolic solution with sodium borohydride and reacted with this adenosine derivative. A product which had the properties expected of an isopropylidene derivative of the coenzyme was isolated from the reaction mixture in poor to moderate yield. Following a suggestion from Dr. V. M. Clark, whom we thank for his interest, we then attempted to reduce the cobalamin not just to the brown vitamin B_{12r} (ref. 5), believed to be a cobalt II derivative, but to the grey-green compound containing cobalt in a lower oxidation state[6]. This compound can be prepared under anaerobic conditions from hydroxocobalamin with sodium borohydride, chromous acetate in ethylenediamine tetra-acetic acid buffer at *p*H 9·5, or with zinc dust and dilute acetic acid. Under acid or alkaline conditions it reacts rapidly with a range of alkylating agents to give good yields of coenzyme analogues. In contrast, vitamin B_{12r}, the exclusive product obtained from hydroxocobalamin and chromous acetate at *p*H 5, is quite unreactive.

The reaction product from fully reduced hydroxocobalamin and 5′-tosyl-2′,3′-isopropylideneadenosine was purified via phenol extraction and passage of an aqueous solution through columns of DEAE-cellulose and CM-cellulose in series. The product passed rapidly through the former and more slowly through the latter as an orange zone which emerged as a red effluent. This was concentrated and crystallized from aqueous acetone. Mild acid hydrolysis (0·1 *N* hydrochloric acid for 16 hr. at room temperature or 15 min. at 90°) gave the vitamin B_{12} coenzyme as the main product. It was fully active in the enzyme assay of Abeles and Lee[7]; the absorption spectra in neutral and acid solution and the R_F value on paper chromatography with wet *sec.*-butanol were the same as those of the natural B_{12} coenzyme. On treatment with cyanide it yielded adenine and dicyanocobalamin.

Analogous experiments using 5′-tosylinosine or 5′-tosyluridine yielded crystalline products with absorption spectra and other properties similar to those of the coenzyme. In the enzyme assay, however, these analogues behaved as powerful competitive inhibitors of the coenzyme. Furthermore, alkyl halides and sulphates, for example, ethyl iodide and dimethyl sulphate, react with fully reduced cobalamin in aqueous or alcoholic solutions, the colour changing almost instantly from greenish-black to red or orange. Crystalline products have been isolated which presumably contain the alkyl group linked to cobalt. $^{14}CH_3I$ yielded the methyl analogue of the coenzyme, which had the same specific activity as the methyl iodide. Similar products have been obtained from reactions with ethylene bromhydrin, chloracetic acid, acetyl chloride and benzyl chloride. Some of these products also inhibit the natural coenzyme in the enzyme system.

Work is in progress to characterize these and related compounds and their degradation products, to measure their inhibition indices and to study their effects in biological systems.

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Acetylcholinesterase Activity in *Nitella*

It is generally accepted that the process of excitation starts with a localized depolarization of the conducting cell membrane. The adjacent region is then depolarized by the resultant current flow. This process repeats itself at successive points and thereby the impulse is propagated. These electrical phenomena have been widely investigated, but the underlying mechanism is still under discussion. Nachmansohn[1] has suggested a theory according to which the acetylcholine system plays an essential part in bioelectrogenesis. Much evidence has been accumulated in favour of this theory during recent years. The evidence is based on experiments with animal cells, mostly nerve and muscle fibres of vertebrates and invertebrates. However, there are plants which resemble nerve and muscle in being able to propagate impulses by electric currents. There is an excitable membrane separating the cell from its environment, in which a sharp decrease in electrical impedance occurs during the passage of an impulse.

Nitella flexilis is a freshwater alga with large single cells. When an internode is stimulated, an action current is generated and conducts the impulse[2]. It appeared of interest to investigate whether the acetylcholine system is present in *Nitella*. There is a considerable number of difficulties to be overcome for testing this problem. But it appeared possible to test whether acetylcholinesterase is present. If the presence of this enzyme were established, this would be an indication that the substrate may also be present and may be hydrolysed there. It has been found that *Nitella* indeed is capable of hydrolysing acetylcholine. This communication is a preliminary report on the properties and concentration of the cholinesterase in *Nitella*.

The branches were dissected in small particles, dried on filter paper and pre-soaked in the incubation fluid for 1 hr. In previous experiments with other tissues it had been observed that in the first hour the rate of hydrolysis seemed to be much higher than in the following hours. Apparently some substrate is taken up by the tissue. After the pre-incubation the tissue was dried, weighed, homogenized, and then placed into the incubation fluid. Following such treatment the rate of hydrolysis was always linear. The substrates tested were acetylcholine bromide and acetyl β-methylcholine bromide. The latter substrate was selected because it is considered to be specific for acetylcholinesterase. The esterase activity was tested by Hestrin's colorimetric method[2,3] in which the disappearance of the substrate is followed.

Nitella hydrolysed 6·5 μmoles/gm./hr. of acetylcholine, while acetyl β-methylcholine was hydrolysed at a rate of 3·8 μmoles/gm./hr. The hydrolysis of the latter substrate indicates that the enzyme present is essentially the specific acetylcholinesterase. The rate of hydrolysis of acetylcholine in the giant axon of squid is in the order of 40–60 μmoles/gm./hr. It has to be kept in mind that the speed of propagation in *Nitella* is about 1,000 times slower than in nerve fibres. In control solutions without the substrate present some substance was released, presumably an ester. The nature of this substance is still unknown. The finding requires much more investigation before any final conclusions can be drawn about its significance. However, the presence of acetylcholinesterase in a plant cell capable of propagating electric currents suggests the possibility of a fundamental similarity of the mechanism of bioelectrogenesis in plant and animal cells.

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In vitro Examination of the Ribonucleic Acid–Deoxyribonucleic Acid–Deoxyribonuclease System in Mammals

THERE is a considerable number of substances which inhibit the activities of deoxyribonuclease (DNase). Some of the natural inhibitors appear to have a relative specificity of action; these are substances of protein nature active against the DNase of yeast[1] or against the DNase of the bovine pancreas[2,3], or ribonucleic acids (RNA) extracted from certain species of micro-organisms and specifically active against a very small number of bacterial DNases[4–6].

The inhibitory effect of the RNA of yeast on pancreatic DNase is rather slight[7].

We thought it of interest to carry out similar investigations on an isologous enzymatic system, that is, one in which use is made of an enzyme and the substrate from the same animal species. We have therefore examined the effect of calf-liver RNA on the degradation of calf-thymus DNA brought about by neutral crystallized DNase of Kunitz[8], obtained from pancreas of the same species of animal.

The DNase (Worthington Co.) was re-crystallized three times.

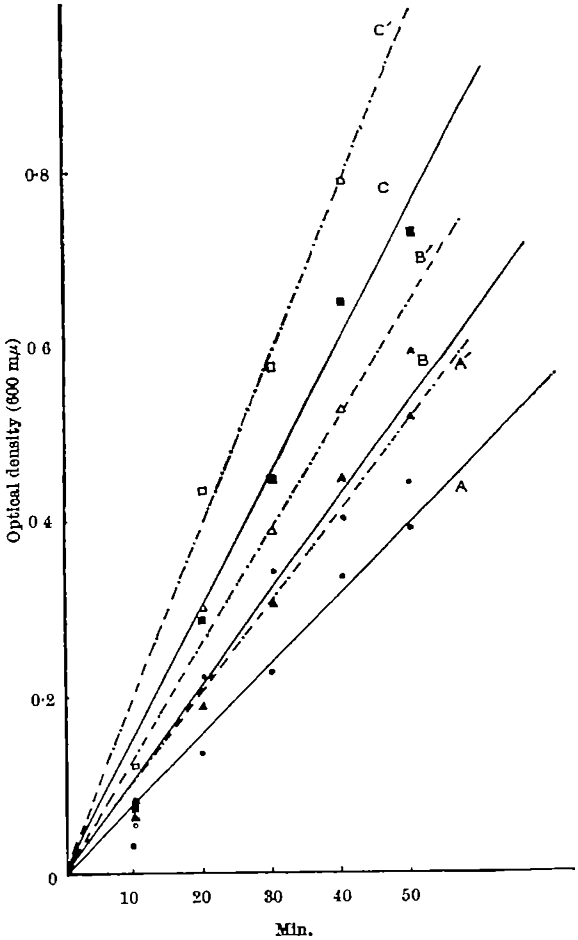

Fig. 1. Influence of calf liver RNA on calf-thymus DNA–Kunitz pancreatic DNase system. Determination of DNase activity was by spectrophotometry. The solutions contained 0·125, 0·166, 0·250 mgm./ml. DNA in *tris*-buffer medium 0 066 *M* at *p*H 7·5, magnesium chloride 0 025 *M* either in the presence of 0·833 mgm./ml. RNA in EDTA 10^{-4} *M* (curves *A'*, *B'* and *C'*) or EDTA 10^{-4} *M* without RNA (curves *A*, *B* and *C*). The concentration of DNase was 0·083 μgm./ml.

The DNA was prepared by the method of Kay *et al.*[9]. It contained less than 0·3 per cent protein. Its molecular weight was 6 × 10^6 (light-scattering data). Its intrinsic viscosity was 5·300 c.g.s. units. $E_{(P)}$ was 6·600.

The RNA was prepared by the method of Laskov *et al.*[10], by precipitation with 3 *M* sodium chloride, and preserved in 10^{-4} *M* ethylenediamine tetraacetic acid. Its molecular weight was 9 × 10^5 (light-scattering data). It contained only traces of protein.

The RNA contained no DNA, showed no DNase activity and was completely precipitable by acid under the experimental conditions used.

The DNA contained less than 0·9 per cent RNA and showed no RNase activity.

The principle of these experiments consisted in the comparison of the degradation-rates of increasing quantities of substrate (DNA) in the presence of an unchanged quantity of enzyme (DNase) with and without RNA. Several experiments were carried out with different RNA/DNA weight ratios. The activity of deoxyribonuclease was determined under optimal conditions as regards *p*H (7·5) and magnesium concentration (0·025 *M*) either by a spectrophotometric method derived from that of Kunitz[8]—determination

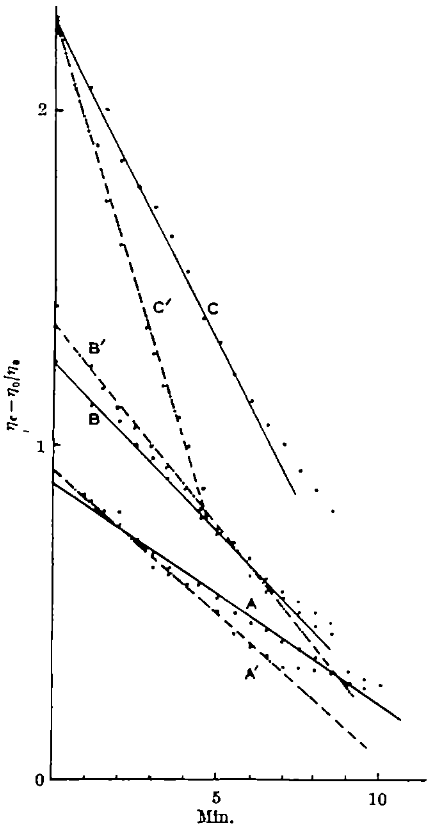

Fig. 2 Determination of DNase activity by viscosimetry. Conditions as for Fig. 1

of the acid-soluble derivatives by quantitative determination of deoxyribose—or by a viscosimetric method at a constant rate of shear.

The results with either method were practically the same (Figs. 1 and 2). The calf-liver RNA definitely had not the same effect as that obtained with bacterial compounds; on the contrary, it slightly enhanced the degradation-rate of the calf-thymus DNA under the influence of crystallized DNase obtained from pancreas of the same species of animal. Preliminary findings (substitution to the RNA of hydrolysates by ribonuclease, or of its calcination residue) suggest that this non-specific activation is due to the presence of mineral substances accompanying the RNA.

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Borate Inhibition of Chymotryptic Proteolysis

In attempts to duplicate the experiments on the rate of hydrolysis of bovine growth hormone by α-chymotrypsin[1] a pronounced inhibitory effect of borate ion on chymotryptic proteolysis was observed.

The bovine growth hormone, isolated in this laboratory, was of high purity and comparable with that reported in the literature[2,3] in regard to both biological activity and physicochemical properties. The human serum albumin had been isolated here some years ago by one of E. C. Cohn's alcoholic fractionation procedures. The α-chymotrypsin was a three-times crystallized product obtained from Worthington and was further characterized by the determination of its specific activity against acetyl-tyrosine ethyl ester and benzoylarginine ethyl ester. The protein substrate was dissolved at *p*H 9·5 in a buffer containing either glycine or 2-amino-2-methylpropane-1,3-diol; the ionic strength ($\Gamma/2$) was 0·1. The chymotrypsin was dissolved in $M/10$ sodium chloride containing 10^{-3} M hydrochloric acid. One ml. of the protein solution was added to 5·9 ml. of each of various buffers of *p*H 9·5 containing different concentrations of borate and sodium chloride to make $\Gamma/2 = 0{\cdot}1$. To this mixture 0·1 ml. of enzyme solution was added from a calibrated microsyringe. The final concentration of substrate was 1 per cent; the enzyme to substrate ratio (E/S) was 1/300 for the digestions of growth hormone and 1/100 for those of serum albumin. The buffer compositions were appropriately modified when ionic strength rather than borate concentration was the variable examined. In every case the *p*H of the reaction mixture was found to be 9·5 at the start of the digestion. Aliquots of the digests were removed at 30-min. intervals and precipitated with an equal volume of freshly prepared 10 per cent trichloroacetic acid. The precipitate and supernatant were separated by centrifugation for 10 min. at 2,000*g*. The colour developed in each fraction with Folin's phenol reagent by the procedure of Lowry *et al.*[4] was measured in the Beckman *DU* spectrophotometer and converted to 'protein' by using charts calibrated with the appropriate protein substrate. The percentage digestion was calculated as 100 times (protein in supernatant)/(sum of protein in supernatant and precipitate). All curves are free-hand constructions.

Since the colour intensity developed in this procedure does not follow the Lambert–Beer law and, moreover, varies with the protein used, and since the protein digests are no longer the native protein, an appreciable error is introduced. For a given sample of digest the specific colour yield varies non-linearly and inversely with the concentration of protein used in the determination. This was especially true for the 'protein' soluble in 5 per cent trichloroacetic acid in contrast to the insoluble protein. To minimize this error each sample was analysed at three low levels of concentration and the curve visually constructed on these values was extrapolated to zero concentration. The degree of inhibition of the chymotryptic hydro-

Table 1. BORATE INHIBITION OF CHYMOTRYPTIC PROTEOLYSIS OF HUMAN SERUM ALBUMIN

Digestion time (min.)	Per cent hydrolysis*	
	Without borate†	With borate‡
60	17·8	8·6
90	21·6	11·8
120	24	11·9

* Both digestions at *p*H = 9·5, $\Gamma/2 = 0{\cdot}1$; $E/S = 1/100$.

† Buffered with 2-amino-2-methylpropane-1,3-diol

‡ Borate concentration of 0·06 M.

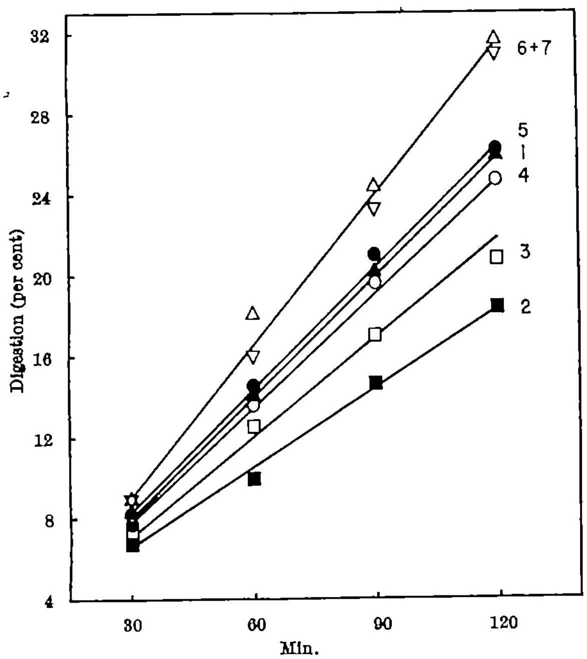

Fig. 1. Borate inhibition of chymotryptic hydrolysis of bovine growth hormone. Curves 2–5, digestion-rate at Γ/2 of 0·1 and borate concentration of 0·113, 0·055, 0 081 and 0 016 *M*, respectively. Curve 1, Γ/2 of 0·04 and borate 0 025 *M*. Curves 6 and 7, digestion-rate in glycine buffer of Γ/2 0 1 (▽) and 0 05 (△) with no borate. The *p*H in all cases was 9·5, the substrate concentration 1 per cent and the ratio of enzyme to substrate 1 : 800

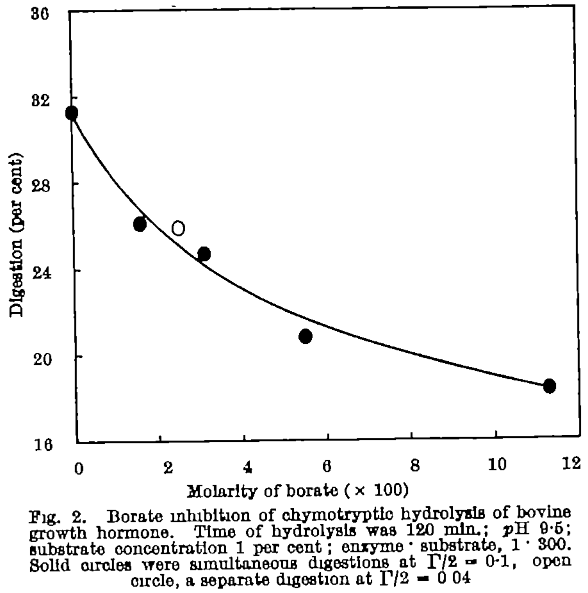

Fig. 2. Borate inhibition of chymotryptic hydrolysis of bovine growth hormone. Time of hydrolysis was 120 min.; *p*H 9·5; substrate concentration 1 per cent; enzyme · substrate, 1 · 300. Solid circles were simultaneous digestions at Γ/2 = 0·1, open circle, a separate digestion at Γ/2 = 0 04

lysis of bovine growth hormone varied from 16 per cent at a borate concentration of 0·016 *M* to 42 per cent at a concentration of 0·113 *M* (Fig. 1). The inhibition is not a linear function of borate concentration (Fig. 2). The adverse effect of borate is not dependent on some peculiarity of bovine growth hormone. Thus Table 1 illustrates the inhibition of chymotryptic hydrolysis of human serum albumin. Since the two protein substrates are quite different in most respects, the inhibition by borate would seem to be a general property of this ion in chymotryptic proteolyses.

Variations of ionic strength independent of borate ion are without effect, within the limits studied, as shown in Figs. 1 and 2. In the latter the digestion carried out at Γ/2 of 0·04 (open circle) falls in line with the curve of borate inhibition at Γ/2 of 0·1. Curves 6 and 7 of Fig. 1 show that the digestion-rate in glycine buffer with no added borate was essentially identical at Γ/2 of 0·1 and of 0·05. A series of four digestions at ionic strengths varying from 0·04 to 0·24 was carried out in borate-free buffers of 2-amino-2-methylpropane-1,3-diol and yielded results which did not differ significantly from those shown for glycine in Fig. 1. Whether or not borate is inhibitory to other hydrolases and whether the inhibition is produced by a complexing with the substrate, the enzyme or both is not known. Osaki[5] has very recently reported on the borate inhibition of the oxidase activity of ceruloplasmin, and proposed the likelihood that the borate combined with one of the carbohydrate residues of that enzyme.

Hofstee[6] recently has reported that salts reverse the inhibition produced by serum albumin on the chymotryptic hydrolysis of synthetic substrates. Schlamowitz and Peterson[7] have shown that ionic strength markedly affects the course of proteolysis by pepsin. The entirely different experimental conditions in both these reports, however, preclude any comparison with the lack of effect of ionic strength described here.

I thank Drs. C. H. Li and H. Papkoff for their co-operation, Mrs. Jessie Ozmun for her assistance, and Dr. H. V. Anderson and Mr. C. Vander-Kolk for the bovine growth hormone.

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In vitro Synthesis of Phosphoarginine by Blowfly Muscle

IN a recent review dealing with respiratory metabolism in insect flight-muscle Sacktor[1] directed attention to the fact that the synthesis of phosphoarginine has not been demonstrated *in vitro* in any insect tissue.

While investigating the conditions necessary *in vitro* for the demonstration of respiratory control in flight-muscle of *Calliphora erythrocephala* we have examined the effect of arginine on the oxygen uptake by suspensions of thoracic muscle. These were prepared at 0° C. by crushing thoraces from 50 flies in 3 ml. of a medium containing 0·32 *M* sucrose, 10^{-2} *M* *tris*/hydrochloric acid buffer (*p*H 7·3), 10^{-2} *M* ethylenediaminetetra-acetate, and squeezing the muscle pulp through six layers of muslin. The oxygen uptake was measured with a vibrating platinum electrode after the addition of 0·1 ml. of the suspension to 2·1 ml. of the following medium: 0·18 *M* sucrose; 2·1 × 10^{-2} *M* potassium dihydrogen phosphate/*tris* buffer (*p*H 7·0); 10^{-3} *M* ethylenediamine tetraacetate; $6{\cdot}3 \times 10^{-3}$ *M* magnesium chloride; $4{\cdot}8 \times 10^{-4}$ *M* adenosine triphosphate; $1{\cdot}3 \times 10^{-2}$ *M* pyruvate; $1{\cdot}3 \times 10^{-3}$ *M* fumarate. Under these conditions the addition of adenosine diphosphate caused a six-fold increase in the rate of oxygen uptake. Similarly the addition of arginine hydrochloride (20 µl. 1 *M*) was followed by a three-fold increase in the rate of oxygen uptake, and it seemed likely that this increase could be attributed to the formation of adenosine diphosphate through the arginine phosphokinase reaction. Since this would result in the simultaneous formation of phosphoarginine, the demonstration of this substance in the incubated suspension was attempted. The reaction mixture was deproteinized with 2 ml. of ethanol 5 min. after the addition of the muscle suspension. For the zero-time control 2 ml. of ethanol was added to the reaction medium before the muscle suspension. The deproteinized mixtures were centrifuged at 0° C. for 10 min. at 2,000*g*, and 50 µl. samples of the supernatants were chromatographed on strips of Whatman No. 1 paper either in water-saturated phenol in an atmosphere of ammonia or in propanol/ammonia (sp. gr. 0·88)/water (60 : 30 : 10).

Duplicate chromatograms were tested either with ninhydrin or with the Sakaguchi reagent. Both tests showed that the supernatant from the reaction mixture contained, in addition to arginine, a compound running with the same R_F as phosphoarginine in both solvent systems (0·3 in the phenol solvent, 0·17 in the propanol solvent), and that this compound was not present in the zero-time control. A sample of the supernatant from the reaction mixture was brought to 0·1 *N* with respect to hydrochloric acid and kept for 1 min. at 100° C. The sample was then neutralized with 3 *N* ammonia and 50 µl. aliquots were chromatographed in the phenol solvent. Treatment of the chromatograms with ninhydrin showed that the slower running compound (R_F 0·3) was no longer present, a finding which supports the supposition that this compound is phosphoarginine.

In further experiments muscle suspensions were incubated in Warburg flasks, 0·1 ml. of suspension being added to 0·9 ml. of the following medium: 0·18 *M* sucrose; $5{\cdot}6 \times 10^{-3}$ *M* potassium dihydrogen phosphate/*tris* buffer (*p*H 8·0); 10^{-3} *M* ethylenediaminetetraacetate; $7{\cdot}3 \times 10^{-3}$ *M* magnesium chloride; $5{\cdot}5 \times 10^{-4}$ *M* adenosine triphosphate; $1{\cdot}3 \times 10^{-2}$ *M* pyruvate; $1{\cdot}3 \times 10^{-3}$ *M* fumarate; $5{\cdot}5 \times 10^{-2}$ *M* arginine hydrochloride. Each flask also contained 25 µc. of [^{32}P]orthophosphate. Suspensions treated similarly except for the omission of arginine were used as controls. After incubation at 25° C. for 30 min. 1 ml. of ethanol was added to each flask and the contents were centrifuged at 0° C. The supernatants were chromatographed in the phenol solvent and phosphorus-32 located by scanning the chromatograms radiometrically[2]. About 67 per cent of the phosphorus-32 from the suspension incubated with arginine appeared at R_F 0·3, where a positive reaction was obtained both with ninhydrin and with the Sakaguchi reagent. On chromatograms from suspensions incubated in the absence of arginine only about 10 per cent of the phosphorus-32 was found at this R_F and the ninhydrin and Sakaguchi tests were negative. The orthophosphate in the supernatants was estimated[3] both before and after hydrolysis for 1 min. at 100° C. in 0·1 *N* sulphuric acid. The suspensions incubated with and without arginine contained 4·3 and 0·6 µmoles of '1-minute-hydrolysable' phosphate respectively.

The supernatant from the suspension incubated with arginine was chromatographed, admixed with phosphoarginine, in the phenol solvent, the amount of supernatant being such that compounds labelled with phosphorus-32 were below the threshold for chemical detection. The labelled compound running at R_F 0·3, detected radiometrically, was found to coincide with the phosphoarginine detected chemically. These compounds on the corresponding area of a parallel chromatogram were eluted and chromatographed in the propanol solvent, when again there was coincidence between the labelled compound and the phosphoarginine.

Finally the phosphorus-32-labelled compound in question, isolated chromatographically in the phenol solvent, was hydrolysed *in situ* (1 min. at 100° C. after spraying paper with 0·1 *N* hydrochloric acid) and the hydrolysis products were eluted and rechromatographed. Both ninhydrin and Sakaguchi tests showed a single compound running with the R_F of arginine in the phenol solvent (0·9) and in the propanol solvent (0·39), and the phosphorus-32 ran with the R_F of inorganic phosphate. Thus it appears that the compound produced on incubating the muscle suspensions with arginine is in fact phosphoarginine.

Sacktor's failure to demonstrate the formation of phosphoarginine by isolated sarcosomes[4] may have been due, as he suggested, to the fact that arginine phosphokinase is associated with some other fraction of the insect muscle. A preliminary examination of the sarcosomal and 'soluble' fractions from suspensions of thoracic muscle has shown that on incubation with arginine and adenosine triphosphate the amount of phosphoarginine produced by the 'soluble' fraction is about ten times that produced by the sarcosomes. Thus, although phosphagens other than phosphoarginine may be present in insect muscle, our findings are in keeping with the accepted view that phosphoarginine is of prime importance.

We thank Dr. P. C. Caldwell for a gift of phosphoarginine.

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Binding Site of Flavin Adenine Dinucleotide combining with Sulphydryl Group of D-Amino-acid Oxidase Apo-enzyme

In previous papers[1,2] it was demonstrated, by a kinetic method using indicators, that the adenylic acid part of flavin adenine dinucleotide combines with the sulphydryl group of the apo-enzyme of D-amino-acid oxidase. The changes of the absorption spectra of flavin adenine dinucleotide, riboflavin-5′-monosulphate and adenosine-5′-monosulphate, observed on adding the apo-enzyme of D-amino-acid oxidase and/or benzoate as a substrate-substitute, revealed that the adenylic acid part of flavin adenine dinucleotide readily combines with the apo-enzyme, whereas the isoalloxazine nucleus of flavin adenine dinucleotide combines with both the apo-enzyme and the substrate-substitute[3,4]. Thus, the combination between adenylic acid part of flavin adenine dinucleotide and sulphydryl group of the apo-enzyme was considered to be important for reconstruction of the holo-enzyme. We wished to determine which group of the adenylic acid part of flavin adenine dinucleotide can actually combine with the sulphydryl group of D-amino-acid oxidase apo-enzyme.

As already mentioned previously[1,2], the NH_2-group in adenine nucleus of flavin adenine dinucleotide was first noticed. To investigate the meaning of the NH_2-group, we examined flavin hypoxanthine dinucleotide, which possesses an OH-group instead of an NH_2-group in adenine nucleus of flavin adenine dinucleotide.

Flavin hypoxanthine dinucleotide was prepared from flavin adenine dinucleotide by the method of Fujisawa[5]. Flavin adenine dinucleotide was diazonized with sodium nitrite in cold 2 *N* acetic acid and the flavin hypoxanthine dinucleotide that resulted was separated by paper chromatography using 5 per cent sodium phosphate as a mobile phase, eluted from the paper with water, saturated with ammonium sulphate, and transferred to phenol. The yellow phenol layer was collected, dried with sodium sulphate and flavin hypoxanthine dinucleotide was extracted with a small amount of water, washed with ethyl ether several times and then precipitated with 95 per cent ethyl alcohol. The absorption spectrum of the solution of this powder has a shoulder at 255 mμ, the ratio E_{260}/E_{450} being 2·8.

D-Amino-acid oxidase apo-enzyme was prepared by a method that we have described elsewhere[4]. Flavin adenine dinucleotide was prepared by the method of Yagi *et al.*[6]. The coenzyme action of flavin hypoxanthine dinucleotide was tested by a conventional manometric method using a reaction mixture of apo-enzyme 1×10^{-7} mole/l., flavin hypoxanthine dinucleotide 3×10^{-6} mole/l. and an excess of D-alanine in pyrophosphate buffer (*p*H 8·3, *M*/10). The reaction mixture showed no uptake of oxygen. It was also observed that flavin hypoxanthine dinucleotide has no inhibitory action on D-amino-acid oxidase at concentrations of 3×10^{-5} mole/l. and 8×10^{-8} mole/l., of flavin hypoxanthine dinucleotide and flavin adenine dinucleotide respectively. These results indicate that flavin hypoxanthine dinucleotide has neither coenzyme activity nor inhibitory action for D-amino-acid oxidase.

The absorption spectrum at visual wave-length region of flavin hypoxanthine dinucleotide was measured, and was found to be similar to that of flavin adenine dinucleotide. It was not changed on adding the apo-enzyme. The influence of both the apo-enzyme and benzoate, a substrate-substitute of this enzyme, on the absorption spectrum of flavin hypoxanthine dinucleotide was investigated. The absorption spectrum at wave-lengths in the visual region of flavin hypoxanthine dinucleotide was not changed at all.

It has been noted[3,4] that the absorption spectrum of flavin adenine dinucleotide at wave-lengths in the visual region was slightly modified by the apo-enzyme and markedly changed on adding both the apo-enzyme and benzoate.

Comparing these data on flavin hypoxanthine dinucleotide with those of flavin adenine dinucleotide, it can be concluded that the NH_2-group of adenine part of flavin adenine dinucleotide is essential for the reconstruction of the holo-enzyme and is one of the most important binding sites of the coenzyme in D-amino-acid oxidase activity.

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PHYSIOLOGY

Sodium–Potassium-activated Adenosine Triphosphatase in the Squid Giant Axon

OUR understanding of the recovery mechanism involved in the active transfer of sodium out of, and of potassium into, the nerve cell has been increased considerably in recent years through the examination of giant axons like those of the squid. Hodgkin and Keynes[1] showed that the efflux of sodium-22 and the influx of potassium-42 were both markedly reduced by exposing the nerves to 0·2 m.mol./l. dinitrophenol, 2 m.moles/l cyanide or 3 m.moles/l. azide. The passive movements of the ions down their concentration gradients were little affected, as was also the electrical activity of the nerves, which involves the latter movements. The inhibition of the active cation transport was largely reversed when the inhibitors were washed away.

Caldwell[2,3] was able to show a parallelism between the axoplasmic levels of adenosine triphosphate (ATP) and related compounds and the sodium-22 efflux from squid axons on exposure to cyanide and during recovery after removal of the inhibitor. Injection of ATP, and also of arginine phosphate (AP) and phosphoenolpyruvate (PEP), into the axoplasm of a squid axon exhausted of endogenous ATP through prior exposure to cyanide caused a transient increase of the sodium-22 efflux[4,5], but external application of ATP and AP had no effect. Injections of creatine phosphate, arginine, adenosine monophosphate, inorganic orthophosphate and pyrophosphate did not change the sodium-22 efflux. It was also shown that within minutes after injection of AP and PEP into a cyanide-poisoned squid axon the internal ATP level had returned to normal[6]. This indicates that the active cation transport is driven by ATP or a related high-energy phosphate compound. In a potassium-free medium the sodium efflux was reduced by 70 per cent[1,6].

These findings assume particular importance in the light of the discovery of a cardiac glycoside-sensitive sodium–potassium-activated ATPase in crab nerve by Skou[7,8], and the subsequent demonstration by Post *et al.*[9] and Dunham and Glynn[10,11] that this enzyme is an integral part of the active sodium–potassium transport mechanism in erythrocyte membranes. Caldwell and Keynes[12] showed that the cardiac glycoside ouabain (10^{-5} mole/l.) applied externally to a squid axon reduced the sodium-22 efflux by 82 per cent, with the remaining sodium-22 efflux being insensitive to cyanide or to injection of ATP. Unlike cyanide. dinitrophenol and azide, ouabain had no effect when injected into the axon[12], nor did it significantly reduce the levels of ATP and AP in the axoplasm[3]. It has now been possible to demonstrate the presence of a cardiac glycoside-sensitive sodium–potassium-activated ATPase in the squid axon and to compare its activity in the sheath on a surface area basis with the ion flux data obtained by Caldwell and Keynes[13].

The giant axons were obtained from the squid *Loligo pealii*, caught at the Marine Biological Laboratory, Woods Hole (Mass.). After dissecting the axon free from the animal and measuring its length and diameter, the axoplasm was separated from the sheath[14]. Sheath and axoplasm were placed on a glass slide, and immediately frozen and stored in dry ice until lyophilization. The material was collected over a 30-hr. period, flown to the laboratory in Bethesda, and frozen-dried within 18 hr. after collection. The length of the sheath was measured again to allow correction for shrinkage during freeze-drying. Sections, approximately 0·7 mm. long, were cut from the sheath, their length was measured microscopically, and the dry weight (1·8–3·3 μgm.) was determined on a quartz fibre ultramicro balance[15]. Samples were taken from the dried axoplasm and weighed in similar fashion (3·0–5·0 μgm. dry weight). Each sample was inserted into a 5 μl. aliquot of each of two substrate media by means of a glass needle under stereomicroscopic observation. The rest of the assay procedure was as described previously[16] except 30 μl. (instead of 50 μl.) 10 per cent w/v trichloroacetic acid was added after incubation. The substrate media used were the complete medium *A* (for total ATPase activity) and the inhibiting medium *B* (containing 0·1 m.mole/l. ouabain for complete inhibition of sodium–potassium ATPase). Six samples of each of five sheaths and two axoplasm specimens were assayed in each medium.

The results, expressed in m.moles/kgm. dry wt./hr., in percentage of total ATPase activity and in moles/cm.^{2}/sec., are shown in Table 1. It will be noticed that the sodium–potassium ATPase activity is present in both structures, representing about 24 per cent of the total ATPase activity. On a dry weight basis, the average sodium–potassium ATPase activity in the axoplasm is only about half as large as in the sheath or really only about one-third as high as in sheaths Nos. 1 and 2, from which the axoplasm specimens were extruded. On a surface area basis, the average sodium–potassium ATPase activity in the sheath amounts to 9·6 (± 1·9 *S.E.*) × 10^{-12} mole/cm.^{2}/sec. Caldwell and Keynes[13] have recently determined that the total resting influx of potassium-42 in the cleaned squid axon is 16·3 (± 2·2 *S.E.*) × 10^{-12} mole/cm.^{2}/sec. Poisoning by 2 m.moles/l. cyanide reduced the potassium-42 influx to 4·3 (± 0·3 *S.E.*) × 10^{-12} mole/cm.^{2}/sec. (ref. 6), indicating that the active potassium-42 influx is 12·0 × 10^{-12} mole/cm.^{2}/sec. The molar ratio of active potassium-42 influx to sodium–potassium ATPase activity is then 1·25 (± 0·34 *S.E.*). This is in good agreement with the ratio of 1·46 moles sodium-22 extruded per mole of ATP injected, determined by Caldwell *et al.*[6]. This approximate equivalence of action flux and sodium–potassium ATPase activity has been found to hold over a 30,000-fold range in cation flux in six different tissues[17].

Table 1. ATPase Activity in Squid Axon Sheath and Axoplasm

Specimen	Ratio area/dry wt. 10^{3} cm.^{2}/gm.	Total ATPase m.mol./kgm. dry wt./hr.	Na–K m.mol./kgm. dry wt./hr.	ATPase activity Percentage of total	ATPase activity 10^{-12} mol./cm.^{2}/sec.
Sheath 1	5·96	857	286	33	13·3
Sheath 2	6·43	854	319	37	18·8
Sheath 3	8·51	664	124	19	4·1
Sheath 4	5·51	824	126	15	6·2
Sheath 5	5·24	1,421	200	14	10·6
Average	—	924	211	23·6	9 6
Axoplasm 1	—	498	109	22	—
Axoplasm 2	—	400	107	27	—
Average	—	449	108	24 1	—

The similarity between the properties of the sodium–potassium ATPase and the active sodium–potassium transport mechanism in the squid axon (ATP requirement, inhibition by ouabain in medium, requirement for potassium in medium, location in sheath), as well as the equivalence of the enzyme activity and the active potassium-influx, seem to indicate that this enzyme is an integral part of the active sodium–potassium transport mechanism in the squid axon[18].

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Inhibitory Effect of Some Mucopolysaccharides on the Lipolytic Activity of the Aorta of Animals

THE acid mucopolysaccharides (MPS) as macroanions form complexes with proteins and thus they change the isoelectric point of the latter. Based on this property some MPS's are able to inhibit or activate many enzyme systems[1].

We previously reported[2,3] on the interaction between MPS's isolated from human aortic intimas and β-lipoprotein fraction as well as fibrinogen of human plasma. The fact was established that one group of the aortic intimal MPS's formed specific complexes with these plasma constituents. Zemplényi *et al.*[4,5] demonstrated the lipolytic activity of the aorta of various species and recently established the inhibitory effect of a raised calcium concentration on the lipoprotein–lipase activity.

We have now demonstrated that some intimal MPS's may be of importance in the mechanism of lipid deposition by their inhibitory effect on the lipolytic activity of the vessel wall.

The lipolytic activity of the aortas of 10 rabbits, 6 dogs and 4 rats was investigated by incubating the aortic homogenates with human lipæmic serum according to the method of Zemplényi *et al.*[4]. The effect of an MPS mixture isolated from the intimas of pooled atherosclerotic human aortas on the lipolytic activity of the vessel wall of the animals was examined. The method of isolation was based on that of Dyrbye and Kirk[6]. Our preparations contained, besides the acid-MPS's, sialic acid and other glycoprotein components.

Fig. 1 shows a typical pattern of the inhibitory effect of increasing concentrations of human aortic MPS mixture on the lipolytic activity of rabbit-aortas. With an increasing concentration of the MPS mixture the lipolytic activity decreased and by 5 mgm. previously incubated mixture the grade of inhibition was complete in all cases investigated.

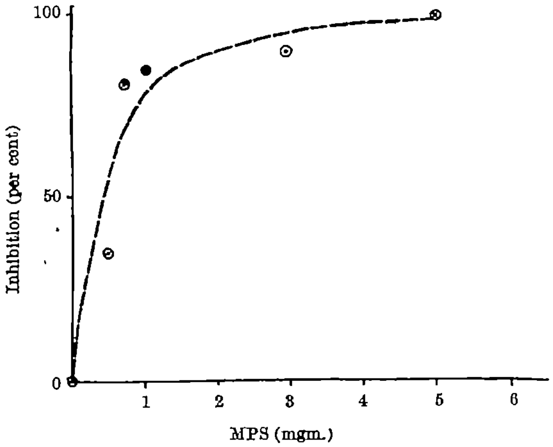

Fig 1. Inhibitory effect of human aortic MPS mixture on the lipolytic activity of rabbit aorta

Next we compared the inhibitory effect of 5 mgm. crystalline hyaluronic acid and that of 5 mgm. chondroitin sulphuric acid (L. Light and Co., Ltd., Colnbrook) with the same quantity of human aortic MPS mixture (which completely inhibited the lipolytic activity of the aortic tissues). Fig. 2 demonstrates that under the same experimental conditions the inhibition of the lipolytic activity of chondroitin sulphuric acid and hyaluronic acid was definitely lower than that of the aortic MPS preparation used by us.

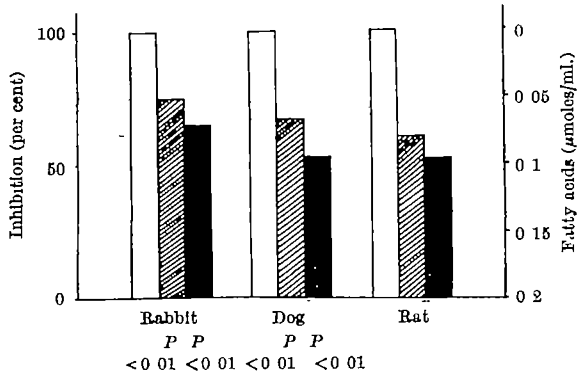

Fig. 2 Inhibitory effect of 5 mgm. aortic MPS, hyaluronic acid and chondroitin sulphuric acid on the lipolytic activity of the aorta. White, human aortic MPS mixture; shaded, hyaluronic acid; black, chondroitin sulphuric acid

Results were similar with all species investigated, differences being significant with rabbits and dogs.

These findings concerning the lower inhibitory activity of chondroitin sulphuric acid and hyaluronic acid support the view that some other MPS components of the vessel wall, even more the interaction of the different components, may be significant.

Based on our previous[2,3] and present investigations —which reflect a part of the possible biochemical processes in the arterial wall—we can assume that the deposition of lipids in the intima may be explained by some chemical or physicochemical changes in the MPS composition of the intimal ground substance. These changes may partly facilitate the formation of MPS-β-lipoprotein complexes and partly inhibit the lipolytic activity of the vessel wall. Both processes may be important in the chain of events which leads to atheromatosis.

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Inhibition of Anaphylactic Histamine Release by 2-Deoxyglucose

The inhibition of anaphylactic histamine release by lack of oxygen[1-3] suggested the possibility that the metabolism of glucose by the oxidative pathway might be involved in the process. However, glucose itself caused no stimulation of histamine release in my experiments[4], although such stimulation has been reported by others[5,6]. It is possible that the stimulant effect of glucose is observed only when the tissue is depleted of endogenous substrate during its preparation It should then be possible to inhibit histamine release even in the presence of endogenous substrate with 2-deoxyglucose, which blocks glucose utilization beyond glucose-6-phosphate stage[7]. Moreover, it has been shown that the inhibition of histamine release by anoxia can be partially reversed by glucose[6,8]. If this reversal depends on the glycolytic breakdown of glucose, 2-deoxyglucose would be expected to block it. In the present investigation, therefore, the effect of 2-deoxyglucose on anaphylactic histamine release was studied both under aerobic and anaerobic conditions.

Anaphylactic reaction was produced *in vitro* using minced and washed lungs from guinea pigs sensitized to egg albumin. The histamine release and oxygen uptake during 20-min. periods after the addition of antigen were determined as described elsewhere[4]. The inhibitor, 2-deoxyglucose, was added 30–50 min. before contact with antigen. The incubation medium routinely used (modified Krebs–Ringer solution[9] at

Table 1. Reversal by Pyruvate and Acetate of 2-Deoxyglucose induced Inhibition of Anaphylactic Histamine Release

Exp.	Substrate	2-Deoxy-glucose (m.mol.)	Oxygen uptake (Q_{O_2})	Histamine release (μgm./gm. dry wt.)	
1	0	0	3·7 ± 0·08	2·8 ± 0·21	(4)
	0	5	3·2 ± 0·07	1·4 ± 0·13	(3)
	Pyruvate, 9 m mol.	5	4·3 ± 0·05	2·0 ± 0·20	(4)
				($P < 0·05$)	
2	0	0	4·4 ± 0·18	8·6 ± 0·49	(3)
	0	5	4·3 ± 0·04	1·9 ± 0·24	(3)
	Pyruvate, 40 m mol.	5	5·6 ± 0·04	5·2 ± 0·38	(4)
				($P < 0·001$)	
3	0	0	4·3 ± 0·05	11·4 ± 0·49	(4)
	0	10	3·9 ± 0·01	3·6 ± 0·21	(4)
	Acetate, 13 m mol.	10	5·2 ± 0·05	5·2 ± 0·34	(4)
				($P < 0·01$)	

Mean histamine release (as base) and standard error of the mean shown. Number of samples in parentheses in the last column. P relates to the two immediately overlying values.

pH 7·3–7·4) was free from substrate. When a substrate was used, the tissue fragments were placed in this medium, in aerobic experiments, about 30 min. before the addition of the inhibitor. In anaerobic experiments the substrate (glucose) and the inhibitor were added at the same time. Anaerobiosis was produced with helium.

In the absence of added substrate 2-deoxyglucose causes pronounced inhibition of histamine release as shown in Fig. 1. The respiration was, however, very little affected—the oxygen uptake corresponding to the histamine values shown in Fig. 1 varying from 86 to 97 per cent of normal. Indeed, the inhibition of histamine release may be associated with an unchanged ($P > 0·5$) uptake of oxygen (Exp. 2, Table 1).

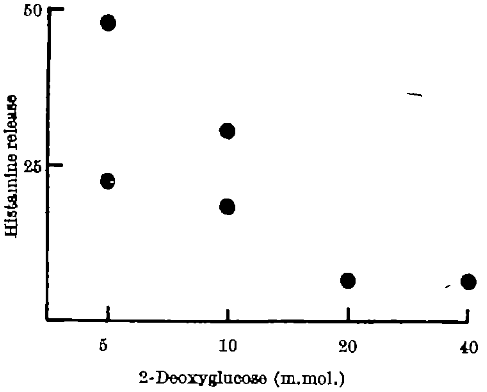

Fig. 1. Inhibition of anaphylactic histamine release by 2-deoxyglucose. Histamine release with inhibitor is expressed as a percentage of the release without inhibitor (control). The points—showing the mean values from 2–4 samples with the inhibitor and an equal number of controls—represent separate experiments except for the two highest concentrations

If the dependence of anaphylactic histamine release on oxygen is due to the oxidative metabolism of glucose, it may be assumed that the blocking effect of 2-deoxyglucose would be reversed by supplying a substrate distal to the block. As shown in Table 1 pyruvate and acetate were indeed effective in reversing the inhibition caused by 2-deoxyglucose. However, the reversal was incomplete even when eight times higher molar concentration of one of the substrates (pyruvate) was used. The penetration of the cell membrane could be a limiting factor, although enough substrate entered the cell to cause a stimulation of respiration.

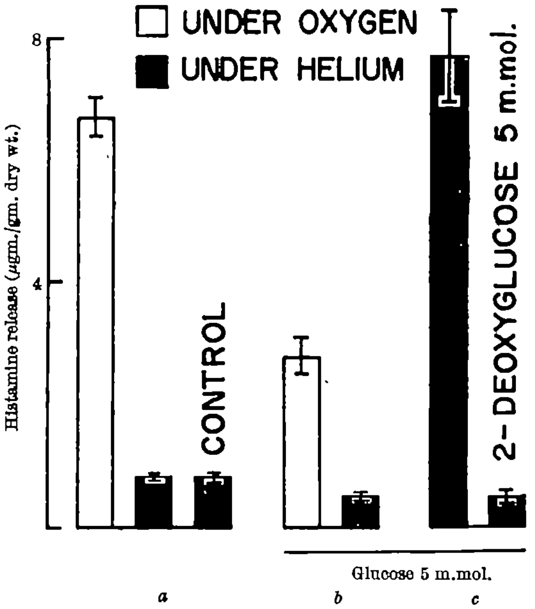

Fig. 2. Effect of 2-deoxyglucose on anaphylactic histamine release (expressed as base) in anoxia. All samples were incubated with antigen except the control of experiment a. Spontaneous histamine release is included in the values of a and deducted from those of b and c. Mean and $S.E.$ of three samples for a and four samples for b and c. a shows that anoxia completely inhibits histamine release in a substrate-free medium; b, glucose partially restores histamine release blocked by anoxia; c, the glucose-effect is inhibited by 2-deoxyglucose

The experiments shown in Fig. 2 were performed to explore the effect of 2-deoxyglucose on histamine release in anaerobic condition. Histamine release was completely inhibited by lack of oxygen in a substrate-free medium (Fig. 2a), and the inhibition was partially restored by supplying glucose (Fig. 2b). As shown in Fig. 2c the reversing effect of glucose could be blocked by equimolar amount of 2-deoxyglucose (5 m.mol.); when 10 m.mol. of 2-deoxyglucose was used in the same experiment histamine release was completely inhibited.

2-Deoxyglucose is phosphorylated by hexokinase but the product, 2-deoxyglucose-6-phosphate, accumulates inside the cell and causes a block in the glycolytic pathway. In rat diaphragm the phosphorylated glucose analogue reaches a concentration of 0·02 M and inhibits the penetration of glucose[10]. This seems to be the mechanism by which the restoring effect of glucose on anaphylactic histamine release in anoxia is blocked by the analogue: 2-deoxyglucose-6-phosphate, formed from the analogue by inhibiting glycolytic utilization of glucose, blocks histamine release in the presence of glucose under anoxia. In case of *in vitro* anaphylaxis under oxygen, where no external substrate was used, the breakdown of glycogen in the cell was apparently blocked by the accumulated 2-deoxyglucose-6-phosphate.

The inhibition of histamine release by 2-deoxyglucose under oxygen was not associated with a remarkable effect on respiration, although only one-third of the oxygen uptake is sufficient for the optimal anaphylactic histamine release[4]. This suggests the possibility that some specific reaction in the metabolic pathway of glucose may be concerned with the histamine releasing process.

It is of interest to note that 2-deoxyglucose has been found to inhibit anaphylactoid œdema of rats caused by dextran and ovomucoid, but the reactions to compound 48/80 were unaffected[11]. The latter observation fits in with the finding[12] that 2-deoxyglucose does not inhibit histamine release from minced rat lung incubated under oxygen with compound 48/80.

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PHARMACOLOGY

Analysis of the Vasodilator Effect of Adrenaline on the Skeletal Muscle Vessels of Man

INTRA-ARTERIAL infusions of small amounts of adrenaline cause a marked but transient increase in the flow of blood in the calf of the leg; this is followed by a return to the base-line or even by a decrease in flow of blood, although the infusion continues[1]. The biphasic effect of adrenaline may be explained by interaction with the different adrenergic receptors[2], the primary vasodilatation being mediated by the β-receptors and the secondary vasoconstriction being brought about by action on the α-receptors. Supporting such an explanation is the observation that the decrease in flow of blood in the muscles of the fore-arm due to adrenaline can be abolished by an intra-arterial infusion of dibenyline which is known to inhibit the action of adrenaline on the α-receptors[3]. Proof of the existence of β-receptors in blood vessels of man has so far not been reported.

In order to elucidate the increase in flow of blood in muscle due to intra-arterially administered adrenaline in man, we used the dichloro analogue of isoproterenol (*DCI*, Lilly Compound 20522) which in experiments on animals was found to abolish almost exclusively the action of adrenaline on the β-receptors[4]. This compound, therefore, can be considered a useful tool for the qualitative separation of α- and β-adrenergic receptors. Measurements of flow of blood were made in the calves of healthy male volunteers, 20–25 years of age, using the venous occlusion plethysmography[5]. All substances were subjected to infusions in the femoral artery by means of an electrically driven infusion pump. The transient vasodilatation in the calf caused by a 3 min. infusion of 1·76 μgm./min. 1(–)adrenaline, which is easily reproducible in intervals of 3–8 min., could be abolished by a preceding infusion of *DCI*.

Fig. 1 shows a typical experiment. After a dose of 22 mgm. *DCI* within 5½ min., which produced some vasodilatation, the increase in flow of blood due to adrenaline was not diminished, but modified so that a secondary vasoconstriction followed (cf. A_1 and A_2, Fig. 1). After a further dose of 20 mgm. *DCI* within 2 min., however, no increase in flow of blood was observed during the following infusion of adrenaline (A_3); a decrease appeared instead. A small vasodilator effect was produced by adrenaline again 9 min. later, which was followed by a vasoconstrictor action (A_4).

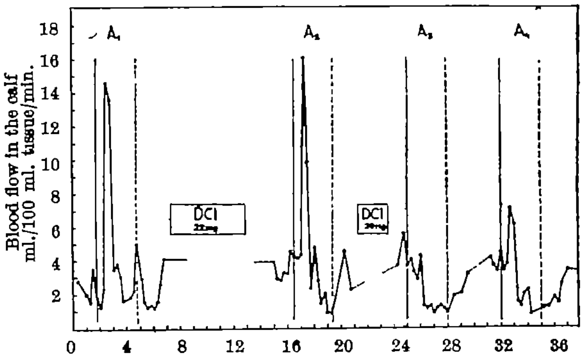

Fig. 1. A_1, A_2, A_3 and A_4, intra-arterial infusion of 1·76 μgm./min. 1(–)adrenaline. *DCI*, 22 mgm.; intra-arterial infusion of 4 mgm. *DCI*/min. for 5·5 min. *DCI*, 20 mgm.: intra-arterial infusion of 10 mgm. *DCI*/min. for 2 min.

The transient vasodilator effect of 0·8 μgm./min. isoproterenol (isopropyl-noradrenaline) (3 min. infusion time) on skeletal muscle vessels, which is similar to that of adrenaline[6], was also blocked by 37·5–62·5 mgm. *DCI*. The increase of flow of blood in the calf due to synthetic bradykinin[7], however, was not influenced by *DCI*.

It can be concluded from these experiments that the transient increase in flow of blood in the skeletal muscle vessels of man caused by adrenaline (and isoproterenol) is mediated by an interaction with the adrenergic β-receptors. If these are blocked by *DCI*, an increase in flow of blood does not occur, and sometimes even a decrease could be observed during the infusion of adrenaline. This vasoconstrictor effect may be explained by unmasking the action of adrenaline on the adrenergic alpha receptors.

Note added in proof. Similar findings have been reported by U. R. Bharadwaj and R. G. Shanks (*J. Physiol.*, 160, 5*P*; 1961) on the human fore-arm.

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Effect of 'Busulphan' ('Myleran') on the Spermatogenic Cell Population of the Rat Testis

THE ability of alkylating agents to produce selective effects on spermatogenesis and sperm in rodents has been reported in several publications[1-3]. A comparison of the timing of sterile periods after treatment with the accepted duration of spermatogenic events in the testis has enabled the phase of spermatogenesis affected by particular compounds to be located.

Following the intraperitoneal administration of 'Busulphan' (10 mgm./kgm. in arachis oil) normal fertility is retained for about 50 days, after which sterility soon develops associated with oligo- or

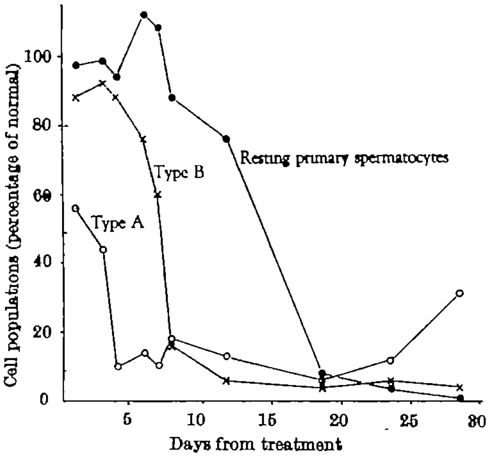

Fig. 1. Consecutive disappearance of spermatogonia (types *A* and *B*) and resting spermatocytes from the seminiferous epithelium of the rat after treatment with 'Myleran' (10 mgm./kgm.)

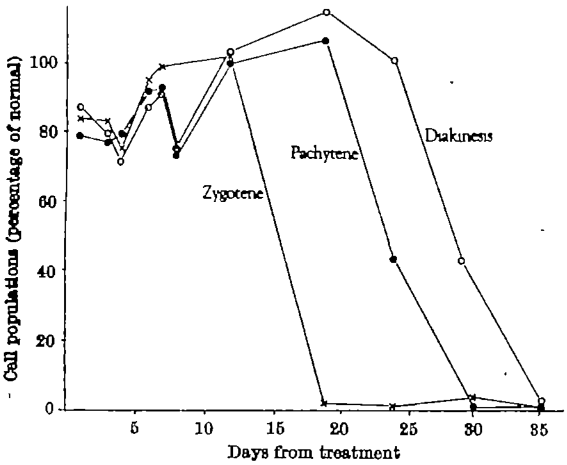

Fig. 2. Progressive depletion by maturation of zygotene, pachytene and diakinesis spermatocytes following treatment with 'Myleran'

aspermia[1,2]. An analysis of the cell population of the testis in animals so treated was undertaken in order to obtain more precise information concerning the identity of susceptible cells. In the rat, any seminiferous tubule section contains up to four successive generations of developing cells, and those in stages V, VIII and XIII[4,5] were selected for counting. Adult male rats were treated and killed at intervals of time from 24 hr. to 35 days. Sections of the testes were examined histologically, and the number of cells of various types counted in the stages referred to here.

The result confirms our earlier belief that this substance acts in a specific manner at a very early phase of spermatogenesis. A block is imposed resulting in the progressive depopulation of the spermatogenic epithelium due to continued maturation of the remainder of the cells into spermatozoa which are shed normally. Thus the number of type *A* spermatogonia falls within 4 days to a low level and remains so for about 24 days, after which slow recovery follows (Fig. 1). The majority of type *A* spermatogonia present at the time of treatment apparently continue to develop but are not replaced. Hence the level of type *B* spermatogonia remains normal for about 6 days before declining. Type *B* cells present at the time of treatment also appear to be unaffected and continue to transform into resting primary spermatocytes. However, the maturation depletion process duly affects these cells after a further lapse of time (Fig. 1). The same applies successively to the various stages of spermatocyte development (Fig. 2) until, about 34 days after the dose, the epithelium is virtually denuded of spermatocytes by conversion to spermatids.

It has been estimated that the testicular phases of spermatogenesis in the rat require exactly 48 days[5], of which 27 days are required for the conversion of type *A* spermatogonia into spermatocytes in diakinesis (calculated from results published by Leblond and Clermont[4], see Jackson, Fox and Craig[3]). From our present results, 26 days elapse between reduction of the number of type *A* spermatogonia and spermatocytes in diakinesis, respectively, to 50 per cent of normal. This is in good agreement with the duration of spermatogenesis from early spermatogonial development to the end of spermatocytic divisions.

It may be concluded, therefore, that 'Busulphan' suppresses spermatogenesis at the earliest phase of the process. A few sperm mother cells remain in a state of arrest for about 30 days, after which spermatogenesis is resumed with progressive repopulation of the seminiferous tubules and ultimate restoration of normal fertility. The evidence suggests that treatment with 'Myleran' selectively inhibits in some way the development of early type *A* spermatogonia, but does not significantly modify the overall time relationships of spermatogenesis. It appears not to interfere with the progressive development of other cell stages present at the time of treatment or with the function of the spermatozoa into which they ultimately differentiate.

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PATHOLOGY

Tumour-promoting Action of 4-Ethylsulphonylnaphthalene-1-Sulphonamide

A two-stage mechanism in carcinogenesis in the skin of the mouse was described by Berenblum[1] and termed initiation and promotion. Added evidence for this mechanism has been supplied by Salaman and Roe[2] for mouse skin, by Jull[3] for mouse breast, and by Peirce[4] for mouse fore-stomach.

It was observed that 4-ethylsulphonylnaphthalene-1-sulphonamide (HPA) and two of its analogues caused hyperplasia of urinary tract epithelium in the mouse and rat[5]. The hyperplasia persisted so long as the administration of the chemicals was continued, but quickly regressed on discontinuation of the chemicals. It was, therefore, decided to test whether HPA would act as a promoter in combination with a known weak carcinogen (initiator). In this experiment the bladders of female stock mice 12 weeks old

Table 1. Incidence of Bladder Tumours

	No of mice at start	Mice dead during experiment	Duration of experiment (weeks)	Tumours of urinary bladder					
				Adenoma	Papilloma		Carcinoma		Carcinomas in survivors to 40 weeks (per cent)
					Single	Multiple	Gd. I	Gd. II	
2-amino-1-naphthol hydrochloride + normal diet	39	9	40	4	–	–	4	1	16·7
2-amino-1-naphthol hydrochloride + HPA in diet	49	10	40	2	2	6	15	5	51·3

were implanted with paraffin pellets containing 2-amino-1-naphthol hydrochloride by the method of Jull[6] as modified by Allen *et al.*[7]. Then the animals were divided into two groups, one receiving normal diet and the other a diet containing HPA in a concentration of 0·01 per cent. The results of the experiments are summarized in Table 1.

The tumours were usually transitional cell in type, either adenoma, papilloma or carcinoma. The latter were divided into grades I and II, according to whether the musculature was invaded or not. Only one squamous cell carcinoma was found in the group with HPA in the diet, while one hæmangiosarcoma was observed in the dome in the other group.

The yield of bladder cancers in the group implanted with 2-amino-1-naphthol hydrochloride was 5 out of 30 animals at 40 weeks (16·7 per cent), an incidence similar to that observed by Bonser *et al.*[8]. In the group fed with HPA there were 20 tumours in 39 animals at 40 weeks (51·3 per cent). In this latter group there was also a greater number of papillomas. HPA, therefore, significantly enhances the yield of cancers in the bladder when combined with a weak carcinogen and thus acts as a promoter.

Oral administration of HPA to 79 mice for periods of up to 40 weeks resulted in urinary tract epithelial hyperplasia but no tumours[5]. It is thus clear that further work is needed to determine whether oral HPA is carcinogenic if the treatment is continued for a longer period and whether pellets of paraffin wax without a carcinogen could act as the initiator.

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Preservation of Living Metacyclic Forms of the *Trypanosoma brucei* Sub-group

Although it has been known for some time that it was possible to preserve alive blood forms of the African trypanosomes at the temperature of sublimation of carbon dioxide (approximately – 79° C.[1,2]), no reports have appeared on the preservation of the metacyclic forms. This report indicates that it is possible to preserve live metacyclic forms of the *T. brucei* sub-group at a similar temperature.

Metacyclic trypanosomes were obtained in the following manner. 158 wild *G. pallidipes* were caught near Lugala on the North-east shore of Lake Victoria. They were brought to the laboratory and maintained as described by Cockings[3]. These flies were fed on defibrinated bovine blood obtained from a cow maintained in an area free of tsetse fly. It was established that the cow was free from trypanosomiasis by blood-film examination and by the inoculation of blood into mice at weekly intervals over a period of several months. All the flies were given the opportunity of feeding through a guinea pig skin membrane on the defibrinated blood maintained at 30° C. in a capsule. After 1 hr., the blood remaining in the capsule was removed; 0·5 ml. was injected intraperitoneally into each of five mice, all of which developed a patent parasitæmia by the fifth day after inoculation.

The remaining 5 ml. of capsule blood was frozen as follows. To the blood 0·67 ml. of glycerol was added and ten 0·5 ml. aliquots of the mixture sealed in glass ampoules. The ampoules were then placed in a boiling tube containing absolute alcohol at 0° C. The boiling tube was securely corked and wrapped in a jacket of cotton wool 1 cm. thick. The insulated boiling tube was immersed in solid carbon dioxide in a 2 pint thermos jar, which was sealed, and rotated for 2 hr. to ensure uniform cooling of the ampoules. The ampoules were thereafter stored at approximately – 79° C. in an insulated cabinet containing solid carbon dioxide. After 3 weeks, 0·1 ml. of this blood mixture was injected intraperitoneally into each of five mice, all of which developed a patent parasitæmia by the sixth day after inoculation.

The organism produced very high parasitæmias in all the mice. In Giemsa-stained blood films, the trypanosomes were polymorphic, approximately 14μ to 30μ in length, and the majority had long free flagella. These features are characteristic of the *T. brucei* sub-group.

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IMMUNOLOGY

Antigen–Antibody Bands in the Micro-Double-Diffusion Agar Technique

Many useful variations of immunodiffusion techniques have been reported[1]. In particular the micro-adaptation has gained wide usage, and the utilization of thiazine red dye to stain the bands *after* completion of the reaction has helped make the lines visible[2]. Yet, after extensive use of these modifications in attempts to reveal anti-spermatogenic antibodies in the sera and tissues of aspermatogenic guinea pigs, our results have been disappointing. First, lines of precipitation were only rarely seen, and when they were observed, they were so faint or faded so rapidly as to instil doubt of their reality. Secondly, the use of thiazine red or other stains in the recommended manner did not improve the visibility or preservation of the lines.

We, therefore, approached the problem from an entirely different point of view. Reasoning that if we could tag one of or both the reactants (antigen and/or antibody) with dyes, we might be able not only to observe the formation of the antigen–antibody lines, but also to enhance the visibility of the lines formed so as to make them easier to photograph and preserve.

The procedure is as follows. A 1 per cent solution of crystalline hæmatoxylin is added to an antiserum or tissue homogenate in the proportion 1 : 2. This mixture is dialysed in Visking dialysis membranes against distilled water in the refrigerator to remove all uncoupled dye (usually 12–24 hr.). The dialysed dye-tagged preparation was placed in peripheral wells of a template on the agar-coated slide as described elsewhere[2]. Antigen was added to the central well. The antiserum had been obtained by cardiac puncture from guinea pigs immunized with homologous testicular homogenate, sperm, or purified aspermatogenic factor as described elsewhere[3–5]. Periodic examination of these slides held at refrigerator temperature disclosed that the reactions became visible within 4 hr.; reactions with other antigens (for example, ovalbumin) require 24 hr. for them to appear.

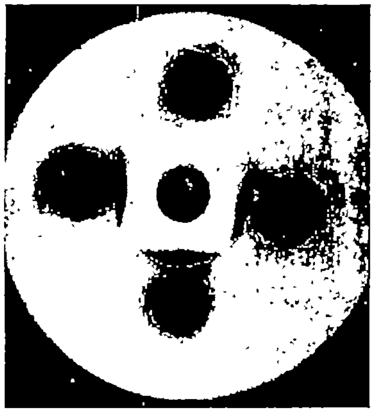

Fig. 1 Reactions between hæmatoxylin-dyed guinea pig aspermatogenic sera (3-, 6-, 9-o'clock wells) and aspermatogen (centre well); control guinea pig serum (12 o'clock well) fails to react

The results are illustrated in Fig. 1, which is a photograph of reactions between the hæmatoxylin-tagged antisera of 3 aspermatogenic guinea pigs (at the 3-, 6-, and 9-o'clock wells) and guinea pig aspermatogen in the centre well. The lack of reaction of a control guinea pig serum (12 o'clock well) is obvious. Comparable tests set up without pre-tagged antisera developed no precipitin bands. The findings illustrated in Fig. 1 were also obtained when the following materials were used: antibody-containing homogenates of spleen, lymph node, ileum and uterus of guinea pigs immunized with testicular antigens; rabbit antisera to heterologous sonicated spermatozoa; sera of infertile human males and females.

Interpretation of the mechanism of action of the tagged dye awaits further exploration. However, there is a suggestion that this technique may be useful for studying non-γ-globulin antibodies and very low molecular weight antigens (for example, insulin). Moreover, the dye-tagging approach provides a means for observing the precipitin lines as they form so that the dynamics of the reactions can be investigated.

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RADIOBIOLOGY

Absorption of Radiostrontium by the Gills of Freshwater Fish

In the course of investigations dealing with the fate of radio-nuclides in fresh waters, we wished to find the site of direct absorption of dissolved radioactive strontium by fish. Several experiments were carried out in which the amount of radiostrontium accumulated by goldfish (*Carassius auratus*), with only the head and gills immersed in distilled water containing strontium-90, was compared with that accumulated by goldfish totally immersed in the same solution.

Other workers[1–4] have studied the absorption of dissolved substances using a divided chamber which separates the head and gills from the rest of the body. Several difficulties are introduced by the use of this method—the excretory products contaminate the chamber, the sealing at the body-surface is difficult, and the tightly fitting diaphragm may damage the fish.

The apparatus used in the present experiments was an elliptical tube fitted with an open grid at the lower end. For a 10-gm. fish, tubes 4 in. long with a 1¼ in. major radius and a ¾ in. minor radius were used. Tubes were made from 'Lucite', polythene, or brass mesh. The last-mentioned were found to be most suitable because it was possible to shape them to fit fish of different sizes.

Each fish was placed in a tube with its head resting on the open grid. The tubes were then suspended vertically in the strontium-90 solution to the required immersion-level. The upper ends of those tubes containing the fish with only the head and gills immersed were plugged with damp cotton wool. The entire tank was covered with polyethylene sheeting to prevent the drying of the body surfaces which were not immersed. Continuous aeration of the strontium-90 solution was provided. Preliminary tests showed that fish were undamaged when placed in this assembly and held in a vertical position with either the head and gills or the whole body immersed for periods up to 72 hr.

Using this apparatus and various concentrations of strontium-90 in distilled water, we compared the accumulation of the isotope by fish that were partially immersed with that accumulated by those that were totally immersed. Both groups of fish were exposed at the same time in a single tank containing the strontium-90 solution. Within each experiment, therefore, the temperature conditions were identical for each group. The fish used each weighed approximately 10 gm. and were not fed during the experiments.

At the end of the experiment all fish were removed from the tubes and weighed immediately. Each whole fish was wet-ashed, using concentrated nitric

Table 1. ACCUMULATION OF RADIOSTRONTIUM BY GILL-IMMERSED FISH COMPARED WITH TOTALLY IMMERSED FISH

			Gill immersion		Total body immersion	
Exp. no.	Strontium-90 in water (units of 10^{3} c.p.m. per ml.)	Uptake period (hr.)	No. of fish	Mean c.p.m. (units of 10^{3}) per gram wet weight ± *S.E.*	No. of fish	Mean c.p.m. (units of 10^{3}) per gram wet weight ± *S.E.*
1	1·1	5	5	2·8 ± 0·5	4	4·1 ± 1·8
2	0·8	20	5	6·6 ± 0·9	5	7·4 ± 0·8
3	0·8	24	5	10·1 ± 1·0	6	11·5 ± 0·5
4	1·9	24	4	46·9 ± 9·2	4	56·3 ± 8·3

acid and hydrogen peroxide until oxidation was complete. The resulting solution was prepared for counting by evaporating an aliquot to dryness on a stainless steel planchette.

After 21 days the equilibrium mixture of strontium-90 plus yttrium-90 was measured using a counter equipped with an anthracene crystal for the detection of β-particles. The efficiency of the counter for a standard mixture of strontium-90 plus yttrium-90 varied from 17 per cent to 21 per cent. All counting-rates shown in Table 1 have been corrected to an efficiency of 20 per cent.

Table 1 presents the results obtained in four experiments in which the accumulation of strontium-90 by the fish was measured after immersion for different lengths of time in various concentrations of the isotope. If the mean counts per minute per gram of the gill-immersed fish (Col. 5) are compared with those of the totally immersed fish (Col. 7) it can be seen that the exposure of the additional body surface in the latter group results in only a small increase in the amount of strontium-90 accumulated.

Using the Mann–Whitney *U* test[5,6] in which the counts per min. per gram of the 38 individual fish samples from both groups are ranked in order of increasing amount of strontium-90, a value of $U = 146$ was obtained. With this value of *U* there is no significant difference (at the 0·05 level) between the concentrations of strontium-90 accumulated by gill-immersed fish and totally immersed fish under the same experimental conditions.

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Skin Decontamination in Presence of Thallium-204

THE problem of decontamination of the skin is now important with the increased use of isotopes in laboratories, and also the possible contamination from fall-out. Tests have been made with isotopes used for clinical purposes such as phosphorus-32 and iodine-131[1], fall-out[2] and fissible materials.

The ingredients used most are water and soap, detergents, complexing agents, chelating compounds and commercially available hand-cleaning products[3]. It has been shown[4] that the use of some decontaminating solutions may enhance the penetration of the contaminant into the skin, not taking into account the mechanical abrasion of the upper layer of the epidermis through rubbing with brushes or pads. This can be influenced by various factors, such as the length of time taken for decontamination, the amount of water, the nature of the decontaminating agents used, and possible irritation of the skin. The tests I have made were performed with a continuous-flow decontamination system, where rubbing, time, and possible spreading of the contaminant are reduced to a minimum.

The contaminant is a solution of thallium sulphate in water, with a specific activity of 5 mc./ml. of thallium-204. 10λ are dropped on the shaved abdominal skin of Wistar albino rats to the extent of 10 mm. diam. and allowed to dry for 30 min. There are four spots on each rat.

Three decontaminating solutions are used: soap, and sodium and calcium ethylenediamine tetraacetic acid solutions at 1 per cent; their action is compared with that of water. The device used consists of two coaxial glass tubes, of which the inner one is connected to the supply of a decontaminating solution, and the outer one to a venturi (Fig. 1).

The decontaminant is brought on to the skin through the inner tube and re-aspirated through the outer one. The amount of decontaminating solution flowing through can be adequately adjusted by pressure with a finger on the soft rubber exhaust duct, so that at any time one is able to control whether all the fluid is re-aspirated.

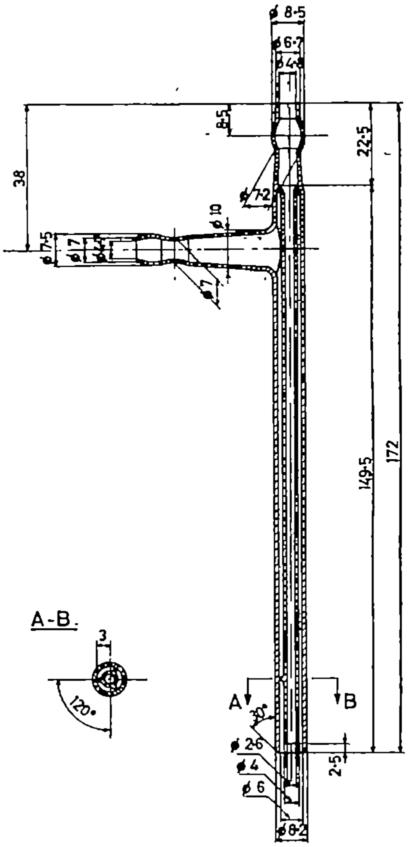

Fig. 1

Measurements have been made with a Geiger counter type Nō. 7 *E* 51 and a scalar model 'C.E.N. Automatic'. The amount of solution used is about 200 c.c. for 30 min. After counting, the procedure is immediately repeated once. Results are given in Table 1.

Table 1. PERCENTAGE OF INITIAL COUNTS AFTER TWO DECONTAMINATIONS

		I	II	III
A	1	7·00	14·93	9·36
	2	7 82	15·10	11.80
	3	12·20	17 10	8·30
	4	7·17	10·21	8·66
	Av..	10 80		
B	1	4·7	5 7	4·5
	2	4·70	6·70	4·90
	3	5·14	5·21	5·90
	4	6·16	5·56	3·80
	Av	5·24		
C	1	3·90	6 82	6·90
	2	4·60	5·20	2 00
	3	8·70	7·40	8·90
	4	9·30	5 20	5·10
	Av.:	6·16		
D	1	13·9	12 7	6·50
	2	8·6	8·20	4·50
	3	18·30	9 60	10·16
	4	8·50	9 06	7·33
	Av.:	9·77		

I, II, III, No. of animal, 1, 2, 3, 4, No. of spot. Decontaminating solutions: *A*, water; *B*, soap, *C*, sodium ethylenediamine tetraacetic acid; *D*, calcium ethylenediamine tetraacetic acid.

The results seem to indicate that in this case a solution of water and soap is the most effective agent for rapid skin decontamination. The total time could be reduced by suppressing the time lost for intermittent countings. I intend to accelerate this technique by monitoring the effluent through a liquid flow counter connected to a ratemeter which indicates almost immediately when contaminant is no longer removed: at this moment, the procedure becomes useless and even harmful for skin penetration. The same instrument can be used with a rubber or plastic cord at the end, for decontamination of hard surfaces.

I thank J. Deman, L. Malfait and J. Van Der Meeren for their help in this work.

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BIOLOGY

Responses of *Calanus finmarchicus* (Gunnerus) to Changes of Hydrostatic Pressure

MANY planktonic species show marked responses to changes in hydrostatic pressure[1-3], but there has been some doubt about the behaviour of *Calanus* in this respect. Field observations by Hardy and Paton[4] suggested that the behaviour of *Calanus finmarchicus* might be affected by the change of pressure with depth, but laboratory experiments by Hardy and Bainbridge[1] and by Knight-Jones and Qasim[3] failed to confirm this. Recent books and reviews[5-10] have stressed the apparent absence of a demonstrable pressure response in *Calanus*.

Recent experiments at Port Erin have now shown that definite responses to changes in pressure can be invoked in *Calanus finmarchicus helgolandicus* (Claus) (identification confirmed by Dr. D. I. Williamson). In neither of the previous laboratory investigations on the pressure responses of *Calanus finmarchicus* was the subspecies used specified, but from the known distribution of the two forms it seems probable that Hardy and Bainbridge's animals were mainly *finmarchicus*, while those used by Knight-Jones and Qasim were mainly *helgolandicus*.

The animals used in the present work were mainly adult females and stage V copepodites with a few adult males, and were collected either at about 6 fathoms or at about 35 fathoms off the west coast of the Isle of Man. The results so far obtained do not indicate any major differences between the pressure responses of animals of different sex, stage or origin. The copepods were placed in a 'Perspex' observation tank, 60 cm. long and with a 5 cm. square cross-section, which was completely filled with sea water; within the tank the pressure could be regulated by means of an adjustable mercury column. The experiments were conducted at temperatures between 12° and 16° C. In Figs. 1–3 pressures are given in millibars in excess of atmospheric pressure. The animals used were mainly negatively phototactic and remained close to the bottom at atmospheric pressure with overhead light, but some photopositive specimens were also used.

With the observation tank vertical and illuminated from above (about 1 k.lux at the top of the tank), an increase of pressure produced no migration in any of the photopositive specimens, but about half those which were initially photo-negative exhibited one of two main types of response:

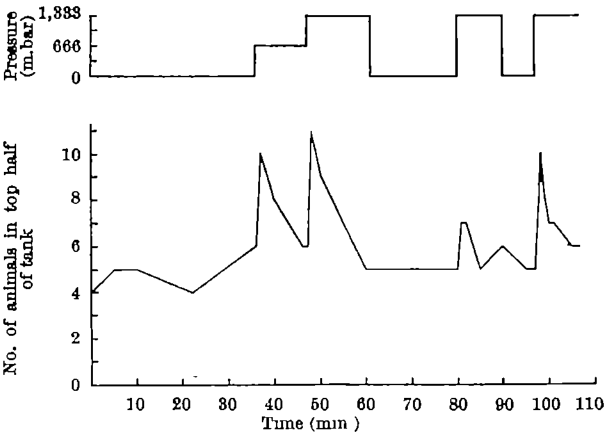

Fig. 1. Distribution during pressure changes of twenty *Calanus finmarchicus helgolandicus* illuminated from above

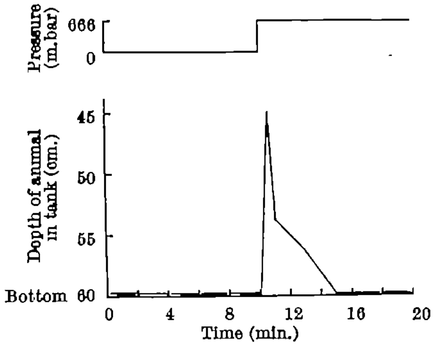

Fig. 2. Movement in response to pressure change of a single *Calanus finmarchicus helgolandicus* illuminated from above

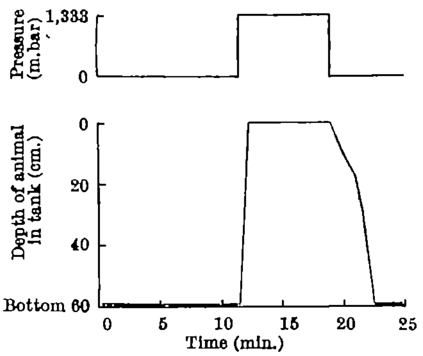

Fig. 3. Movement in response to pressure change of a single *Calanus finmarchicus helgolandicus* illuminated from above

(1) The most frequently observed response (Figs. 1 and 2) was an upward swimming movement towards the light, lasting as a rule for less than a minute. In this time the copepods moved upwards 15–30 cm., then the steady upward swimming ceased and the animals began to sink, with intermittent short periods of upward swimming. The original distribution was usually re-established within 4–5 min. of the pressure change.

(2) A more lasting response to pressure increase was observed in less than 5 per cent of the specimens tested (Fig. 3). In these cases the copepods reacted to an increase in pressure by swimming rapidly to the surface and remained there swimming actively for 15 min. or more if the pressure was not decreased. The upward migration was usually much more rapid than in the previous type of response, with the copepods covering the 60 cm. from the bottom to the top of the tank in 15 sec. or even less. Such specimens would move down again if the pressure was decreased, mainly by passive sinking but also sometimes by intermittent downward swimming.

With illumination from above, a decrease in pressure following an initial increase produced responses in about half the specimens which were originally photopositive. Most of those which reacted sank passively for a period, but swam back to the surface within 1 min. (a reaction comparable with response 1). A few individuals swam actively to the bottom and remained there until the pressure was increased again (see response 2).

In experiments in which the light was directed from below or horizontally, reactions were again recorded in only about 50 per cent of individuals. These usually responded to increased pressure by swimming towards the light, and to decreased pressure by either passive sinking or active swimming away from the light. Another type of response was observed in photo-negative animals in a few of the experiments in which the tank was illuminated from below; in these, increased pressure caused passive sinking towards the light and decreased pressure caused swimming upwards away from the light. The relation between the active and passive phases and the pressure changes in these cases is the reverse of that observed with overhead light.

The few results so far obtained show that the ability to perceive, and respond to, changes in hydrostatic pressure is definitely developed in *Calanus finmarchicus helgolandicus*, but the nature of the response is very variable under laboratory conditions.

Digby[11] suggested that the pressure sensitivity shown by many marine organisms might reside in the properties of a very thin film of electrolytically produced gas; he has since (personal communication) found that carefully handled *Calanus* show definite pressure responses, and suggests that rough handling of the material used might destroy such a mechanism. The animals used in the present work were collected in short hauls, usually with a metre tow-net, and were treated with great care in the laboratory, so that this might explain the differences between previously published results and those presented here.

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Effect of Thyroxine on the Permeability of the Isolated Toad Bladder

VIRTUALLY nothing is known of the role of thyroxine in the cellular metabolism of adult Amphibia. Though insulin[1] and vasopressin[2] have received much recent attention as hormones which effect permeability of cells, thyroidal hormones have not hitherto been shown to have this effect. The following is a preliminary report showing that both water transport and oxygen uptake of the isolated bladder of the toad *Bufo bufo* are influenced by thyroxine.

Bladders were removed from the toads and mounted in a manner similar to that described by Bentley[3] except that a modified saline was used. All experiments were performed at 25° C. Thyroxine when dissolved in the normal Ringer solution on the serosal side of the membrane increased the transfer of water from the mucosal to the serosal surface of the bladder. The loss of water prior to and after thyroxine treatment was measured over 45-min. periods, normal values of loss of water being of the order of 11·3 μl./cm.^{2}/hr. The concentration of thyroxine used varied from 10^{-8} M to 10^{-5} M (Fig. 1) and a log dose response curve was obtained; these concentrations may be considered as approaching a physiological level.

When the bladders were everted with the serosal surface inside and the experiment performed in the usual manner with normal Ringer solution outside and Ringer solution diluted 1 : 5 inside the bladder the increased effect of loss of water after thyroxine treatment was again observed. Concentrations of thyroxine used and water-losses measured were of the same order as those shown in Fig. 1 for non-everted bladders. This latter observation stands in contradistinction to all previous observations made on the toad bladder using vasopressin where it has been shown that the hormone exerts its effect on loss of water only from the serosal side. The fact that thyroxine can act when on either side of the mem-

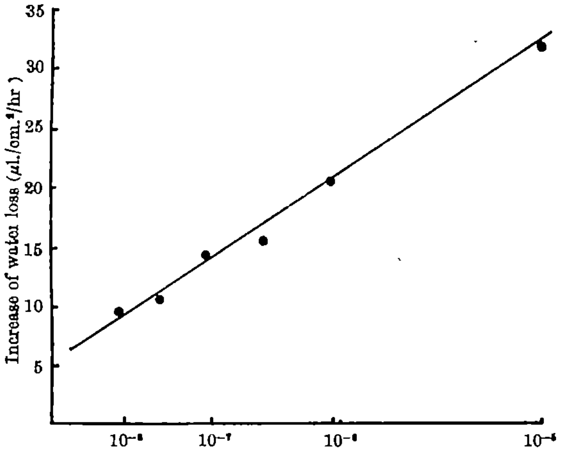

Fig. 1. Log dose response curve of thyroxine on loss of water from isolated toad bladder

brane is probably due to the small size of the molecule compared with that of vasopressin.

The effect of certain inhibitors of oxidative phosphorylation in combination with thyroxine was also examined. 2,4-Dinitrophenol (DNP) at a concentration of 10^{-4} *M* decreased the effect of added thyroxine to a value approaching that in Ringer solution alone. Sodium arsenate at the same concentration had a similar effect on loss of water as DNP. Sodium cyanide at a concentration of 10^{-5} *M* differed from the foregoing two inhibitors in that the effects observed were extremely variable; sometimes reversibility to normal values did not occur whereas in other cases the bladder behaved as though in a thyroxine solution alone. A further investigation of this effect is being pursued. Nitrogen when bubbled through the Ringer solution instead of air inhibited the thyroxine effect on loss of water as did lowering the temperature from 25° C. to 4·5° C. In all experiments using some kind of inhibitor one half of the bilobed bladder was used as a control and treated in a normal way. These inhibitory results obtained from DNP, arsenate, lowering the temperature, and bubbling with nitrogen were not obtained when similar experiments[4] were performed with vasopressin in order to investigate its mechanism of action. It therefore would appear that with thyroxine we are dealing with a different mechanism of water transport. This effect of thyroxine on increased water movement through the membrane and the arrest of this movement by substances which are known to inhibit metabolic activity within the cell suggests that thyroxine is increasing the rate of this metabolism.

In order to elucidate this view, respiration measurements were performed on isolated whole toad bladders. Oxygen consumptions were measured after 40 min. incubation in 10^{-4} *M* thyroxine or in normal Ringer solution alone while bubbled with air. The measurements were made polarographically at 5 min. intervals over 20 min., by a technique to be described elsewhere. The mean value obtained for six control bladders in Ringer solution was 1·21 ± 0·012 (*S.E.*) µl./mgm. dry wt./hr., and for six thyroxine-treated bladders, 2·47 ± 0·022 µl./mgm. dry wt./hr. This result is highly significant ($P < 0 \cdot 001$), the thyroxine more than doubling the normal rate of oxygen consumption of the bladder.

From these results it appears that thyroxine increases the metabolism of the cells and hence the water transport.

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Pigment of the Hermaphrodite Gland of *Arion ater rufus* L.

THE hermaphrodite gland of the slug *Arion ater rufus* is pigmented, and changes in the degree of pigmentation are correlated with the sexual stages in the gland[1]. In the early stages of development the hermaphrodite gland is translucent, but during spermatogenesis it becomes opaque and grey in colour, at the oocyte stage dark brown and ultimately, after oviposition, black. The final dense pigmentation is due partly to a gradual increase in the amount of pigment, partly to concentration of existing pigment by shrinkage of the gland.

The pigment was studied in hermaphrodite glands taken from animals a year or more old which had reached the end of their reproductive life. The glands were fixed in 'Formol'-calcium or aqueous Bouin and gelatine and paraffin sections were subjected to a number of the tests described by Lillie[2], Lison[3] and Pearse[4]. The solubility, bleaching properties and staining properties of the pigment were investigated. Special colour tests were also performed for carotenoids and glycerol-mounted sections were tested for porphyrins in ultra-violet light.

In sections treated for general histological examination, dark zones of pigment are present in the connective tissues of the tunica and around and between the acini of the hermaphrodite gland. The pigment resists the usual procedure of embedding in paraffin and is visible in unstained sections as an accumulation of granules of different sizes which vary in colour from yellow to dark brown or black. It is unaffected by 48 hr. treatment with 5 per cent sulphuric acid, 5 per cent concentrated acetic acid, 5 per cent potassium hydroxide, acetone or chloroform, though it is partially soluble in hot pyridine and bleaches slowly in hydrogen peroxide (24 hr.) or potassium permanganate (6 hr.). The appearance and reactions of the pigment generally recall those of the melanins and lipofuscins so typical of animal tissues. Certain of these reactions are, however, shared with carotenoids, which have been found in the gonads of both sexes of *Patella vulgata*[5], and porphyrins, which occur in the integument of *Arion ater ater* (ref. 6). The pigment, however, belongs to neither of these groups. It fails to give the specific colour reactions of carotenoids with concentrated sulphuric acid, concentrated hydrochloric acid, iodine or the Carr–Price test with antimony trichloride in chloroform, and in its insolubility differs from the extremely fat-soluble carotenoids or water-soluble chromoproteins. It is not extracted by methanol and sulphuric acid[6] and does not fluoresce in ultra-violet light, both specific properties of porphyrins.

Differentiation between melanins and lipofuscins is not easy since both react positively to a number of tests regarded as specific for one or the other, for example, the pigment reacts positively to Schmorl's test for lipofuscins, but melanins also reduce ferricyanide to ferrocyanide. Strong alkali destroys the tissue but does not dissolve the pigment, which indicates the presence of lipofuscin[5]. More evidence for the lipoidal nature of the pigment is given by its partial solubility in the pyridine and its partial staining with sudan IV and chromalum-hæmatoxylin. Although the pigment is unstained by sudan black *B*, the larger granules are positive to periodic acid–Schiff and all granules stain with aldehyde fuchsin.

Three final tests clearly indicate that the pigment is lipofuscin and not melanin. (1) Using Masson-Fontana's method[4] the pigment does not reduce ammoniacal silver nitrate and remains unstained. It is therefore not a melanin[2,3]. (2) After bleaching in hydrogen peroxide for 24 hr. the pigment stains blue with Nile blue sulphate[7]—melanin would be colourless. (3) The pigment stains deep blue with 0·05–0·1 per cent Nile blue in sulphuric acid and decolorizes in acetone rinse[8].

The slow accumulation of the lipofuscin *pari passu* with age suggests that the pigment is a waste product. It is presumed to be derived by the progressive oxidation and polymerization of lipids, and the non-uniform reaction to some tests can be attributed to the material being at different stages of oxidation. At the more advanced stages of oxidation the pigment is no longer lipid in the accepted sense and responds negatively to the tests for lipids.

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Order of Death as a Test of Therapeutic Effect

In assessing the effect of a treatment on the action of a poison it is usual to measure either the effect on the LD_{50} or on the time to death. In a recent investigation by one of us (D. F. H.) on the effect of treatment with dimethylformamide on the poisoning of male and female rats with dimethylnitrosamine it was found that changes in LD_{50} provided a rather insensitive test of effect, which required large numbers of rats to establish, and that mean times to death in treated and untreated groups were not greatly different after doses which killed all the rats. Dimethylformamide had, however, a very marked effect on the order in which rats died at doses such that a few survived. Times to death cannot be calculated in these circumstances without making arbitrary decisions about "times to death" of the survivors. Orders of death, however, proved susceptible to a more rigid statistical treatment. As this may be of general use it is reported here.

Let there be two groups of n rats, one group receiving the treatment and the other not. Let the order of death be denoted by the rankings: $1, 2, \ldots 2n$. Consider one group. The order in which these die can be denoted by n numbers from the $2n$ possible rankings. On the null hypothesis that the treatment has no effect the average ranking, $\bar{x}$, should be the average of the numbers 1 to $2n$, that is, $n + \frac{1}{2}$. It is required to find the significance of differences between $\bar{x}$ and $n + \frac{1}{2}$.

For $n = 4$, calculation showed that $\bar{x}$ was distributed very nearly normally, and that the normal approximation slightly underestimated the significance of differences. The normal approximation will therefore be adequate for $n \geqslant 4$.

By standard statistical methods, it can be shown that the variance of $\bar{x}$ is given by:

$$V(\bar{x}) = (2n + 1)/12$$

whence, for a normal distribution, and n small, the deviate is given by:

$$d = [\,|\,\bar{x} - (n + \tfrac{1}{2})\,|\, - 1/2n]/\sqrt{(2n + 1)/12}$$

The probability that the deviate will be as great or greater than the experimental value can be found from statistical tables.

In calculating $\bar{x}$ all the animals surviving are given the same ranking, equal to their average rank. This corresponds to the fact that they are experimentally indistinguishable. It follows that the test is most sensitive when the dose is high enough to kill most of one group.

As $\bar{x}$ is distributed normally about $n + \frac{1}{2}$, one must always indicate which group tended to die first, as otherwise confirmatory experiments may give the same probabilities as contradictory ones.

Table 1. EFFECTS OF DIMETHYLFORMAMIDE ON THE TOXICITY OF DIMETHYLNITROSAMINE

		By order of death			By LD_{50}'s	
Sex	$2n$	Order of death	$\bar{x}$	P	No. of rats	P
M	8	*AAAABB*	$(1+2+3+4)/4$	0·015	72	0·006
F	20	7*A*'s,*BABABAB*	$(1+2+\ldots+7+9+11+13)/10$	0·0005	140	0·0002

Rats were given 700 mgm./kgm. of dimethylformamide intraperitoneally 30 min. before dimethylnitrosamine, given intraperitoneally to females and intravenously to males. Compounds were in normal saline. Order of death is indicated, *A* representing a rat which received dimethylnitrosamine only, and *B* a rat also receiving dimethylformamide treatment. The probabilities of the results on the null hypothesis are compared with probabilities estimated from LD_{50}'s (calculated by probit analysis) determined using the number of rats shown

The results of two experiments are shown in Table 1. In both experiments small numbers of rats demonstrated with almost as much certainty as LD_{50}'s determined on much larger numbers that dimethylformamide protected rats from the lethal effects of dimethylnitrosamine.

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Production of Insect Juvenile Hormone by the Microsporidian Parasite *Nosema*

WHEN microsporidian parasites of the genus *Nosema* infect insects, abnormal growth and metamorphosis are commonly observed. Heavily infected pupæ of saturniid Lepidoptera moult to adults which retain many pupal characters[1]. When flour beetles of the genus *Tribolium* are similarly infected, they appear larger than normal and die at or before pupation[2]. We have found that the larger size of such infected beetle larvæ is a result of more rapid growth and of supernumerary moults. All these effects resemble those which can be produced by injection of insect juvenile hormone or by implantation of corpora allata, the endocrine gland which produces this hormone[3-6]. Three hypotheses could explain this similarity of parasitic infection and endocrine activity: (1) The parasite might cause hypertrophy of, or hypersecretion by, the corpora allata of the host. (2) The parasite might cause other tissues of the host to produce juvenile hormone. (3) The parasites, themselves, might produce materials with juvenile hormone activity. We have carried out experiments by which we can decide among these alternatives.

Our source of parasites was a group of infected cultures of *Tribolium castaneum*, for which we are indebted to Prof. A. E. Bell, of the Population Genetics Institute, Purdue University. Parasites from this species were successfully transmitted to several insect orders, including the following species: Coleoptera, *Tribolium confusum*, and *Tenebrio molitor;* Blattaria, *Periplaneta americana*, *Blaberus craniifera*, and *Byrsotria fumigata;* Lepidoptera: *Galleria mellonella*, *Bombyx mori*, and *Hyalophora* (*Platysamia*) *cecropia*. In all these species the parasite induces more rapid growth of the nymph or larva and inhibits metamorphosis to the adult stage.

When *Bombyx mori* or any of the Blattaria were allatectomized as nymphs or larvæ the intermoult period was extended by 30–50 per cent, and they moulted to premature adults. Following sham operations the intermoult period was also extended, but only by 15–40 per cent. When *Nosema* was implanted into allatectomized hosts the intermoult period increased 15–40 per cent, but the animals moulted to another nymphal or larval stage. These experiments rule out the first hypothesis since it is apparent that the effects of the parasite are expressed even in the absence of corpora allata and, in fact, the parasite substitutes for the endocrine gland.

The second hypothesis was ruled out by placing parasites in chambers attached to allatectomized hosts. In some experiments the chambers were in direct contact with the hæmocœl of the host; in others, they were separated by a 'Millipore' filter with a pore size of 0·47μ. This pore size is too small to permit the passage of the microsporidia. In no case did the tissues of the host show signs of infection or hypertrophy. Infective parasites were recovered from the chambers. In seven of ten such experiments the response of the host was consistent with production of juvenile hormone by the parasites. In sixty experiments in which the chambers contained tissues of insects free of parasites, a juvenilizing effect was never noted. These experiments show that juvenile hormone is not produced by non-endocrine host tissues.

Finally, 3 gm. of *Nosema* spores were isolated from 48 gm. of dried, infected *Tribolium*, and, by standard techniques[8] used for the purification of juvenile hormone, 11 mgm. of a yellow oil was recovered. This hormone preparation showed activity when assayed on *Hyalophora cecropia*[9]. An extract prepared in the identical fashion from uninfected *Tribolium* displayed no activity.

We believe that these experiments provide strong evidence that this parasite displays a new mode of parasite pathology; that is, production, in excessive amounts, of a substance normal to the host by a parasite. This substance then acts to upset the normal mechanisms of control of growth and differentiation. We know of no previous description of such a pathogenic action by a parasite.

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Changes in Transpiration induced by Ethyl Alcohol

ETHYL alcohol was used as an auxiliary solvent in the preparation of auxin solutions for a series of transpiration experiments with plants in water culture when the roots were supplied with different auxins at varying concentrations. This method appears to be fairly common for auxin studies[1,2]. After weighing, the auxin is dissolved in a small quantity of ethyl alcohol, and the resulting solution is diluted with water or a nutrient solution to obtain the concentration desired. The small amounts of alcohol used are usually considered to be harmless; nevertheless, it has been shown that ethyl alcohol may have modifying effects in auxin experiments even at low concentrations[3]. In an attempt to determine whether this is also the case in transpiration experiments, some experiments were carried out with nutrient solution to which had been added varying amounts of ethyl alcohol.

The rate of transpiration was measured by means of the corona hygrometer[4], which allows continuous recordings of the transpiration through the determination of the water vapour pressure (pH_2O) in the air which has passed the plant chamber. The following transpiration conditions were used: illumination of about 10,000 lux from a photo lamp of the *B*-type at a distance of 45 cm., the light having been filtered through 10 cm. of cooled water; atmospheric air free of carbon dioxide of a relative humidity of 50 per cent and a temperature of 20° C. being passed along the leaves at a rate of about 1·3 m./sec. When the plants (6–7 days old barley seedlings grown in water culture) had established constant transpiration under these conditions, the nutrient solution surrounding the roots was replaced by a mixture of ethyl alcohol and

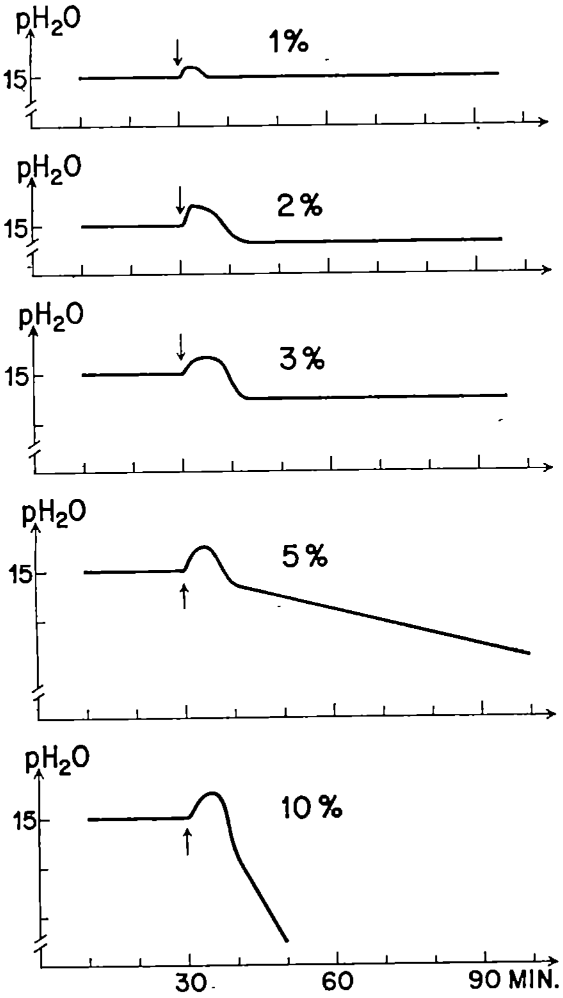

Fig. 1. Transpiration changes in barley seedlings induced by different concentrations of ethyl alcohol in the nutrient solution surrounding the roots. At the times indicated by arrows the plain nutrient solution was replaced by nutrient solution containing the amounts of ethyl alcohol shown

nutrient solution. Past experience has shown that a mere replacement of the nutrient solution does not affect transpiration. Fig. 1 shows that even 1 per cent ethyl alcohol induces a transient, but, nevertheless, distinct and reproducible, change in transpiration. An addition of 2–3 per cent alcohol causes an additional more lasting effect, the rate of transpiration becoming established at a level lower than that maintained prior to replacement of the nutrient solution. An addition of 5 per cent alcohol causes transpiration to decrease slowly following the above brief increase. A concentration of 10 per cent alcohol in the nutrient solution induces a similar reaction which, incidentally, corresponds to the reaction caused by cutting plant stems in air[5], or by treatment of roots with strong auxin solutions.

Due to the fact that the effect of ethyl alcohol reported here sets in so rapidly, in fact almost instantaneously, it appears to be reasonable to assume that it is localized in the roots, and it probably consists in changes of the water permeability of the root cells in one form or another. This must provide the possibility of a brief increase in the water supply to the leaves, allowing an increase in transpiration. At the higher concentrations of 5 and 10 per cent of alcohol, a lasting (irreversible) change in the properties of the root cells appears to occur after a certain period of time, causing a more or less pronounced decrease in transpiration to replace the momentary increase.

It appears that even at the low concentrations of 1–2 per cent occurring in the case of auxin solutions, ethyl alcohol is not an inactive component. Even in cases where growth experiments are not involved, the use of alcohol in the preparation of auxin solutions is inadvisable. Instead, the auxin should be dissolved in basic solution which is then adjusted to the desired *p*H value by means of the appropriate amount of acid, as recommended by Larsen[6].

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Translocation of Captan by Broad Bean Plants

CAPTAN (*N*-trichloromethylthio*cyclo*hex-4-ene-1 : 2-dicarboxyimide) is widely used as a protectant fungicide and since its introduction in 1952 claims have been made for a systemic action. Applications of captan to the roots of cucumber[1], broad bean[2], rose[3] and pea[4] plants have been reported to give increased fungal control on the foliage although the results with broad bean have not been confirmed by later workers[5]. Acetone extracts of the leaves and stems of plants the roots of which had been treated with captan have been shown to be more toxic to *Ceratocystis ulmi* than extracts from untreated plants[6,7].

Systemic activity may be demonstrated unequivocally by the chemical identification of the fungicide in parts of the plant not in direct contact with the fungicide. Wallen and Hoffman[4] found, by chemical analysis, up to 0·85 p.p.m. (fresh weight) of captan in pea seedlings the roots of which had been watered with captan solutions. More sensitive analysis is provided by the use of a radio-tracer and we have used this technique to follow the translocation of captan, labelled with sulphur-35, in the broad bean.

Broad bean plants (var. Early Giant Windsor) grown in John Innes compost, in 4-in. pots, were treated with captan solutions when four weeks old. 50 ml. of an aqueous chloroform (at 0·15 per cent) solution containing 375 μgm. captan was applied daily to each pot and after nine days the plants were harvested above the second pair of leaflets. Each pot received a total of 3,375 μgm. of captan. The main stem was discarded and the leaflets, petioles, apical bud and flower buds weighed and rapidly chilled to − 18° C. After 2–3 hr. at − 18° C. the leaves were ground with solidified benzene in a chilled mortar. The warmed mixture was filtered through Whatman No. 54 paper, the filtrate shaken for 20 min. with 4 gm. anhydrous sodium sulphate and 0·5 gm. charcoal and then filtered through Whatman No. 42 paper. This extraction procedure gave 99 per cent recovery of added ^{35}S-captan.

The benzene extracts were evaporated to dryness in a current of clean, dry air and the residue dissolved in 1 ml. benzene. The radioactivity of the sample was determined by spotting a number of 3 μl. aliquots of the extract, to a total volume of 0·5–0·7 ml., on to the same position on a filter paper strip and counting under an end-window Geiger–Müller tube. Each 3 μl. drop spread to a maximum diameter of 16 mm. and was completely dried off before the next drop was added.

The maximum radioactivity found in any plant was equivalent to 0·45 ± 0·06 μgm. captan or 0·09 p.p.m. (fresh weight). Many plants gave lower counting values and some gave counts not significantly different ($P = 0·05$) from that of the background. These values assume that the benzene-extractable sulphur-35 is wholly in the form of captan; any volatile compounds labelled with sulphur-35 produced by the decomposition of captan would be removed in the evaporation process. The presence of such minute amounts of captan in the bean leaves after the application of relatively heavy doses to the roots suggests that any chemotherapeutic effect of captan in these circumstances cannot be attributed to its systemic activity. Even the maximum concentration of captan found in bean leaves is only a small fraction of that required to inhibit the *in vitro* spore germination of plant pathogens; for example, the median effective dose (ED_{50}) of captan against conidia of *Botrytis fabae* Sardiña is approximately 0·7 p.p.m. In view of the known chemical reactivity of captan[8,9] and its capacity to inhibit metabolic processes in plants[10], it may be suggested that interference with host metabolism is responsible for the fungal control observed in foliage after root application.

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Inverse Ammonia–pH Relationship in Grasses

Two perennial grasses, *Brachiaria ruziziensis* and *Setaria sphacelata*, were sown at the same time in adjacent field plots. The first samples were taken after six weeks and later at weekly intervals during the next 16 weeks. The whole plant was taken from approximately 1 in. above the soil at different growth stages. The samples were dried at 50° C. under forced draught, milled and analysed.

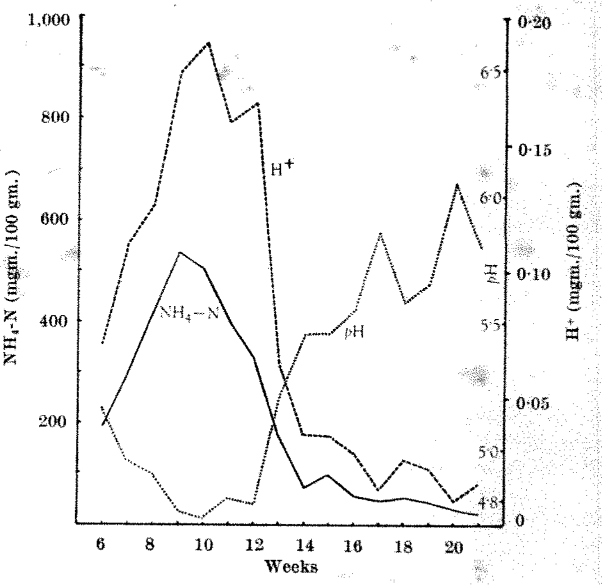


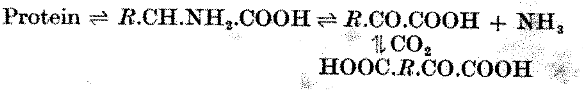
Fig. 1. Ammonia, *p*H and H ion fluctuations during growth of *Setaria*

Throughout the period (6–21 weeks) the *Brachiaria* samples showed little variation in water-extractable ammonia (approximately 30 mgm. NH_4–nitrogen per 100 gm. air-dry material) or *p*H, which fluctuated between 6·2 and 6·4. With *Setaria* a completely different pattern of behaviour occurred, as shown in Fig. 1, in which *p*H values have also been converted to mgm. H^+ ions extracted per 100 gm. material. The regression (equation 1) of H^+ on NH_4–nitrogen (each as mgm./100 gm. plant material) was positive, linear and highly significant (r^2 is equal to 0·948):

$$H^+ = 0·000355\ NH_4\text{–nitrogen} + 0·0053 \qquad (1)$$

The regression line passes almost through the points of origin, showing that there was an almost constant proportionality between H^+ and ammonia during their rise and decline. Further, it was found that the amount of ammonia extracted was significantly and directly related to titratable acidity (r^2 is equal to 0·88) such that each equivalent of ammonia was associated with approximately one equivalent of titratable acidity.

From these results and the dissociation constants of the acids, it is considered that in *Setaria* some protein breakdown occurred between the sixth and tenth weeks, as follows:


$$\begin{array}{r}\text{Protein} \rightleftharpoons R.\text{CH.NH}_2.\text{COOH} \rightleftharpoons R.\text{CO.COOH} + \text{NH}_3\\ \upharpoonleft\!\downharpoonright \text{CO}_2\\ \text{HOOC.}R.\text{CO.COOH}\end{array}$$

leading finally to the formation of the acid ammonium salt of the dicarboxylic acid. This satisfactorily explains the equivalence found between ammonia and titratable acidity and the inverse ammonia–*p*H relationship. During protein regeneration (tenth–fourteenth weeks) the proportionality between ammonia and acid was maintained, indicating that the reverse reactions then occurred.

There is evidence that with *Brachiaria* some protein breakdown occurred so far as the amino-acid stage but no further due to lack, or inactivation, of the required enzymes. It is considered unlikely that the difference in behaviour between *Setaria* and *Brachiaria* was due to greater uptake of ammonia by *Setaria* since both grasses were very similar in chemical constitution, including ash contents, indicating similar abilities to take up cations and ammonia. Further, assuming greater ammonia uptake by *Setaria*, it is improbable that this would be paralleled by equivalent acid production within the plant.

It is not known to what extent the behaviour noted with *Setaria* is of rare or common occurrence in plants, as it has not been a general practice to determine ammonia and *p*H simultaneously in aqueous plant extracts. Since, however, the analytical techniques are so simple and the relationship so well defined it should not be difficult to study the frequency of occurrence and the causes involved. If of frequent occurrence, ammonia so derived would complicate the interpretation of trials involving ammonia uptake by plants following various applications of ammonium salts or urea. The occurrence or otherwise of the relationship would also give some insight into the enzyme systems operative during plant growth. Further applications of this relationship, for example, in relation to ammonia detoxication in plants, also suggest themselves, and it is concluded that it would be a good standard practice to include *p*H determinations whenever ammonia is determined in aqueous plant extracts.

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Secretion from Pores of Surface Wax on Plant Leaves

THE source of surface wax deposits on plants and their method of formation have been investigated since 1871 by optical microscopy[1] and more recently by electron microscopy. Mueller, Carr and Loomis[2] considered that the wax was probably secreted from pores in the leaf surface, but no evidence of them was seen. A study of leaf surfaces by Juniper and Bradley[3] using carbon films evaporated on the leaf surface also failed to reveal their presence.

Recent work on clover (*Trifolium repens*) at higher magnifications (80,000–200,000 diam.) carried out by us has established that pores are present beneath each particle of wax. Since they have not been seen elsewhere on the leaf it seems reasonable to assume that they are the source from which wax is secreted. Moreover, if leaves are placed in a wax solvent for progressively longer periods, the last wax to disappear is that immediately above the pores.

The only other plant leaves investigated were those of *Brassica oleracea* and *Poa colensoi* and pores were observed in each case. Our present study of the waxy surface of apples has also shown that similar pores are present in the cuticle of fruit.

The pores were recorded only when suitable replica preparations were used, and the following was soon found to be the most satisfactory method. The leaf was immersed in a wax solvent, such as chloroform, for sufficient time to remove most of the wax. Clover leaves required over-night immersion whereas the wax on *Brassica* was removed to the same degree within 10 min. When the wax was not dissolved completely the thin remaining deposits helped in locating the pores. A shadowing layer of uranium oxide was applied directly to the leaf surface, stripped off with collodion and then coated with a carbon film. The plastic was dissolved in amyl acetate leaving a pre-shadowed carbon replica.

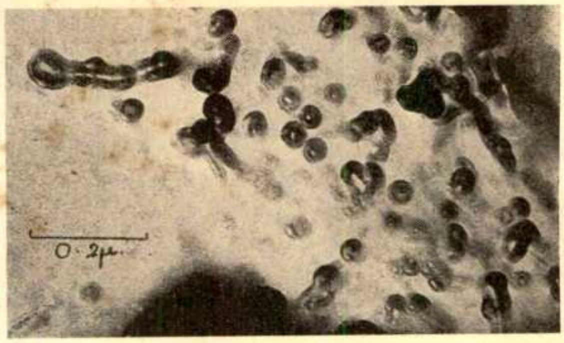

Fig. 1. Surface of a *Brassica* leaf showing pores

It will be seen from Fig. 1 that each wax-free pore appears as a faint circular area in the centre of which is a small depression that could be an opening in the leaf surface. Most of the pores in this micrograph are partially or wholly concealed by incompletely dissolved wax deposits each having a central hole immediately above the depression in the pore. The central hole has a mean diameter of 6 mμ for *Trifolium repens* and 7 mμ for *Brassica oleracea*, and the outer diameters of these wax residues on the two plant leaves are 38 mμ and 45 mμ, respectively.

The form taken by the wax on the surface of the leaf appears to be determined by the number of pores associated with each waxy structure. On white clover, for example, the wax secreted from a group of up to nine pores combines to form a discrete wax particle. The number of pores in each group on the surface of the *Brassica* leaf, however, varies from a few to several hundred under the larger pieces of wax. The small rod-like structures of wax formed on tussock leaves suggest that only one pore is associated with each extrusion, but we have insufficient evidence to be sure. Where a number of pores are inter-connected at the surface (as in Fig. 1), there is evidence that the wax is extruded along the length of this connexion.

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MICROBIOLOGY

A Change in Phenotype associated with the Bacteriophage Carrier State in a Strain of *Proteus mirabilis*

Proteus phage *57* forms clear plaques on *Proteus mirabilis* strains *12*, *55*, *57* and *63*[1]. On strains *12* and *57* the central clear hole is surrounded by a spreading halo of altered bacterial growth due to the liberation of a lysin by the phage-infected organisms[2]. This lysin lyses versene-treated suspensions of these strains[3]. No free phage is present in the haloes, and thick saline suspensions of the organisms present in these zones do not adsorb phage *57* under conditions where controls with organisms scraped from unaffected areas of the plate show 99·9 per cent adsorption. This change in surface structure is the only effect of the lysin on untreated cells demonstrated to date. While the organisms in haloes have an obvious alteration in their optical properties they appear normal microscopically and do not show increased osmotic fragility. They are viable and, after a period of growth in the absence of lysin, the progeny are again susceptible to phage *57*.

Phage *57* was isolated[1] by the use of an enrichment technique (with *Proteus 57*) from sewage. Further work on it and *Proteus 57* has shown that phage *57* is liberated spontaneously by *Proteus 57*. It is a virulent variant of a temperate phage for which *Proteus 57* is lysogenic and is able to overcome the immunity conferred by the prophage[4]. Strains *12*, *55*, *57* and *63* are indol-negative, do not ferment maltose or salicin and ferment sucrose late. The late fermentation of sucrose is due to mutants which arise at low rates and rapidly ferment sucrose[5].

When a culture of *Proteus 57* is spread on Bacto nutrient agar containing 1 per cent Seitz-filtered sucrose and Bacto neutral red indicator at *p*H 7·1, the haloes of plaques formed either by the spontaneous liberation of phage *57* or by spotting with this phage are red. The colour which indicates acid production from sucrose never extends beyond the halo and is observed with phage *57* lysates prepared on any of its host strains. No change in colour occurs when the sucrose is replaced by lactose, maltose or salicin nor in plaques of phage *57* on the other 3 strains on media containing any of the four sugars mentioned. If the red growth of *Proteus 57* bordering on the central hole, and therefore contaminated with phage *57*, is streaked on the sucrose medium (on which *Proteus* does not swarm) two types of colony develop overnight. The majority are bright red and have a nibbled edge.

The remainder are pale and entire. On sub-culture the former always yield both varieties of colony while the latter only produce pale entire colonies.

The behaviour of the red colonies is unlike that of sucrose-positive mutants of *Proteus 57* (or other *mirabilis* strains) which are stable *in vitro*[5]. The red colonies are invariably contaminated with phage *57* while the pale ones are free of it. When red nibbled colonies are suspended in phage *57* antiserum and then plated on the sucrose medium all the colonies which arise are pale and entire. These phage-free colonies are sensitive to phage *57*. The red colonies where phage and bacteria exist in a more or less stable equilibrium are called carrier colonies[6]. The organisms in these colonies may owe their immunity to the lysin liberated by the small number of bacteria to which the phage succeeds in adsorbing.

Sucrose fermentation by carrier colonies was thought to be due to phage conversion but in view of the fact that *Proteus 57* is lysogenic for a temperate variant of phage *57* it is unlikely that the virulent phage will convert when its temperate counterpart does not. Conversion was excluded by further observations. The first is that young broth cultures of *Proteus 57*, sonically lysed and sterilized by Seitz filtration, cleave sucrose into glucose and fructose. This was demonstrated chromatographically. Unlysed and unfiltered aliquots are unable to do this. The enzyme system has not yet been identified but is active in a phosphate buffer at *p*H 7·4 and inactive at *p*H 5 with a citrate buffer. Cultures of *Proteus 12*, *55* and *63* treated similarly do not cleave sucrose. The other observation concerns the anomalous behaviour of *Proteus 57* on the sucrose medium and this medium plus 1 per cent Bacto bile salts.

Colonies of *Proteus 57* are pale on the sucrose medium and when replicated to both the latter and the medium with bile, all the new colonies are again pale on the sucrose medium but red and larger on the bile medium. Colonies of the other three strains are pale on both media. Surface-active agents do affect the cell wall of *Proteus*[7] and saline suspensions containing 10^9 organisms/ml. of the four strains undergo rapid and marked clearing in the presence of 1 per cent bile salts. It is suggested that strain *57* differs from strains *12*, *55* and *63* in being genetically sucrose-positive. Whether this is due to lysogenic conversion by the temperate variant of phage *57* cannot be stated at this stage. However, phenotypically it is sucrose-negative. Decryptification is brought about by a change in surface structure rendering the organism permeable to sucrose. This can be done with bile salts or by the surface-modifying effect which the lysin, liberated by phage-lysed organisms, has on neighbouring bacteria.

Recently it has been demonstrated that another virulent phage for *Proteus 57*, phage *69*[1], can enter into a carrier state with *Proteus 57*. The carrier colonies also ferment sucrose. Phage *69* is serologically unrelated to phage 57, and lysing complexes liberate a lysin which also changes the surface of strain *57*.

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Sialic Acid in *Pasteurella pestis*

Sialic acid is widely distributed in the animal kingdom as a constituent of mucoproteins, mucolipids and mucopolysaccharides[1-3]. A search among the bacteria[4-13] has disclosed its occurrence in many Gram-negative bacteria[7,10,11]. A polymer of *N*-acetylneuraminic acid called colominic acid[4], a neuraminic acid peptide resembling the cell wall constituents and the corresponding uridine nucleotide which is the biosynthetic precursor[5], and *N*-acetylneuraminic acid and *N* : 7-*O*-diacetylneuraminic acid[6,12] in the hydrolysate of the cell mass with 0·05 *N* sulphuric acid have been reported in *Escherichia coli*. From group *C Neisseria meningococcus*, a group-specific haptenic material has been isolated which contains 3 units of hexosamine and 7 of sialic acid[8,9]. *Citrofactor freundii* has yielded a serologically active polysaccharide which contains 36 per cent sialic acid, 21 per cent hexosamine, and the rest unidentified (no uronic acid and only traces of protein)[11]. Sialic acid appears to be a constituent of the endotoxin[6,12,13], capsular antigen or some surface layers of the cell wall[5,12,13] of some bacteria.

After the detection of sialic acid in the cell mass of *Pasteurella pestis* in preliminary fractionation experiments, we tried to find out whether this acid is connected with the capsular material, protective antigen, and virulence of *P. pestis*. For this, six strains of *P. pestis* were selected with different characteristics given in Table 1. The bacteria were grown in casein hydrolysate medium, routinely used in this Institute for the preparation of the vaccines[14], and at 37° C. and 27° C., since it is known that the production of the protective antigen is maximum at 37° C. and very little at 27° C.[15-17]. After the growth period, the micro-organisms were killed with 0·1 per cent formalin (which did not interfere with the estimation of sialic acid by the thiobarbituric acid method, but did in reaction with orcinol), the cell mass collected by centrifugation, washed with saline, and acetone powders prepared by treating with cold acetone. For each experiment, 50–100 mgm. of the dried material was used. Sialic acid in the extracts was estimated by the thiobarbituric acid method of Warren[18,19] taking the optical densities at 532 and 550 mμ. In case of strong interference with deoxyribose, a preliminary extraction with isoamyl alcohol was carried out which removed most of the chromogen due to deoxyribose[18,19]. The absorption spectrum of the chromogen extracted with *cyclo*hexanone was also taken to check that it tallies with that of sialic acid[18].

Treating the bacterial mass with boiling water[7] extracted less than 25 per cent of the sialic acid; the aqueous extract after hydrolysis with sulphuric acid showed strong interference due to deoxyribose. Heating the bacterial mass with 0·1 *N* sulphuric acid at 80° C. (ref. 6) released about 50 per cent of the sialic acid obtained by the other method given below. Fractionation of the cell mass of two strains (120/5*H* and 139/*L*) successively with 2·5 per cent saline, cold trichloroacetic acid (TCA), 75 per cent alcohol and hot TCA, showed that sialic acid was present in the hot TCA extract and the hydrolysate of the residue with 0·1 *N* sulphuric acid. The routine method adopted finally was to extract the cell mass with cold TCA for 10 min. followed by hot 5 per cent TCA at 90° C. for 30 min. Sialic acid was present in the latter extract and the quantity of deoxyribose varied. Hydrolysis of the residual mass with 0·1 *N* sulphuric acid also furnished some sialic acid. The results obtained are given in Table 1.

No sialic acid was detected in Fraction I (ref. 20), which is supposed to contain the protective antigen and the mucoid substance surrounding the micro-organism[21].

Table 1. SIALIC ACID CONTENT OF THE CELL MASS OF VARIOUS STRAINS OF *P. pestis*

No.	Strain	Virulence	Protection to mice on immunization	Presence of capsule	Temp. of growth (°C.)	Sialic acid content (μgm./100 mgm.) Hot TCA extract	Sialic acid content (μgm./100 mgm.) Hydrolysate of residue
1	139/*L*	Virulent	+	+	27	253	62
2	139/*L*	Virulent	+	+	37	575	226
3	139/*L*	Virulent	+	+	37	127	20
4	195/*P*	Virulent	+	+	27	350	90
5	195/*P*	Virulent	+	+	37	205	14
6	53/*H*	Avirulent	+	+	27	230	108
7	53/*H*	Avirulent	+	+	37	337	40
8	*EV*	Avirulent	+	+	27	632	140
9	*TRU*	Avirulent	0	–	27	384	223
10	120/5*H*	Avirulent	0	–	27	421	87

All strains were grown in Haffkine flasks with 1 l. of medium in each. No. 3 was grown in a shaker for 2 days while the rest were grown in stationary cultures for 6 to 9 days to get significant growth.

The culture filtrates from Exps. 1, 4, 8 and 10 contained respectively 3·1, 3·5, 4·6 and 2·3 μgm. of sialic acid per ml.

No quantitative significance is attached to the figures for comparison since they are likely to change with differences in growth conditions and periods of collecting.

The results show that sialic acid is present in all the different strains of *P. pestis* grown under different conditions and that there is no correlation between its occurrence and the virulence of the organism, presence of capsule, or protective antigen.

Further work is in progress to isolate the macromolecular complex containing sialic acid and to find out its location in the cellular fractions.

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CYTOLOGY

Cell Retention and Incidence of Carcinoma in Several Portions of the Gastrointestinal Tract

THE epithelial cells of the stomach, small intestine and colon are subject to rapid renewal[1]. In the mucosa of these organs, cells are produced in proliferative zones which are rather sharply defined. Maturing cells and fully mature cells move in coherent sheets towards the lumen of the gastrointestinal tract or, in one area of the stomach, into the depth of the glands. Generation times of the epithelial cells vary from about ½ day to a few days.

When tritiated thymidine is injected into an animal, the label is available for only a short time, and is incorporated specifically into DNA in process of formation. The location and amount of label can be studied afterwards by microautoradiographic methods[2]. Following the incorporation of tritiated thymidine, the label disappears from the proliferative zone by migration of labelled and later maturing cells. Label remaining in the cells located in the proliferative zone is reduced by 50 per cent at each cell division. Within one week after injection of thymidine, most proliferative cells in the body of the stomach and in small and large intestine have undergone several divisions, which reduces the label to near background-level. Cells that matured within one or two divisions after labelling and were therefore heavily labelled have migrated to the surface and sloughed off. Therefore, one week after injection, the label is not demonstrable in the majority of the epithelial cells. However, although the majority of cells participate in this general pattern of division, migration and dilution of label, some scattered cells do not[3]. These cells, which can be isolated and identified by microautoradiography, are the topic of this communication.

Sixteen *C57* Brown mice and nine rats were injected with tritiated thymidine (0·7 mc./gm., sp. act. 0·3 c./m*M*), and killed at 1 hr. and 1, 3, 5, 7, 10, 14 and 21 days later. Histological sections were prepared from various parts of the gastrointestinal tract and microautoradiographs were made using Kodak '*NTB*' emulsion. In these sections, the percentages of moderately and heavily labelled cells were determined. A heavily labelled cell (Fig. 1) is one that has undergone one or no division since labelling, as ascertained by comparison with cells from animals killed 1 hr. after labelling. A moderately labelled cell is estimated to have undergone 2 or 3 divisions.

Four days following labelling, differences in the number of heavily labelled cells retained in various portions of the gastrointestinal tract begin to become obvious. These differences are well expressed one week following injection. Labelled cells are removed most rapidly from duodenum and least rapidly from stomach and colon. The results of counts taken in two mice and two rats one week after injection are shown in Table 1. At two weeks, there were almost no recognizably labelled cells in the small intestine, and very few in the colon and stomach. Messier and Leblond have reported persistence of label in rats in the colon, tongue, œsophagus and duodenum (listed in order of decreasing amounts)[3].

The persistence of labelled cells in each area cannot be explained unequivocally at this time. They have no distinctive morphological characteristics. Appropriate cytochemical staining procedures[4] have revealed that they are not argentaffin cells. Some take the periodic acid–Schiff stain which is characteristic of goblet cells, but some do not. They may represent the extreme of the distribution of generation times of the general cell population, or they may be members of a specific population which so far has not been identified. In the colon, they could be slowly migrating descendants of the cells located in the bottom of the crypts[5].

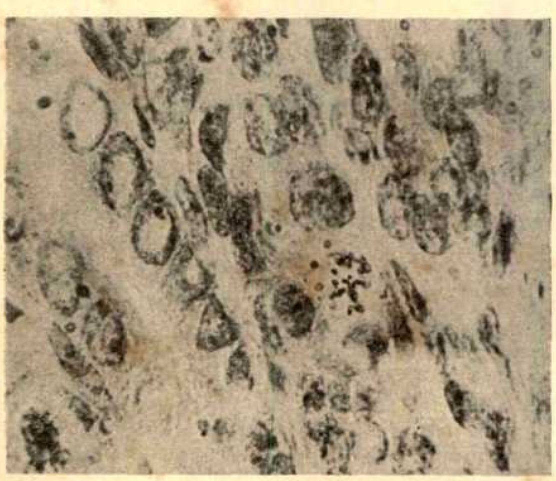

Fig. 1. Microautoradiograph of colonic crypt of mouse one week after injection of tritiated thymidine. One heavily labelled epithelial cell and several nuclei with 1–3 grains

Table 1. RETENTION OF PROLIFERATIVE CELLS IN MAMMALS AND AMPHIBIAN COMPARED TO INCIDENCE OF CARCINOMA IN MAN FOR VARIOUS PORTIONS OF THE GASTROINTESTINAL TRACT

Organ	Occurrence of cells heavily labelled after administration of tritiated thymidine				Frequency of gastrointestinal carcinoma in man in two series	
		Cells labelled (per cent)				
	No. of cells counted in each species	Mouse*	Rat*	*Rana pipiens*†	Ref. 6	Ref. 7
Sigmoid colon	1,000	2·4	4·3	8·4	416	52
Ascending colon	2,000	1·2	5·9		48	11
Jejunum	2,500	0·1	0·3	1·3	9	1
Duodenum	3,000	0	0		2	1
Stomach (mid-portion of body)	1,000	4·0	3·2	5·1	1,405‡	70‡

*, † Mice and rats killed 1 week and frogs 3 weeks after injection of label.

‡ Entire stomach.

The phenomenon is not restricted to mammals. In *Rana pipiens*, there appears to be retention of heavily labelled cells in the colon contrasted with more rapid dilution of label in the jejunum, when tritiated thymidine has been similarly injected and the frogs maintained at room temperature. The results of counts taken in three frogs two weeks after injection of tritiated thymidine are also shown in Table 1. In addition, data in two hamsters at one week revealed retention of heavily labelled cells: sigmoid, 2·9 per cent; ascending colon, 11·6 per cent; jejunum, 0·2 per cent; duodenum, 0·6 per cent; stomach, 2·2 per cent.

It is not known whether in man a similar distribution of retained epithelial cells occurs. We note with interest, however, that the rank order found for retention of labelled epithelial cells in these areas of the species examined is the same as the rank order of frequencies of precancerous and cancerous lesions in the corresponding areas of the gastrointestinal tract of man[6,7]. The retention of labelled and therefore potentially proliferative cells in these areas of the gastrointestinal tract could be associated with malignancy. In general, carcinomas arise frequently in tissues capable of rapid growth. A notable exception is the small bowel. Here, proliferation occurs very rapidly, yet tumours are rare; they are virtually absent in the duodenal bulb (Table 1). This could conceivably be due to a mechanism by which pathological or potentially pathological cells are swept out of the proliferative region in small intestine, while remaining for longer periods of time in other areas of the gastrointestinal tract.

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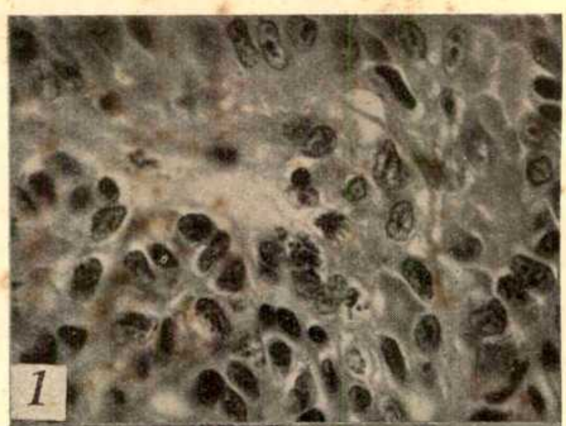
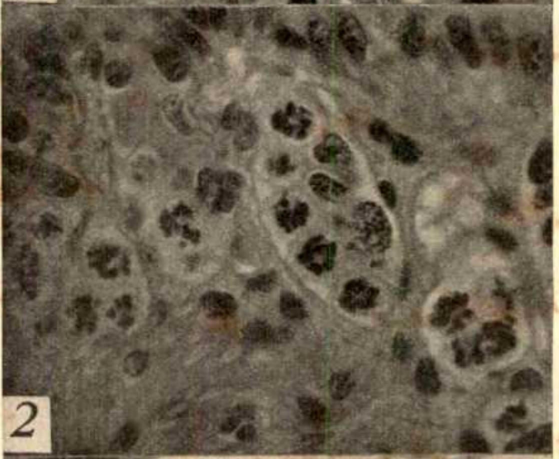
Kinetin, as a Mitotic Stimulant in *Triturus viridescens*

Miller *et al.*[1-3] presented evidence that kinetin when used together with indolyl-3-acetic acid increased cell proliferation in tobacco callus tissue. These authors isolated kinetin from the DNA of herring sperm and calf thymus. Since that time additional evidence indicating that kinetin acts as a mitotic stimulant on plant tissues has been presented. Guttman[4] reported a larger proportion of division figures in the meristematic region of roots of *Allium cepa* following 4 and 24 hr. treatment with kinetin. Torrey[5] noted that kinetin together with 2,4-*D* initiated frequent mitoses in tetraploid nuclei in excised callus tissue from mature pea roots. In the absence of kinetin the tetraploid nuclei remained in the resting state. The action of kinetin on animal tissues is still controversial. Cudkowicz[6] treated mice with kinetin following irradiation and compared these with mice that were simply irradiated. He noted that irradiation inhibited mitoses in the crypts of Lieberkühn. The return to the normal mitotic rate was enhanced by treating the mice with kinetin following irradiation.

Ogawa, Abe and Fujioka[7] and Ogawa[8] examined the effect of kinetin on the mitotic activity of Yoshida sarcoma cells transplanted to Wistar-strain rats. A significant increase in the frequency of mitotic figures was found in the tumour cells following injection of a solution of kinetin into the abdomen. This occurred only if the sarcoma cells were transplanted. Lettré and Endo[9], however, found no changes in cell division or tumour growth following treatment with kinetin in cultures of normal or malignant human and animal cells. Ogawa[10] raised embryos of *Triturus pyrrhogaster* in kinetin solutions of varying concentrations. Kinetin significantly increased the rate of early embryonic development. Guttman and Back[11] noted an increase in the mitotic divisions in *Paramecium caudatum* as the concentration of kinetin was lowered from 4 to 1·5 mgm./l. Moewus[12] reported an increase of mitotic activity by kinetin in *Polytoma uvella*.

Because of the interesting results reported by Ogawa[10] and since *Triturus* is good cytological material, the effect of kinetin on the mucosa of the alimentary tract of *Triturus viridescens* was examined. Adult specimens of *Triturus viridescens* collected in Poughkeepsie, New York, were used in these experiments. The animals were immersed in varying concentrations of kinetin for varying periods of time. In the initial experiments 5 mgm. and 10 mgm. per cent solutions were used alone and in combination with equal amounts of alpha naphthalene acetic acid in the concentration of 5 parts per million in tap water. Two hundred c.c. of each solution were placed in finger bowls together with the animals. The best results were evident after treating the salamanders with a 10 mgm. per cent solution of kinetin for 7–9 days. During the period of treatment the animals were not fed and the solution was changed every third day. The animal was killed after 7 or 9 days of treatment. Portions of the stomach, small intestine, large intestine and cloaca were fixed, sectioned and stained with Heidenhain's hæmatoxylin using standard techniques. Sections of untreated tissue were prepared at the same time.





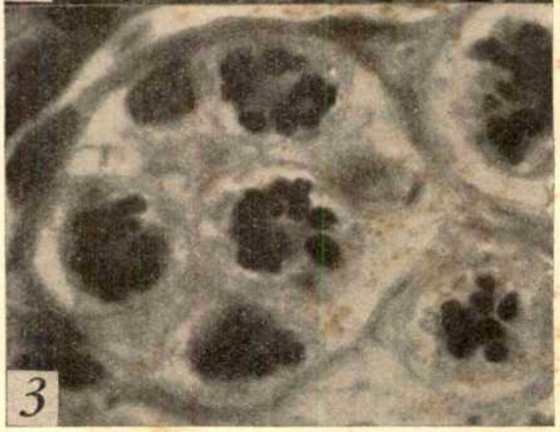


Fig. 1. Photomicrograph of a section of normal stomach mucosa (× 400). Fig. 2. Photomicrograph of a section of treated (kinetin and alpha naphthalene acetic acid) stomach mucosa (× 400). Fig. 3. Photomicrograph of a section of a cell nest in the gastric mucosa following treatment with kinetin and naphthalene acetic acid (× 1,000)

In the normal epithelium of the stomach, small intestine, large intestine and cloaca a relative lack of mitotic activity was found. No divisions were found in the surface epithelium and divisions were uncommon in the nests (Fig. 1). This lack of mitotic activity in the nests was noted earlier by Patten and Andrew[13]. According to their findings normal mitotic activity within the cell nests was present in 0·57 per cent of the cells. In the present work following 9 days exposure to kinetin a marked increase in the mitotic activity was noted. This was evident in the stomach, small intestine, large intestine and the cloaca. No divisions were found in the surface epithelium. The increased activity was noted in the nests. This mitotic activity seemed to be localized in the different areas of the intestinal mucosa. In some areas of the stomach and intestine every cell in the nests was in division. In other areas relatively few cells were in division. The statistical details will be given in a subsequent paper. The divisions in all cases were typical diploid divisions. The animals behaved normally during the treatment. This stimulatory effect of kinetin appeared to be further enhanced when it was combined with alpha naphthalene acetic acid (Figs. 2 and 3).

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Two Types of Islet α Cells in Different Parts of the Pancreas of the Dog

A CRITICAL evaluation of the argyrophil reaction in the islets of Langerhans in different vertebrates revealed that only a certain number of the α cells are argyrophil (= α_1 cells), while the rest (= α_2 cells), like all the β cells, lack this property[1–7]. Differences were demonstrated between the two types of α cells also with regard to their localization within the islets, the size and eccentricity of their nuclei and the metachromatic reaction with toluidine blue and azure *A*[4–7]. The reports of Bencosme *et al.*[8,9] that the uncinate process of the dog pancreas is devoid of α cells and contains practically no extractable hyperglycæmic activity has lent strong support to the view that the α cells are a source of glucagon. The present investigation is an attempt to find which type of α cell is possibly associated with glucagon secretion by analysing how the α_1 and α_2 cells are regionally distributed within the pancreas of the dog.

Biopsies were taken from the uncinate process, the body and the tail of the pancreas in 8 adult dogs of both sexes, anæsthetized with 'Nembutal^R'. The pancreatic samples were fixed in Bouin's solution and, after dehydration and clearing, embedded in paraffin. Serial sections, 4μ thick, were alternately granule stained with Gomori's chrome-hæmatoxylin or aldehyde fuchsin using phloxine or ponceau fuchsin as counter-stains for the α cells or silver impregnated as previously described[1–2]. Since the initial pre-oxidation in the specific granule staining procedures results in a complete removal of the silver, the islet cells in a section could be examined first in a silver impregnated state and then afterwards granule stained without any alteration of their original staining properties, as shown by comparisons with the alternately directly stained sections.

While the β cell granulation was found to be characterized by the usual staining reaction with aldehyde fuchsin or chrome-hæmatoxylin, the remaining islet cells could be divided into two major fractions because of their affinity to phloxine or ponceau fuchsin. One type displayed the heavy red cytoplasmic staining as seen in the α cells in other species, and the other had a more pink cytoplasmic tinge. The latter cells appeared to be the only argyrophil islet cells in the dog and have therefore been regarded as corresponding to the α_1 cells of other vertebrates. In addition to these types of cells some non-argyrophil islet cells lacking apparent cytoplasmic staining were occasionally observed.

There was a different appearance of the islet tissue in the uncinate process as compared with the remaining parts of the pancreas. In the former region the islets were not only smaller but their cellular composition was also different. While the islets of Langerhans in the uncinate process were devoid of α_2 cells, the contribution of the silver-positive α_1 cells was, on the other hand, comparatively very high in this particular part of the pancreas (Fig. 1). The different arrangement of the islets was also reflected in the position of the elongated argyrophil α_1 cells, which were packed together, mainly in the central parts of the islets in the uncinate process, but more diffusely distributed within the islets of the body and the tail.

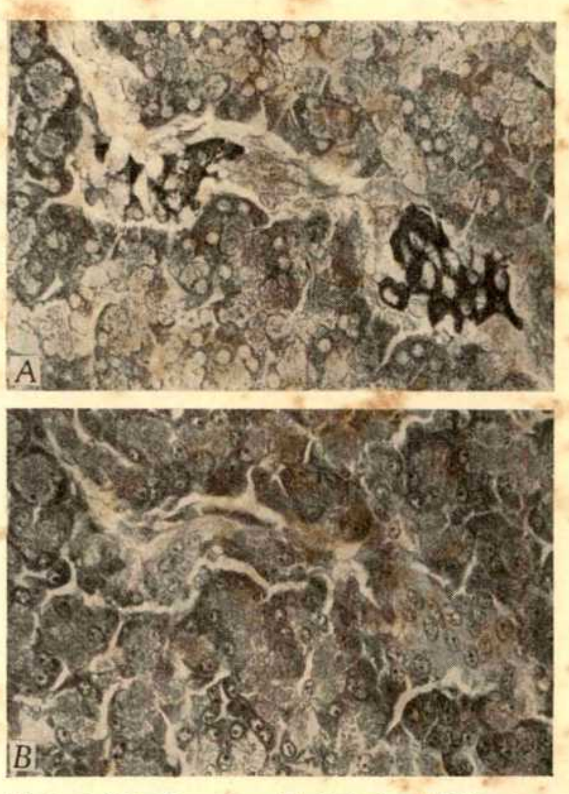

Fig. 1. An islet of Langerhans of typically elongated shape from the uncinate process. (× *c*. 350.) *A*, Islet in a silver impregnated state. The numerous blackened silver cells are characteristically packed close together. *B*, Islet after removal of the silver and stained with aldehyde fuchsin combined with ponceau fuchsin. The formerly argyrophil cells now exhibit a lighter staining than the β cells. In the left upper part of the picture it can be seen that the islet is connected to a small excretory duct. No α_2 cells can be recognized

While in some other species, as in the rat, both types of α cells are equally well stained with phloxine or ponceau fuchsin, it is worthy of note that the α_1 cells in the dog pancreas have lower affinity to these stains. This might well explain why the uncinate process has been considered erroneously to be devoid of α cells in spite of the relatively very high contribution of α_1 cells demonstrated by us in the islets of this particular region. The finding that the uncinate process of the dog pancreas was characterized not only by extremely low or absent extractable hyperglycæmic activity but also by a different cellular composition of the islets due to absence of α_2 cells suggests that this latter type of α cell is the source of glucagon. The recent demonstration[10] of pronounced regressive nuclear changes in the α_2 cells after repeated injections of glucagon to rats supports such a hypothesis.

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STATISTICS

Spherical Errors

I HAVE read with interest the recent article by N. Singh[1] concerning spherical errors. Work of a similar nature has been done in this laboratory.

It is assumed here that the components of the total errors in the position of an object along three cartesian co-ordinates (x, y, z) are normally distributed with variances σ_x^2, σ_y^2, σ_z^2, and mean at the origin. That is, the probability density of the position of the object in question along the x co-ordinate is:

$$\frac{1}{\sqrt{2\pi\sigma_x^2}}\exp[-x^2/2\sigma_x^2]$$

and similarly for the other co-ordinates. The probability $P(R)$ that the object is within a sphere of radius R about the origin is then given by:

$$P(R) = \frac{2\pi^{-3/2}}{\sigma_x\sigma_y\sigma_z}\iiint_R \exp[-x^2/2\sigma_x^2 - y^2/2\sigma_y^2 - z^2/2\sigma_z^2]\,\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z$$

where the integration is carried out over the volume of the sphere in question. Unfortunately, this expression for $P(R)$ cannot, in general, be integrated in closed form, nor is it a tabulated function.

In order to avoid the difficulty of a triple numerical integration, it is usual to assume that $\sigma_x = \sigma_y = \sigma_z = \sigma$, whence the expression for $P(R)$ becomes (after conversion to spherical co-ordinates):

$$P(R) \approx \frac{\pi^{-1/2}}{\sigma^3}\int_0^R \exp[-r^2/2\sigma^2]\,r^2\mathrm{d}r$$

If the substitution $r^2/2\sigma^2 = u$ is made, this can be expressed as

$$P(R) \approx \frac{2}{\sqrt{\pi}}\int_0^{R^2/2\sigma^2} e^{-u}\,u^{1/2}\,\mathrm{d}u$$

which is the incomplete Γ function[2], and is tabulated.

In making the aforementioned assumption, personnel normally choose a value for σ^2 which is equal to the sum of the variances of all the errors involved, regardless of their direction. This naturally results in a value of $P(R)$ which is smaller than the actual value. The transition from ellipsoidal to spherical symmetry suggests that a better alternative than the above might be to assume:

$$\sigma^2 = \tfrac{1}{3}(\sigma_x^2 + \sigma_y^2 + \sigma_z^2)$$

As the values of σ_x, σ_y, and σ_z diverge, the spherical approximation to the real case becomes less valid. It is therefore worth while to establish some limits on the actual value of $P(R)$ to increase the confidence in any approximate answer obtained. On examination of the expression for $P(R)$ it is obvious that:

$$\frac{2}{\sqrt{\pi}}\int_0^{R^2/2\sigma_M^2} e^{-u}\,u^{1/2}\,\mathrm{d}u \leqslant P(R) \leqslant \frac{2}{\sqrt{\pi}}\int_0^{R^2/2\sigma_m^2} e^{-u}\,u^{1/2}\,\mathrm{d}u$$

where σ_M is the maximum of $(\sigma_x, \sigma_y, \sigma_z)$ and σ_m is the minimum of $(\sigma_x, \sigma_y, \sigma_z)$. Thus, a set of limits on the actual value of $P(R)$ is established.

Another set of limits may be established by integrating the expression for $P(R)$ over the volume of a cube rather than a sphere, that is:

$$\frac{2\pi^{-3/2}}{\sigma_x\sigma_y\sigma_z}\int_{-R/\sqrt{3}}^{R/\sqrt{3}}\int_{-R/\sqrt{3}}^{R/\sqrt{3}}\int_{-R/\sqrt{3}}^{R/\sqrt{3}} \exp[-x^2/2\sigma_x^2 - y^2/2\sigma_y^2 - z^2/2\sigma_z^2]\,\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z \leqslant P(R)$$

$$\leqslant \frac{2\pi^{-3/2}}{\sigma_x\sigma_y\sigma_z}\int_{-R}^{R}\int_{-R}^{R}\int_{-R}^{R} \exp[-x^2/2\sigma_x^2 - y^2/2\sigma_y^2 - z^2/2\sigma_z^2]\,\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z$$

The two cubical regions of integration are taken as the cubes that circumscribe and inscribe the actual region of integration. The limits are then given by the product of three tabulated functions (error functions). This latter set of limits is the more useful when the values of the variances are widely divergent. Taking the larger of the two possible lower limits and the smaller of the two larger limits should establish a fairly good bracket on the value of $P(R)$. A graph of the limits of $P(R)$ versus R then establishes limits for the radius of the 50 per cent probability sphere.

C. F. EATON

Advanced Development Laboratory,
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Bedford, Mass.

[1] Singh, N., *Nature*, **193**, 605 (1962).
[2] Pearson, K., *Tables of the Incomplete Γ Function* (Camb. Univ. Press).

FORTHCOMING EVENTS

Monday, June 25

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Annual General Meeting and Presidential Address.

Monday, June 25—Tuesday, June 26

INSTITUTE OF RURAL LIFE AT HOME AND OVERSEAS (at the Department of Technical Co-operation, 6 Carlton House Terrace, London, S.W.1), at 10.30 a.m. on Monday and 10.15 a.m. on Tuesday—Twelfth Annual Conference—"Training the Farming Community at Home and Overseas".

Tuesday, June 26

BRITISH SOCIETY FOR RESEARCH ON AGEING (at the Zoological Society of London, Regent's Park, London, N.W.1), at 2 p.m.—Informal discussion on "Age and Cancer".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE (in Committee Room 11, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Problem of Diseases Arising out of the Use of New Materials and Processes in Industry and Agriculture" introduced by Prof. H. G. Sanders, Mr. T. A. Lloyd Davies and Dr. Donald Hunter.

Wednesday, June 27

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. I. S. E. Carmichael: "The Crystallization of Feldspar in Volcanic Acid Liquids".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting.

Friday, June 29

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at Rothamsted Experimental Station, Harpenden, Herts), at 11 a.m.—Annual Summer Meeting.

Monday, July 2—Friday, July 13

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY (at Imperial College, London, S.W.7)—Summer School in "Health Physics (Radiation Protection)".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with special interest in teaching and research in the general field of the strength, elasticity and plasticity of engineering materials) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 29).

ASSISTANT LECTURER (preferably with an interest in cryptogamic botany) in BOTANY—The Registrar, The University, Nottingham (June 30).

ASSISTANT LECTURER (with special interest in palæontology and stratigraphy) IN GEOLOGY—The Registrar, The University, Sheffield (June 30).

ESSO RESEARCH STUDENT IN THE DEPARTMENT OF CHEMISTRY, to undertake research in the field of chemical crystallography—O.S. Mills, Department of Chemistry, The University, Manchester 13 (June 30).

LECTURER IN EDUCATIONAL PSYCHOLOGY—The Secretary, The University, Exeter (June 30).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (June 30).

RESEARCH DEMONSTRATOR (with good honours qualifications in organic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Registrar, University College, Singleton Park, Swansea (June 30).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF METALLURGY—The Registrar, The University, Liverpool, quoting CV/105/T (July 1).

ASSISTANT IN GEOGRAPHY—The Secretary of University Court, The University, Glasgow (July 2).

ASSISTANT LECTURER IN SOCIOLOGY—The Registrar, The University, Sheffield (July 2).

LECTURER IN PSYCHOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 2).

JUNIOR RESEARCH OFFICER (with an honours degree in economics, statistics or agriculture with agricultural economics) IN THE FARM ECONOMICS BRANCH—The Secretary, School of Agriculture, Cambridge (July 4).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Leeds 2 (July 7).

RESEARCH ASSISTANT (with a good degree, preferably in physics or electrical engineering, and several years postgraduate experience in the following or related fields: electronics, microwave techniques, or semiconductor technology) at the Nuffield Radioastronomy Laboratories, Jodrell Bank, to take a leading part in the existing programme of developing parametric amplifiers for use in conjunction with the 250 ft. radio telescope—The Registrar, The University, Manchester 13 (July 7).

LECTURER or ASSISTANT LECTURER (with an honours degree in chemical engineering or an equivalent qualification, and evidence of research ability) IN CHEMICAL ENGINEERING at Houldsworth School of Applied Science—The Registrar, The University, Leeds 2 (July 9).

READER (preferably with interests in inorganic or physical chemistry) IN CHEMISTRY at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 11).

ASSOCIATE PROFESSOR OF MEDICAL BIOCHEMISTRY IN THE DEPARTMENTS OF MEDICINE AND BIOCHEMISTRY, The University, Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 13).

LECTURERS (2) IN PURE MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 13).

CHAIR OF THEORETICAL PHYSICS in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 16).

RESEARCH ASSISTANT, for investigation of aspects of zooplankton metabolism under the direction of the Professor of Zoology—The Secretary and Registrar, The University, Southampton (July 16).

CHAIR OF GEOGRAPHY in Monash University, Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (July 27).

ASSISTANT LECTURER (with at least a good B.A. honours degree or its equivalent in psychology, and preferably a knowledge of philosophy or physiology) IN PSYCHOLOGY IN THE DEPARTMENT OF PHILOSOPHY, University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, July 31).

CHAIR OF THEORETICAL PHYSICS in the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 3).

CHAIR OF LARYNGOLOGY and OTOLOGY at the Institute of Laryngology and Otology—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 16).

CHAIR OF BIOCHEMISTRY—The Registrar, University College, Cathays Park, Cardiff (September 14).

AGRONOMIST (Soils) (male, between 26 and 40, with an honours degree in agricultural or horticultural science with agricultural chemistry as main subject, experience in field experimental work on soil fertility, and a knowledge of tropical soils and crops) IN THE DEPARTMENT OF AGRICULTURE, Sarawak—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. RC.213/123/01/T.

HEAD OF THE MATHEMATICS DEPARTMENT—The Rector, Glasgow Academy, Glasgow, W.2.

MASTER TO TEACH MATHEMATICS AND PHYSICS—The Headmaster, Lawrence Sheriff School, Rugby.

PRINCIPAL LECTURER (with experience of supervising research of an academic standard) IN THE DEPARTMENT OF PHYSICS—The Clerk, Northern Polytechnic, Holloway, London, N.7.

RESEARCH ASSISTANT (either botanist with a good background in physical chemistry or physical chemist with an interest in entering the field of biophysics) IN THE ASTBURY DEPARTMENT OF BIOPHYSICS, for investigation of the permeability properties of timber with particular reference to permeability barriers in the impregnation of wood by preservatives, using physical techniques including electron microscopy—Prof. R. D. Preston, The Astbury Department of Biophysics, The University, Leeds 2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Tin and Cast Iron. By Dr. D. A. Robins. (Reprinted from *Foundry Trade Journal*, November 23, 1961, Vol. 111, No. 2346.) (Greenford, Mddx.: Tin Research Institute, 1961.) [283

The Library Association. Special Subject List No. 36: Semiconductors and Transistors. Compiled by E. H. C. Driver. Pp. 19. (London: The Library Association, 1962.) 6*s.* (L.A. members 4*s.* 6*d.*) [283

Annual Report of the British Aerosol Manufacturers' Association for the year ended 31st December, 1961. Pp. 12. (London: British Aerosol Manufacturers' Association, 1962.) [283

Royal Observatory Bulletins. (Joint publications of the Royal Greenwich Observatory, Herstmonceux; and the Royal Observatory, Cape of Good Hope.) No. 56. Time Service 1961—April–June. Pp. B101-B118. (London: H.M. Stationery Office, 1962.) 3*s.* net. [283

Report of Her Majesty's Civil Service Commissioners for the period 1st April 1960 to 31st December 1961. (Ninety-fifth Report). Pp. v+50. (London: H. M. Stationery Office, 1962.) 4*s.* net. [303

Chelsea College of Science and Technology. Sixty-sixth Annual Report, Session 1960–61. Pp. 46. (London: Chelsea College of Science and Technology, 1962.) [303

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Droitwich, Abberley and Kidderminster, (Explanation of Sheet 182). By Dr. G. H. Mitchell, the late Dr. R. W. Pocock, and Dr. J. H. Taylor. With contributions based on notes by Dr. T. N. George, Dr. S. E. Hollingworth and T. H. Whitehead. Pp. x+187+8 plates. (London: H.M. Stationery Office, 1961.) 22*s.* 6*d.* net. [303

The Case Against Decimalisation. By Prof. A. C. Aitken. Pp. 22. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 2*s.* 6*d.* [34

Opportunities for Girls and Women in Science and Technology. Pp. 42. (London: British Federation of University Women, Ltd., 1961.) 3*s.* 6*d.* [34

Committee of the Privy Council for Agricultural Research. Report of the Agricultural Research Council for the year 1960–61. Pp. viii+79. (Cmnd. 1661). (London: H.M. Stationery Office, 1962.) 5*s.* 6*d.* net. [34

University of London. University College Annual Report, 1960–1961. Pp. 101. (London: University College, 1962.) [34

Experimental and Molecular Pathology, Vol. 1, No. 1, (February, 1962). Edited by Frederick Coulson and Wilbur A. Thomas. Pp. iii+84. Vol. 1, 1962, (6 issues) 18 dollars. (London and New York: Academic Press, 1962.) [34

Proceedings of the Royal Irish Academy. Vol. 62, Section A, No. 2: Some Special Solutions in Viscous Fluid Motion. By S. K. Lakshmana Rao. Pp. 11–16. 1*s.* Vol. 62, Section A, No. 3: On a Certain Non-Linear Differential Equation. By J. L. Synge. Pp. 17–42. 4*s.* 6*d.* Vol. 61. Section B, No. 20: Cannono Lough, Kilrea, Co. Derry—Stratigraphy and Pollen Analysis. By A. G. Smith. Pp. 369–388+plates 21–24. 3*s.* Vol. 62, Section B, Nos. 2 and 3: Antitubercular Substances—16: Rimino-Compounds and Related Derivatives Synthesised by the Kehrmann–Cordone Method. By J. G. Belton, C. N. O'Callaghan and Dermot Twomey. Antitubercular Substances—17: Some Rimino-Compounds Derived from Glyoxalino-Phenazines and Anilinoaposafranines. By J. G Belton, M. L. Conalty, C. N. O'Callaghan, J. F. O'Sullivan and Dermot Twomey. Pp. 9–22. 2*s.* Vol. 62, Section B, No 4: Winter Dormancy and Vegetative Propagation in Irish *Pinguicula grandiflora* Lamk. By Y. Heslop-Harrison. Pp. 23–30+plate 2. 1*s.* Vol. 62, Section B, No. 5: The Vegetation and Flora of Some Islands in the Connemara Lakes. By D. A. Webb and E. V. Glanville. Pp. 31–54+plate 3. 2*s.* 6*d.* Vol. 62, Section B, No. 6: The Porphyritic Felsite of the Tertiary Ring Complex of Slieve Gullion, Co. Armagh. By C. H. Emeleus. Pp 55–76+plates 4–6. 4*s.* (Dublin: Hodges, Figgis and Co., Ltd., 1962.) [34

Other Countries

Connecticut Agricultural Experimental Station, New Haven. Bulletin 635: The 63rd Report on Food Products; and the 51st Report on Drug Products, 1958. By H. J. Fisher. Pp. 80. Bulletin 636: C2, a New Mosaic Resistant—Connecticut Broadleaf Tobacco. By Seaward A. Sand and Gordon S. Taylor. Pp. 4. Bulletin 637: Commercial Fertilizers—Report for 1960. By H. J. Fisher. Pp. 80. Bulletin 638: Chemical Control of Weeds in Nursery Plantings. By John F. Ahrens. Pp. 41. Bulletin 639: Fungitoxicity of Carbamic and Theocarbamic Acid Esters. By Saul Rich and James G. Horsfall. Pp. 95. Bulletin 640: Chemical Investigations of the Tobacco Plant. XI: Composition of the Green Leaf in Relation to Position on the Stalk. By Hubert Bradford Vickery. Pp. 42. Bulletin 641: Caterpillars on Oaks. By Stephen Hitchcock. Pp. 15 Bulletin 642: Prevention and Control of Crabgrass in Lawns. By J. F. Ahrens and A. R. Olson Pp 8. Bulletin 643. Effect of Sprays on Mite and Insect Species of the Apple Orchard Floor. By Philip Garman. Pp. 16. Bulletin 644. Deep Tillage and Root Growth: Study of Tobacco Growing on Sandy Loam Soil. By H. C. De Roo. Pp. 48. Bulletin 645. Commercial Feeding Stuffs—1960 Report on Inspection. By H. J. Fisher. Pp 143. Bulletin 646: Population Ecology of the Gypsy Moth *Porthetria dispar* L. (Lepidoptera: Lymantridae). By Henry A. Bess. Pp. 43. New Haven, Conn.: Connecticut Agricultural Experiment Station, 1961.) [34

Connecticut Agricultural Experiment Station, New Haven. Circular 215 Control of Leaf Miners. By John C. Schread. Pp. 16. Circular 216: Scales and Mealybugs. By John C. Schread. Pp. 11. Circular 217: Highway Post Survey a 1960 Progress Report. By A. R. Alson and N. Tirner. Pp. 12. Circular 219: Improving Nursery Soil by Addition of Organic Matter. By H. A. Lunt. Pp. 8. (New Haven, Conn.: Connecticut Agricultural Experimental Station, 1961) [34

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New Zealand Department of Scientific and Industrial Research: Geophysics Division. New Zealand Seismological Report, 1955. (Seismological Observatory Bulletin, E-136). Pp. ii+78. Tables to Facilitate the Study of Near Earthquakes. By G. C. Eiby and M. G. Muir. (Seismological Observatory Bulletin, S–109). Pp. 31. (Wellington: Government Printer, 1961.) [34

United States Department of Commerce: Weather Bureau. Technical Paper No. 42: Generalized Estimates of Probable Maximum Precipitation and Rainfall-Frequency Data for Puerto Rico and Virgin Islands for Areas to 400 Square Miles, Durations of 24 Hours, and Return Periods from 1 to 100 Years. Pp v+94. (Washington, D.C.: Government Printing Office, 1961.) 50 cents. [34

Smithsonian Institution: Bureau of American Ethnology. Bulletin 183: Seneca Thanksgiving Rituals. By Wallace L. Chafe. Pp. iii+302. (Washington, D.C.: Government Printing Office, 1961.) 2.25 dollars. [34

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 18, No. 2, (March 1962): Low Birth Rates of European Catholic Countries. Pp. 21–40. (Washington, D.C.: Population Reference Bureau, Inc., 1962) [34

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South African Museum. Guide No. 6: Greek Vases in Cape Town. By John Boardman and Maurice Pope. Pp. 20+16 plates. (Cape Town: South African Museum, 1961.) [34

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Universidade de Sao Paulo. Boletim do Instituto Oceanografico. Tomo 11, Fasc. 3. (1): Algunas Diatomaceas Encontradas sobre Algas Superiores. Por M. B. Kutner. (2). Diurnal Variation of Phytoplankton Production and Solar Radiation on Coastal Water Off Cananéia. By A. Garcia Occhipinti, A. Magliocca and Cló vis Teixeira. (3): Contribuicao para o Conhecimento das Diatomáceas da Regiao de Cananéia. Por C. Teixeira e M. B. Kutner. (4): *Dentostomnia besnardii*, nova Espécie de Foraminifero. Por Walter Narcho. Pp. 76. Tomo 11, Fasc 4. (1). Diatomáceas dos Sedimentos de Doze Estacoes Oceanograficas na Costa Brasileira. Por C. Teixeira. (2): Distribuicao dos Chaetognatha no Atlantico sul Ocidental. Por M. S. de Almeida Prado. Pp. 49. (Sao Paulo: Universidade de Sao Paulo, 1961.) [34

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Physics Letters, Vol. 1, No. 1, (1 April 1962). Edited by G. E. Brown and D. Ter Haar. Pp. 32. Subscription price per volume 36 guilders; 62*s.*; 10 dollars; 40 D.M. (Amsterdam: North-Holland Publishing Company, 1962) [34

Proceedings of the California Academy of Sciences. Fourth Series. Vol. 31, No. 8, (March 7, 1962): One some Earthworms of Eisen's Collection. By G. E. Gates. Pp. 185–225. Vol. 31, No. 9, (March 7, 1962): Pyrgomorphidae (Orthoptera: Acridoidea) Collected in Africa by E. S. Ross and R. E. Leech, 1957–1958, with descriptions of New Species. By D. Keith McE. Kevan. Pp. 227–248. Vol. 31, No. 10, (March 7, 1962): Flora and Vegetation of Clipperton Island. By Marie-Hélène Sachet. Pp 249–307+1 map. (San Fransisco: California Academy of Sciences, 1962.) [34

Physical Oceanography of the Southeast Asian Waters. By Klaus Wyrtki. ("Naga" Report, Vol 2—Scientific Results of Marine Investigations of the South China Sea and the Gulf of Thailand. Sponsored by South Viet Nam, Thailand, and the United States of America.) Pp. 195 (44 plates). (La Jolla, Calif.: University of California, Scripps Institution of Oceanography, 1961.) [34

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Institut des Parcs Nationaux du Congo et du Ruanda-Urundi. Exploration du Parc National Albert et du Parc National de la Kagera. II: Mission K. Curry-Lindahl (1951–1952, 1958–1959). Fascicule 1: Contribution à l'Étude des Vertébrés Terrestres en Afrique Tropicale. Par K. Curry-Lindahl. Pp. 331+22 planches. (Bruxelles: Institut des Parcs Nationaux du Congo et du Ruanda-Urundi, 1961.) [34

I. & E. C. Product Research and Development, Vol. 1, No. 1, (March, 1962). Pp. 64. Published quarterly. Sold only on combination with the general monthly, *Industrial and Engineering Chemistry*.) (Easton, Pa.: American Chemical Society, 20th and Northampton Streets, 1962.) [174

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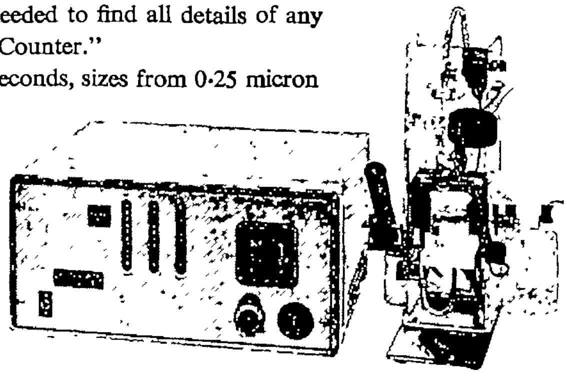

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POSITION OF COAL IN ELECTRIC POWER GENERATION

IN a modern industrial community the generation of electrical power becomes one of the essential pillars of national prosperity. In Great Britain, the standards of efficiency of the generation of such power have changed markedly in the past forty years. This change amounts to an improvement in the thermal efficiency of power generation from some 18 per cent at the end of the First World War to a realizable figure of 36 per cent foreshadowed in new equipment at the present time. The coal now used for this purpose represents 84 per cent of all the fuel consumed by the Central Electricity Generating Board. In 1957 the proportion was 95 per cent. By 1965 it may fall to 80 per cent.

The why and the wherefore of these prospects were explained recently in an interesting lecture by Mr. L. Rotherham, the member for research of the Central Electricity Generating Board, on the occasion of the award to him of the British Coal Utilization Research Association's Coal Science Medal*.

Mr. Rotherham discussed the extent to which nuclear energy and oil are likely to be used within the coming decades, and the technical and economic factors affecting the situation.

In 1965–66 nuclear energy will be contributing an equivalent of 6–7 million tons of coal. This consumption of nuclear energy is justified not entirely on economic grounds, but in order to enable the technology of nuclear energy to be developed. By 1970–71 nuclear energy is expected to be contributing some 20 per cent of the energy output of the Board. With oil keeping its position, coal will only be used for 65 per cent of the station requirements. Nuclear energy might have come into the picture earlier; but its advancement has been affected by three principal factors, namely, a less than anticipated rise in the price of coal, improved efficiency of electricity generation and reduction in capital costs in coal-fired stations. Over the period envisaged the rate of energy consumption is anticipated to increase by some 7 per cent per annum. The position of coal varies regionally. The North-West of England is a high coal cost area. The healthy industry in Yorkshire and the Midlands can maintain a high demand for coal. Oil is competitive on the coast, where seaborne oil can compete favourably with rail-borne coal. If the energy transport problem could be overcome it would assist the use of coal from productive East Midland collieries in respect of London and the South.

Mr. Rotherham discussed the factors affecting the economics of the transport of energy. A basic factor is that three units of heat have to be transported as oil, gas or coal for one of electricity. In regard to the transmission of electricity over great distances, 400-kV. lines are preferred for reasons of amenity. A high-voltage grid is indeed a step in the direction of advancing the economic transfer of power, and favours the greater utilization of coal. The hydraulic transport of coal is being studied, an experimental development being the delivery by such means over a distance of 2,000 yards from a colliery to a power station in Wakefield.

Important technical problems arise in the effects and disposal of the ash from the fuel, a nuisance to be paid for. It has been intensively studied, particularly in respect of overcoming the effects of the deposits on boiler surfaces, and the resultant loss of efficiency and productive plant capacity by interrupted operation. The outcome has been a marked improvement in the time in which a boiler remains on load. In this respect, substantial help in the attainment of this result has been derived from the activities of the Boiler Availability Committee, which is supported jointly by the Central Electricity Generating Board and the boiler manufacturers, the research activities being undertaken in the main by the British Coal Utilization Research Association. The limitations in the properties of materials still constitute a problem. Thus, the vulnerability of superheater metals to the corrosive effects of deposits, even if austenitic steels are used, is the main obstacle to the use of higher steam temperatures and improved efficiency in the generating plant.

The layman will be pleased to know that, although electrostatic precipitators are now used for collecting solid particles in chimney emissions to the extent of recovering 99 per cent of the emission from the boilers, the escape of the remaining 1 per cent is still regarded as unsatisfactory, and attention is being devoted to the difficult problem of its dispersion. The collected ash has useful purposes in brick-making and horticulture. When used for land reclamation the presence of toxic constituents, particularly boron and molybdenum, calls for tolerant crops in the early stages of cultivation.

Improvement in thermal efficiency is inevitably accompanied by increase of cost in the necessary complication of equipment, and new fields of electricity generation are being studied—thus fuel cells, thermionic devices and magneto-hydrodynamics.

The final picture envisaged is that coal has an assured place in the generation of electric power at the present time. The future may well depend on the outcome of research. The coal consumption required annually for electricity supply could be expected to rise above 100 million tons by 1970. Marginal economies in utilization could have great significance. The position of coal must, nevertheless, depend largely on the price of coal. With improved methods of mining and an acceleration of research the position of coal could be maintained. Mr. Rotherham has concluded that an interesting and possibly fruitful field of development exists, given the necessary resources for the purpose.

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* *British Coal Utilization Research Association Quarterly Gazette*, No. 44 (1961).

THE SCIENTIFIC WORKS OF LORD RUTHERFORD OF NELSON

The Collected Papers of Lord Rutherford of Nelson, O.M., F.R.S.

Vol. 1: New Zealand—Cambridge—Montreal. Published under the Scientific Direction of Sir James Chadwick, F.R.S. Pp. 931 + 12 plates. (London: George Allen and Unwin, Ltd., 1962.) 126*s*. net.

IN any age there are but a few scientists who are important enough to make it both useful and fitting that all their scientific works should be collected and republished. Ernest Rutherford is certainly one of these rare beings. This first volume of his collected works covers the period from 1894, when the twenty-three-year-old student of Canterbury College, Christchurch, New Zealand, started experimenting on the magnetization of iron by high-frequency discharges, to the end of his time at McGill University, Montreal, in 1907. By then Rutherford had completed the work on radioactivity for which he was awarded the Nobel Prize for Chemistry the following year. During these thirteen years at Christchurch, Cambridge and Montreal, Rutherford published 65 papers, an average of 5 a year, with an average of 14 pages each—a remarkable output. His earliest papers showed his gift for finding the simple and direct experimental approach. When he suspected that the high-frequency electric discharge only magnetized a thin outer layer of a steel needle, due to the skin effect, he just dissolved off the outer layer with acid while watching the magnetization of the needle with a magnetometer: the magnetization first disappeared and then returned weakly in the opposite sense, so demonstrating a surface layer magnetized one way and an inner layer magnetized the other.

Rutherford's arrival in Cambridge in 1896 coincided with the huge impact on the scientific world of the discovery of radioactivity by Becquerel, and of X-rays by Roentgen. He immediately started, in close collaboration with J. J. Thomson, the study of the process of ionization of gases by X-rays, and made his first important contribution to physics by his measurement of the mobility and rate of recombination of ions. He turned his attention to radioactivity as soon as he arrived in Montreal as MacDonald professor at McGill in 1899, at the age of 28. From then on his scientific life's work was settled: all but one of the papers in this volume relate to radioactivity; this is a paper in 1903 entitled "A Penetrating Radiation from the Earth's Surface", in which he surrounded an electroscope with 5 tons of lead and deduced the existence of a radiation which was more penetrating than any known rays from radioactive substances: this was, of course, one of the early investigations of what later became known as cosmic rays.

The high points of this Montreal period were the discovery of thorium emanation, the elucidation with Soddy of the transformation theory of radioactivity, and the detailed study of the alpha particle; for this particle he was later in life to display an almost paternal affection. His wider interests are shown by a 'popular' article in *Harper's Magazine* in 1905, entitled "Radium as the Cause of the Earth's Heat", and two years later a lecture to the Royal Astronomical Society of Canada, "Some Cosmical Aspects of Radioactivity": both make good reading to-day and show Rutherford's interest in geophysics and astronomy.

Sir Edward Appleton has contributed a valuable essay on "The Young Rutherford", and H. L. Bronson and Otto Hahn have told of the time at Montreal.

The second volume will cover the Manchester period, 1907–1919, the third the Cavendish period 1919 to his death in 1937, and the fourth will contain miscellaneous papers, lectures and letters, together with a short biography.

The scientific world is indeed indebted to all those who made this great publishing venture possible: the scientific editor, Sir James Chadwick, the publishers, George Allen and Unwin, and Dr. Paul Rosbaud, who conceived the project. Generous grants from the Government of New Zealand, the National Research Council of Canada, and the Leverhulme Trust in Britain have greatly assisted publication. Here is a 'must' for every library, and a 'should' for many individuals.

P. M. S. BLACKETT

PHYSICAL METALLURGY IN THE SEMICONDUCTOR FIELD

Metallurgy of Elemental and Compound Semiconductors

Proceedings of a Technical Conference, Boston, Massachusetts, August 29–31, 1960. Edited by Ralph O. Grubel. (Metallurgical Society Conferences, Vol. 12.) Pp. xi + 494. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 13 dollars; 98*s*.

THE fly-leaf of this book identifies the sponsors of the conference, of which it reports the proceedings, as the Semiconductors Committee of the Institute of Metals Division, the Metallurgical Society, and the Boston Section, American Institute of Mining, Metallurgical and Petroleum Engineers. It is a pleasant change to review a book dealing with the metallurgy of semiconductors, as distinct from the solid-state physics approach, although the fundamental background is reviewed in each section in such a way that no physicist would quarrel with it.

The main aspects reviewed are the heavily doped materials for Esaki tunnel diodes, and new techniques of crystal growth. In the latter case, dendritic growth and vapour phase deposition are specifically considered. Part I deals with germanium and silicon for tunnel diodes, with detail of the properties resulting from heavy doping. Patel *et al.* show that the growth of germanium single crystals from the melt can be achieved with a high degree of crystal perfection by using a high concentration of gallium (3×10^{20}/cm.^{3}). The velocity of dislocation movement under shear stress has been measured to assess the effect of thermal stresses or constraint on the crystal perfection. Arsenic doping of germanium decreases the maximum stress for a given dislocation velocity, while gallium doping raises the stress, both compared with the intrinsic crystal. Interesting results on vapour phase deposition of arsenic and phosphorus on silicon are given in a paper from the General Electric Laboratory. Heavily doped layers, pyramids and rods have been made to grow epitaxially on single crystal substrates, or other nucleation sites.

Part 2 is concerned with dendritic growth, with two excellent chapters on fundamentals of such growth. The problem of solute segregation is covered by two further chapters and a final one with the role of surface tension in crystal pulling. Some beautiful pictures of crystal facets and dendrites are presented in this section, and all these papers could be read with advantage by metallurgists concerned with solidification problems.

The third part of this book deals with vapour phase deposition. For silicon, the breakdown of the tetra-iodide, or the disproportionation of the di-iodide can be used, with the addition of impurity elements via their tri-iodides (indium, gallium, aluminium, boron, phosphorus, arsenic and antimony). Several methods are described for the preparation of p–n junctions with controlled impurity concentrations. Similar work is described on germanium, utilizing disproportionation of the di-iodide. Impurities are more difficult to transfer by this method on germanium, but phosphorus, arsenic and antimony are easily transferred. Growth considerations of germanium crystals prepared from the iodide are also covered.

Part 4 deals with compound growth and solubility considerations. This field refers to the more refractory semiconductor materials, which are becoming more important. One method is to grow the compound crystals directly, another to prepare alloys, and a third to utilize solutions of compounds in Group III metals. Germanium–phosphorus, aluminium–arsenic and alloys of germanium–arsenic with aluminium–arsenic are described. The addition of zinc to alloys of gallium–phosphide in silicon is dealt with in one chapter, with interesting structural considerations to explain the properties.

Some consideration was given to other semiconductor materials at the conference, including thermo-electric materials suitable for direct conversion of heat from nuclear reactors. The latter include lead and bismuth telluride, and zinc–antimonide. A final part covers the measurement of Hall effect on tunnel diodide materials and X-ray investigations on the perfection of silicon crystals.

Considering the wealth of information presented in this book and its excellent reproduction of diagrams and photographs, the price is not unreasonable. The title may mislead many who would benefit by reading it, for here is a perfect example of modern physical metallurgy applied to a most fascinating and rapidly expanding field. It deserves to be available in every metallurgical library, not merely those concerned with semiconductor research and development.

C. R. TOTTLE

PLUTONIUM

The Metal Plutonium

Edited by A. S. Coffinberry and W. N. Miner. Pp. xi+446. (Chicago and London: University of Chicago Press, 1961.) 10 dollars; 76*s*.

PLUTONIUM presents a unique combination of properties of scientific, technological and military significance. Since the first minute quantities were prepared in Chicago in 1943, immense efforts have been devoted to the production and fabrication of the metal and to the investigation of its properties and uses. Security requirements have delayed or prevented the general dissemination of this knowledge and experience, and the first comprehensive review of the work concerned was contained in the papers presented, by acknowledged authorities on the subject from the United States, Canada, France and the United Kingdom, at the World Metallurgical Congress in Chicago in 1957. Edited versions of those papers, with some additions covering important work published between 1957 and 1960, have been combined to produce this attractive and interesting book. One can only regret that it was not published immediately after the 1957 conference.

The papers have been grouped under three headings. Of those that constitute the historical review, the accounts of early work in the United States with milligramme quantities or less of the metal are particularly fascinating. Techniques and results included under the heading of plutonium metallurgy deal with radiography, autoradiography and velocity-of-sound measurements as well as physical properties, crystal structure, microstructure, corrosion and alloying behaviour. Conspicuous gaps in the available knowledge and understanding of the behaviour of this extraordinary element are evident from the text; for example, many phase diagrams are ill-defined or unknown, and there is no reference to diffusion coefficients for plutonium in any metal or alloy. The last part of the book is devoted to plutonium in nuclear reactors and presents a useful review of some of the possibilities and problems in the fabrication and use of plutonium, its alloys and compounds in power production.

As a starting point in the study of plutonium metallurgy no better book is available at the present time. For the serious student, the next step will be to read the papers presented at the plutonium conference in Grenoble in 1960. For the general metallurgical reader, parts of this book provide a stimulating indication of the ingenuity, patience and skill that have been devoted to the scientific investigation and safe industrial use of this highly toxic material.

V. W. ELDRED

CURRENT STATUS OF ATOMIC SPECTROSCOPY

Atomic Spectra

By Dr. H. G. Kuhn. Pp. xvi+436+18 plates. (London: Longmans, Green and Co., Ltd., 1962.) 60*s*. net.

ATOMIC spectra have played a large part in the development of modern physics, and the subject was studied most intensively in the 1930's. Interest in the more academic side has since waned because the nucleus has attracted more attention, and, as Condon and Shortley stated in their book *Theory of Atomic Spectra* in 1935, "the interpretation seems to be in a fairly close and highly satisfactory state". (Nevertheless, optical spectroscopy was high on the list of subjects in a recent survey of subjects studied by Ph.D. students.) Its use as a tool either in analytical spectrochemical applications or in the study of plasma physics has, moreover, increased remarkably in the past thirty years, with the recent development of lasers, depending on spectroscopic knowledge of crystals, extending the applied field further. Even for problems connected with nuclear physics the study of hyperfine structure is a powerful means of deriving information on nuclear constants.

In the meantime, steady, although less spectacular, progress has been made in the interpretation of atomic spectra, and Dr. Kuhn's book attempts to present this for the more advanced undergraduate and research worker. As can be seen from the contents list, a wide range is covered. After a short introduction to the subject there appears a theoretical section which surveys the interpretation of spectra in terms of classical and quantum mechanics and the old quantum theory. The various types (and their interrelationship) of spectra from elements throughout the Periodic Table are discussed, ranging from the simple hydrogen atoms to the lanthanide and actinide series. X-ray spectra and calculation of electron structures are treated briefly, while hyperfine structure and isotope shifts are thoroughly discussed. The book ends with a chapter on the width and shape of spectral lines. Tables of intensity ratios in multiplets and information concerning the ground state and ionization of elements and nuclear moments of natural isotopes are included.

The book steers a middle course between the formal treatment given in Condon and Shortley's book and the more experimental approach of White's *Introduction to Atomic Spectra*. In so doing, it gains by the omission of the early historical material which is considered in the latter book and which can often be confusing to the undergraduate reader. It is well produced with clear type and very few misprints which in any event do not give rise to confusion, for example, the misplaced minus sign in the expression $d^{k-2}\,s^{2}$ on page 318. More diagrams would probably encourage the undergraduate.

One reader, at least, is left with the impression both of how many advances have been made in the understanding of spectra and how much more remains to be done on the experimental and theoretical side, particularly in the complex spectra from the lanthanide and actinide elements and which accordingly are only briefly discussed in the book. With the advances in such equipment as high-quality gratings and computers and the powerful techniques based on group theory, one can hope that the graduate reader of this book will have the sound basis to tackle the many unsolved problems in the field. At the undergraduate level, it gives a good summary of the academic side of the subject.

L. Bovey

MALAYAN ORCHID HYBRIDS

Malayan Orchid Hybrids

First Supplement. By G. H. Addison. Pp. viii + 93. (Singapore : Government Printer, 1961.) 21 Malayan dollars.

WITH the steady increase in the production of new orchid hybrids and the continual introduction of fresh species into general breeding it is inevitable that any book providing a comprehensive account of the hybrids cultivated in any given area will soon be out of date. This was recognized by the authors of *Malayan Orchid Hybrids* when they published the book in 1956, and the present work is a first supplement to their previous account, designed to bring our knowledge in this field up to date so far as is possible. This supplement includes illustrations and descriptions of about 90 hybrids additional to the 160 dealt with in the original work. Each hybrid is illustrated by two half-tone photographs showing respectively the general appearance of the inflorescence and a single flower, these being accompanied by a short description and details of the hybrid's origin. Some of the hybrids are illustrated in colour. On the whole the photographs reach a satisfactory, though in my own opinion not such a high, standard as in the original work.

The hybrids figured mostly fall into two groups, the genus *Dendrobium* on one hand, and hybrids produced from various genera belonging to the monopodial *Vandinae*, such as *Vanda*, *Arachnis* and *Euanthe*, on the other. The *Dendrobium* hybrids once again are mostly combinations of species belonging to the two sections *Ceratobium* and *Phalaenanthe*, but with the introduction into the breeding system of further species of both sections a wider range of forms is being produced. Nevertheless, there still seems to be much scope for the improvement of the individual flowers so as to give greater body to the inflorescences without impairing their gracefulness. It would also be interesting to see if some at least of the striking species belonging to such other sections as *Laourea* could be utilized for breeding. Among the monopodial hybrids those in which *Phalaenopsis denevei* is one parent are of special interest botanically, though their short inflorescences are, of course, a disadvantage for cut-flower purposes. Another interesting feature in these monopodial hybrids is the persistent dominance or strong influence of certain floral characters wherever they occur in combinations, such as the flower shapes and colorations in the genera *Arachnis* and *Euanthe*.

Mr. Addison, who is responsible for the preparation of this first supplement, is to be congratulated on such a useful continuation to the original book. Those concerned with orchids everywhere will look forward with interest to the appearance of further supplements in due course.

V. S. Summerhayes

THE CONTROL OF INSECT VECTORS

Insect Control in Public Health

By C. B. Symes, R. C. Muirhead Thompson and J. R. Busvine. Pp. vii + 227. (Amsterdam: Elsevier Publishing Company, 1962.) 60*s*.

BEFORE the discovery of the synthetic insecticides, of which DDT was the first, it was only practicable to control insect vectors of disease very locally, mostly in urban areas. The high toxicity of the new compounds, their relative cheapness, and the long period over which some of them may remain effective as thin residual films has allowed their use in situations in which control was previously impossible. A large number of striking successes has been obtained.

The volume under review is a compendium of the latest information on the chemical methods of controlling all the more important insect vectors of human diseases. The information is detailed and practical and deals with such topics as the constitution and properties of the chief insecticides, the different formulations in which they can be used, the methods of applying them, the dangers to persons using them, and the organization of a campaign for the control of insect vectors. Eleven chapters deal with special features of the principal types of insect-transmitted disease, such as malaria, plague, yellow fever and sleeping sickness. The text is extremely clear and well written so that it will be suitable for all those concerned in controlling insects, even workers with very little specialized training. There are nineteen illustrations, mostly based on excellent photographs. At the end is a short list of selected references, arranged according to the various topics which have been discussed in the text.

The book is very well produced, and the authors state that it is intended to replace a small hand-book of malaria control which the Shell Company produced in 1940. The publishers of the present volume also acknowledge that the Shell International Chemical Company made its publication possible. It is perhaps natural that with this sponsorship the emphasis should be almost entirely on the chemical methods of control.

The information about the insects themselves is very scanty. There is a short but admirable chapter on the development in one species or another of resistance to most of the insecticides. This is now such a menacing development that there is a strong case for devoting more attention to the ecology of the insect vectors and reconsidering the possibilities of some of the older methods of control. It might have been difficult to do this in such a short, concise volume, and what they set out to do the authors have done very successfully. O. W. Richards

FLESH FOOD AVOIDANCES IN THE OLD WORLD

Eat Not this Flesh

Food Avoidances in the Old World. By Prof. Frederick J. Simoons. Pp. xiii + 241. (Madison, Wisc.: University of Wisconsin Press, 1961.) 6 dollars.

This book is the work of a cultural geographer. While travelling across Africa from Ethiopia to Nigeria some years ago, the author noted that there were large areas where a particular flesh food was used and others where the same food was avoided with strong feelings. Following up the interest thus aroused, he examined the cultural and religious prejudices involved in the use of pork, beef and the flesh of horses, camels and dogs. (Curiously, the use of neither goat flesh nor mutton is discussed.) The author exposes the inadequacy of a common assumption that food avoidances are based on rational considerations—he rejects, for example, the thesis that the ancient Jews abstained from pork on hygienic grounds. He points out how in some instances, as abstention by Europeans from the flesh of dogs and cats, there is incompatibility between associating with these animals as domestic pets and treatment of them as a source of food. He lays great stress on the significance of religious belief as an element in food avoidance, and points to the way in which the prophet or other founder of a religion may play a critical part in promoting this. He also indicates how some food avoidances are correlated with the desire of a social group to improve its status relative to others—a process seen in modern India as some caste groups tend to follow the Brahmanical custom of not eating meat.

The general treatment of the book is itemistic, with instances given from a very wide geographical range, without much indication of the social and economic context in which the use or avoidance of the food occurs. Five maps showing the distribution of certain flesh foods are informative, but other maps showing the location of social groups are much less so. About half the book is taken up with maps, notes, bibliography and index.

The study is useful as a general source of information, and stimulates thought about economic and social effects of the great variation in choice of flesh food. The author is less successful in advancing reasons for these food avoidances than he is in describing them. For example, his hypothesis that prejudice against the pig and pork arose as the reaction of Asian pastoralists against an animal alien to their way of life and was then diffused to the sedentary peoples with whom they had contact is not very convincing. But for this he can scarcely be criticized, because of the general lack of historical information about the origins of such customs.

What the book does bring out is the need for more specific analytical studies of food usages directly related in a systematic way to economic, social and ritual considerations in particular communities.

Raymond Firth

DICTIONARY FOR TEXTILE COLOUR CHEMISTS AND PRINTERS

Dictionary of Dyeing and Textile Printing

By H. Blackshaw and R. Brightman. Pp. iv + 221. (London: George Newnes and Co., Ltd., 1961.) 30s. net.

The authors of this little dictionary have spent many years in dyeing and textile printing and so are well qualified for their task. They provide nearly 1,500 definitions relating to dyeing, printing and finishing processes and machines, as well as to the chemicals and auxiliary products used. Other subjects covered are textile fibres, colour terminology, chromophoric systems, natural colouring matters, and fastness testing.

Rather more than 100 of the definitions given are those produced—after much debate—by the Terms and Definitions Committee of the Society of Dyers and Colourists, and these definitions are acknowledged and indicated in the text. Apart from items concerning the Society of Dyers and Colourists, the present dictionary does not overlap to any great extent with the Textile Institute's publication, *Textile Terms and Definitions*, from which the authors have borrowed in a few cases. Sometimes they have preferred to give a fuller definition rather than the very concise official version. It is easy to think of a few more terms that they might have defined, but difficult to think of many.

The authors are very reliable when dealing with their own field, but outside this field they frequently go astray. For example, distillation is confused with fractional distillation, and chelation with intramolecular hydrogen bonding. Proof reading has been adequate, except with empirical and structural formulæ and with equations. It is to be hoped that consultation and careful checking will precede publication of the second edition.

The text is illustrated with thirteen useful diagrams of machines—with the exception of the final one (Fig. 13), which is an unlucky choice. There is, in addition, a "reading list", as well as a useful appendix consisting of an alphabetical index of the more important man-made fibres, although such lists become out of date very quickly.

In view of the slow progress invariably made by committees concerned with terminology, the authors are to be congratulated on their enterprise. Their little dictionary is likely to become popular among those concerned with dyeing and textile printing, very few of whom can be familiar with all the terms defined. C. L. Bird

Advances in Enzymology and Related Subjects of Biochemistry

Vol. 23. Edited by F. F. Nord. Pp. v + 557. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 15.50 dollars.

THIS volume of *Advances in Enzymology* contains nine articles covering a useful range of biochemical topics, but unfortunately some are of very poor quality.

Three contributions deal with particular enzymes: "Pancreatic Lipase" by Desnuelle, "Collagenases and Elastases" by Mandl and "Cytochromes of Group A and Their Prosthetic Groups" by Lemberg, all of which are very thorough surveys of their subject, the last-mentioned being particularly concerned with the chemistry of the active site. An interesting feature of the articles by Desnuelle and Mandl is the emphasis given to applications of enzymes particularly as analytical tools. This feature of enzymology is further developed in the stimulating paper "Denaturation and Inactivation of Enzyme Proteins" by Okunuki, which describes the use of proteinases for investigating changes in the secondary and tertiary structure of proteins.

There are two excellent articles on enzymes in metabolic systems, "The Metabolism of 2-Carbon Compounds by Micro-organisms" by Kornberg and Elsden and "Mechanisms of Synthesis of Adenosine Triphosphate" by Racker. The latter is an important review containing a serious attempt to correlate the conflicting findings by different groups on oxidative phosphorylation in mitochondria. The last article in the volume is a short but very topical one on the "Discovery and Chemistry of Mevalonic Acid" by Wagner and Folkers.

Of the two articles not so far mentioned, one deals with the possibility that the configuration of a peptide link may be determined by the side chains of its constituent amino-acid residues. The other attempts to explain periodic steps found in progress curves of enzyme reactions by the assumption that all the enzyme molecules are acting synchronously, this idea being derived by ignoring the statistical concept of thermodynamic equilibrium.

M. J. SELWYN

Introduction to Advanced Inorganic Chemistry

By Dr. Philip John Durrant and Beryl Durrant. Pp. xv + 1,171. (London: Longmans, Green and Co., Ltd., 1962.) 95*s*. net.

THIS book attempts to provide a comprehensive survey of inorganic chemistry based "on the concepts of theoretical chemistry which have been developed in the last twenty years". Approximately a third of the book deals with the physical aspects of inorganic chemistry such as wave mechanics, spectra and X-ray diffraction, and the remainder to descriptive inorganic chemistry. This type of treatment, in a one-volume book, although more than 1,000 pages in length, almost inevitably falls into the error of presenting a little of a lot of large topics. This fault could have been partially remedied for the student and research worker by including references to original literature. In fact very few references are included, and only at the ends of certain chapters are recommendations for further reading given.

The physical-inorganic section gives a lucid and useful presentation of many modern techniques, but also it has some surprising omissions; for example, in the section on spectra, little is mentioned about the spectra of transition metals. Also there is no adequate treatment of possible reaction mechanisms in inorganic chemistry. The descriptive section of the book is traditional in its presentation. It gives an adequate description of most of the properties of the elements with, however, one surprising omission. There is no detailed treatment of the complexes of the transition metals. The reason for this is given as lack of room (p. 988). Since so much of the solution chemistry of the transition metals depends on the nature of their complexes, this omission is a definite weakness in the book. There is a tendency in this section to be rather non-critical, and statements like "liquid hydrogen fluoride is a better solvent than water" require some justification. A number of chemical errors, such as in the preparation of sodium paratungstate, appear in the text, although this is probably inevitable in a book of this type.

All in all, this book presents in one volume much useful information, but it cannot be regarded as a 'must' for the inorganic chemist.

D. H. BROWN

The Birds of British Somaliland and the Gulf of Aden

Their Life Histories, Breeding Habits, and Eggs. By Sir Geoffrey Archer and Eva M. Godman. Vol. 3: Pp. lxviii + 627–1042 + plates 21–27. Vol. 4: Pp. vii + 1043–1570 + plates 28–34. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 189*s*. net the set of two volumes.

THE first two volumes appeared in 1937 and are now out of print; the second half of the work will therefore appeal mainly to the veterans and librarians who are enabled to complete their sets. The volumes are very handsomely produced, although orthographical errors are rather numerous. Twelve of the colour plates are from originals made many years ago by Archibald Thorburn and are of interest in being apparently the only examples of that well-known artist's work in which he depicted exotic birds. Sir Geoffrey Archer was governor of what was then British Somaliland from 1912 until 1922, and later held similar office in Uganda and the Sudan; his field observations, together with his collection of some 3,500 skins and more than 1,000 clutches of eggs, form the basis of the work as a whole. To Miss Godman, bearer of a famous name in ornithology, has fallen the museum side of the study and the task of putting the material into the shape of a systematic text-book.

The authors have naturally been embarrassed by the changes in taxonomic thought between their two dates of publication; this has to be borne in mind in placing the book in the general pattern of present-day literature on the birds of Africa. They also appear to have been handicapped by a plan of treatment, set in the earlier days, that involves giving much space to extralimital information readily available elsewhere; thus there are descriptions of the breeding habits in Europe of species known only as migrants in Somaliland. The main value of the book lies in the first-hand accounts of habits, as observed in an ornithologically very interesting area in which relatively few others have had the opportunity of doing extensive field work. This is true alike of species that are well known in Europe in very different circumstances, and of those that are native to the Horn of Africa.

LANDSBOROUGH THOMSON

SCIENCE, TECHNOLOGY AND CIVILIZATION*

By PROF. R. V. JONES, C.B., C.B.E.

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THE rise of science and technology is a unique element of our civilization. In earlier times, technology played a relatively small part, and science almost none; and it is worth briefly looking at the two ancient civilizations, those of Greece and China, in which science might have been most expected to flourish.

In Greece, the early philosophers valued the sobering contact of everyday life; and they regarded manual skill as a virtue. The later philosophers developed the conceit that they could think out the whole structure of the natural world from a few axioms; and recourse to experiment was in their view undignified and a sign of intellectual inferiority. This error, perpetuated in our classical education, crippled Greek science.

In techniques, China was far above Greece. Chinese inventions included printing, paper, porcelain, gunpowder, the rocket and the compass; and yet science failed to develop. The explanation may again lie in the surpassing value that the Chinese set on the mind. Scholars were recognized as the top class of the Chinese hierarchy, followed in order by farmers, artisans and traders. The Chinese State was run by civil servants recruited, as in Britain to-day, from the brightest scholars. These could ably govern any situation that they could imagine; but their imagination was never fired by the new experience gained from experiment. The traders or the soldiers might have seen the value of a scientific approach to technology but, if they did, they were not heeded by the scholars. Thus, the two ancient civilizations in which science might have been most expected to flower, because of the value they placed on pure thought, became instead—for this very reason—academic mausoleums.

The heritage of Greece, so prized in Western Europe, almost stifled the development of science here. It would be valuable, incidentally, to know the cause of the emancipation of scientific thought, for in it may lie Europe's claim to a fair share of the world's natural resources. These resources are now being taken over by men who by accident live over them at the present day, and who did nothing either to discover their uses or the ways of winning them. This raises a complex problem of many aspects, which may ultimately be a subject of world argument; but, whatever the reason, the scientific and technical revolution did start in Europe, and in this respect the rest of the world is in its debt.

While the cause is not entirely clear, we can trace one or two steps in the emancipation of science from the arid methods of the medieval school-men, who had said: "If a physical theory is inconsistent with received metaphysical teaching, it cannot be admitted, because metaphysics is the supreme science, not physics"; no progress could be made while such doctrines prevailed. The philosophical break was made by William of Ockham, and the scientific break completed by Gilbert and Galileo.

The philosopher of the scientific revolution was Francis Bacon, who, about 1600, pointed out with comprehensive clarity the advance in knowledge to be gained by adopting scientific method. He valued this knowledge both for its own sake, and for the benefits that would come from its application. Recently we have heard of the "euphoria of gadgets", but Bacon made the valid point that without a "gadget"—the mariner's compass—Columbus might not have discovered America. Macaulay summarized Bacon's contribution: "It was not by furnishing philosophers with rules for performing the inductive process well, but by furnishing them with a motive for performing it well, that he conferred so vast a benefit on society".

Bacon's brilliant advice went almost unheeded for two centuries. In the meantime, science and technology advanced largely through mutual interaction, and thanks solely to a few individuals. Von Guericke's pumps led to Boyle's study of gases. Charles, famous for his gas law, had a vital practical interest in gases—he was in 1783 one of the crew of two on the first hydrogen balloon flight. Gay Lussac had a similar interest—for many years he held the altitude record with a balloon ascent to 23,000 ft. To these men a knowledge of gas behaviour was a technological necessity and a scientific challenge. In this stream we can trace the development of the kinetic theory by Joule—a brewer—its refinement by Maxwell and Boltzmann, and its application back to technology by Langmuir in the high-vacuum pump.

This interaction of science and technology made Britain a great power even after its politicians had lost America. The man who contributed particularly to its recovery was James Watt, at one time instrument maker to the University of Glasgow. Watt there formed, incidentally, a friendship with Joseph Black, afterwards professor of chemistry at Edinburgh, who originated the concepts of specific and latent heats, as a result of being asked by the whisky distillers why they needed much more coal to evaporate the contents of a still than they did to bring these contents to the boil. Watt's engines consummated the Industrial Revolution; and this Revolution, more than anything else, revived Britain after the loss of America.

With no support from the classically educated Government, and in spite of its mistakes, the engineers and scientists built up Britain's industrial pre-eminence to the peak of the 1851 Exhibition, where British firms won nearly all the premier awards. There were even signs that technological education might be taken seriously, although Britain was already lagging behind the Continent. Anderson, professor of natural philosophy in the University of Glasgow, founded in 1798 what is now the Royal

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* Substance of the Brunel Lecture delivered at the Brunel College of Technology on February 14.

College of Science and Technology. The London Mechanics' Institute was founded in 1823 by Birkbeck, who had come to London from Anderson's institute in Glasgow, and by 1850 there were more than six hundred mechanics' institutes in England and Wales with more than a hundred thousand members. Spencer and Ruskin were pressing the merits of science in education; but prospects for science in the schools were dimmed in 1859 by the scandalized reaction of clerical headmasters to Darwin's *Origin of Species*. Moreover, the scientists could not agree among themselves about which sciences should be taught in the schools, and their opponents exploited their differences.

Prince Albert wanted to start a technical university, and he arranged to buy a site in South Kensington with proceeds from the 1851 Exhibition. Unfortunately, with his death in 1861 the prospect for a technical university receded: and there was little drive for science and technology in the existing universities, where Cardinal Newman's "Idea of a University" of 1873 perpetuated the régime so profoundly criticized by Bacon.

At the Paris Exhibition of 1867, however, British complacency—both academic and managerial—was shocked. Lyon Playfair, professor of chemistry at Edinburgh, who had been a judge at the 1851 Exhibition and who was again a judge in Paris, wrote in *The Times* of May 15, 1867:

"With very few exceptions, a singular accordance of opinion prevailed that our country had shown little inventiveness and made little progress in the peaceful arts of industry since 1862. Deficient representation in some of the industries might have accounted for this judgment against us, but when we find that out of 90 classes there are scarcely a dozen in which pre-eminence is unhesitatingly awarded to us, this plea must be abandoned. . . . So far as I could gather them by conversation, the one cause upon which there was most unanimity of conviction is that France, Prussia, Austria, Belgium, and Switzerland possess good systems of industrial education for the masters and managers of factories and workshops, and that England possesses none. A second cause was also generally, though not so universally, admitted, that we had suffered from the want of cordiality between the employers of labour and workmen, engendered by the numerous strikes, and more particularly by that rule of many Trades Unions, that men shall work upon an average ability, without giving free scope to the skill and ability which they may individually possess."

Playfair had pointed out the dangers of British complacency as early as 1852, and over the next forty years he attacked especially the sterility of the classical basis of education, saying, for example:

"Our Latin and Greek education still goes on, though, for its practical uses, it is as much a survival as the two buttons behind our coats. . . . New professions are arising around, but for these our old Universities make no provisions; and old professions have completely changed their aspects, yet the schools and colleges remain as of old. Perhaps the most robust men of our time have been our engineers and mechanicians, for they have given more impulse to civilisation than any other class of men. But I look in vain for a single representative man among our Telfords, Watts, Stephensons, Arkwrights, Wedgwoods, whose intellect was nurtured on ancient classical learning, or who could find anything in school life to aid the development of their genius."

Incidentally, in the same context (*Universities in their Relation to Professional Education*, 1873), there occurs:

"You must not judge of other Universities by Oxford and Cambridge for they are exceptional. The old English Universities have not the same function as the Scotch and Irish Universities. The former teach men how to spend a thousand a year, while the latter aim at showing men how to make a thousand a year."

Academics have tended to be patronizing towards soldiers, but there was one officer who saw far more clearly than did the common rooms of Oxford and Cambridge and the lobbies of the Houses of Parliament what was needed. He was Colonel Alexander Strange of the Royal Engineers, who in a lecture to the Royal United Services Institution in 1871 said:

"The duty of the government with respect to science is one of the questions of the day. No question of equal importance has, perhaps, been more carelessly considered and more heedlessly postponed than this. And, now that a hearing has been obtained for it, neither the governing class nor the masses are qualified to discuss it intelligently; . . . But there is one part so important that I feel called on to name it—I mean the appointment of a Minister of Science. He need not necessarily be exclusively devoted to science; he might perhaps, with advantage, have charge of education and fine arts also; but someone in Parliament, directly representing the scientific branches of the national services, has become absolutely indispensable. Another urgent want which, as its scientific character is not purely physical, will probably not be dealt with by the Royal Commission on Science, is that of a high war council—a council of naval and military officers of the greatest professional attainments and distinction, constituted for the purpose of advising the government on the highest problems of strategical science. At present we have not a vestige of anything of the kind, and are consequently, as a military nation, almost destitute of the basis of the military art.

"When we have all scientific national institutions under one Minister of State, advised by a permanent independent, and highly qualified consultative body; when we have a similar body to advise the Ministers of War and Marine in strategical science, then the fact that, in accordance with our marvellous constitution, these ministers must almost necessarily be men without pretension to a knowledge of the affairs which they administer need cause us no alarm. When these combinations have been, as they assuredly will be, sooner or later, effected, the wealth, resources, and intelligence of the nation, having due scope, will render us unapproachable in the arts of peace, and unconquerable in war, but not till then."

What Strange said about the Government is still true. What he advocated regarding the Minister of Science and the two committees (anticipating the Advisory Council on Scientific Policy and the Defence Research Policy Committee) has only recently come to pass. Indeed, if we distinguish between a Minister of Science and a Minister for Science, we have not yet gone as far as he wished. In intense admiration for his wide insight, we can only wonder that it was all but lost on so many of his unenlightened countrymen.

Now, what came of all this ? Playfair and Strange carried the British Association for the Advancement of Science and the Society of Arts (the record of which in the matter was admirable) with them, forcing a Royal Commission on Scientific Instruction in 1871 and another on Technical Instruction in 1881. The City and Guilds College was inaugurated in 1878. The Central Technical College, now Imperial College, was opened in 1884. Many technical colleges were started, along with several university colleges having some technological bias.

The movement thus had some substantial results; yet they fell far short of what the originators intended, and what was really required. Manufacturers failed to see that the best hope of improving their products and widening their range lay in educating suitable technologists. Headmasters influenced their pupils away not only from technology but also from science, maintaining that "the proper study of mankind is man", and failing to appreciate the force of a later couplet in the same Essay, "Slave to no sect, who takes no private road, But looks through Nature up to Nature's God". Thus there was no supply of students coming up from the schools for higher education in science and technology; and, indeed, if students had been forthcoming in any numbers, they would have found few employers at the end of their courses.

In such a situation, where no section of the community sees the need to act, there exists the unique opportunity for prescient government. But unfortunately, as Playfair and Strange—and after them many others such as Tizard and Lindemann—pointed out, the members of the Government were themselves products of classical education, and were both blind and deaf to what might be achieved, and what ought to be done. So, even after Playfair went into politics and became chairman of the House of Commons and deputy speaker, the lead from the Government never came.

In what respects is the situation of Britain different to-day ? It has lost its easy markets and its cheap supplies of raw materials. Moreover, it has been overtaken notably by the United States and by the U.S.S.R., where Lenin was fired with an appreciation of what technology could do. "Communism", he said, "is a Soviet government plus electrification"; and the huge electrical and educational programmes in the U.S.S.R. were a direct result.

Finally, British survival in 1940 had depended on an exhilarating partnership between the Royal Air Force and some scientists and engineers, in a way that no post-war Government could ignore. This was followed by the challenge of nuclear energy. Plans were made to expand the numbers of graduates in science and technology. Even then the professional humanists succeeded in maintaining their hold in the universities in 1945 by arguing that the non-science departments should be expanded to the same extent, not for such a fundamental reason as that more non-science graduates would be required, but for the sanctimony of "preserving the balance of the Universities".

At last, there are signs that this prejudice is beginning to disappear; but it is still strong. Until recently, Latin lingered as compulsory for entrance to Oxford; it was this together with Greek that kept Sir Winston Churchill out ("I could not contemplate toiling at Greek irregular verbs after having commanded British regular troops"), and after his example can it be maintained that a knowledge of Latin is essential to the writing of good English ? Nevertheless, there is still a compulsory examination in Latin for entrance to the public schools in Great Britain, and optional papers in Greek and in Latin verse; but there is no paper on even elementary science.

The only fundamental differences from the position of 1880 are the facts that the powers bestowed by science and technology have been forcefully demonstrated, and that the economic position of Britain is much weaker. But for these facts, the advocates of science in education might have been as much ignored in the past decade as were Playfair and Strange a century ago.

Assuming that science now wins its proper place in education, it must not in the process suffocate what is valuable in the humane studies in the way that the humanists tried to strangle science and technology. By themselves, science and technology are not enough, and all men need to pay attention to the humanities. All that men of science ask is that never again should the humanists be allowed to warp the education of the whole man by denying him some of his most exciting intellectual activities and from realizing the wider range of his ability by co-ordinating his physical senses with his mind: and if at some future stage some other branch of learning should become of more urgency than a particular science or a particular technology, or than science and technology altogether, scientists and technologists should not seek to cramp it by their vested interests as the so-called humanists for so long did with science.

If science and technology now jointly receive due weight in education and civilization, men of science and technologists should take care not to perpetuate within their own field the prejudice against which for so long they have fought the external battle. It could happen that the pure scientist, and especially the theorist, might assume the mantle of the classical scholar—he is the one whose activity most closely approaches that of the pure intellect. The greatest theorists have been keenly appreciative of the details of practical experimentation. But there are less good theorists who may tend to look down on contact with experiment, and less good experimenters who may rate a moderate experiment above a superb piece of technology. Some British physicists have frowned on the larger machines of America—as though string and sealing wax were just admissible to assist the pure mind, but that resort to a large machine indicated intellectual failure. Happily, this point of view is disappearing: the elegance of experiment lies in achieving results with economy, and this sometimes involves using large machines because small ones are useless. The use of large machines or of elaborate techniques is, of course, not always justified. Sometimes it contributes merely to a sense of self-importance, and it is salutary to remember Rutherford's "We haven't the money, so we've got to think".

The debt of technology is so obvious that it requires no elaboration; but, apart from the deliberate stimulus to technology from pure science, there are many accidental benefits that are impossible to foresee. Röntgen's researches, for example, on electric discharges in gases would not have received support from a medically minded benefactor in 1895, and yet the X-rays that Röntgen discovered were within a few months profoundly influencing medical technology.

But pure science has its own debt to technology; the example of the kinetic theory has already been mentioned, and there are many others. Louis de Broglie described this debt:

"Every important step forward made by Astronomy, Physics, Chemistry or Biology had one essential condition—the previous existence or invention of certain apparatus; and as the sciences sought to extend their advance, so it became necessary for instrumental technique to develop and to expand in its delicate adjustments. . . . Left to itself, theoretical Science would always tend to rest on its laurels; but experiment, by becoming continually more exact and delicate, has shown us more clearly each day that 'there are more things in heaven and earth than are dreamt of in your philosophy'. By pointing to the infinite complexity of Reality, experiment has broken the circle within which speculative thought might easily risk confining itself, if left to its own devices. And since experiments depend on the perfection of experimental technique, the machine to-day is in a sense one of the essentials of intellectual process" (address to the Lycée Pasteur, July 13, 1932).

Examples of de Broglie's thesis continue to occur. The discoveries of cosmic rays and of radio emissions from stars originated in technology.

It is impossible to draw a clear distinction between pure science and technology; they not only interact, but they also overlap. Playfair saw a happy analogy in an anecdote concerning Faraday:

"Faraday's first experiment, made as a newsboy while waiting outside for a paper, was to put his head through a railing and then speculate on which side he was! Here we see the philosopher acknowledging the head as of preponderating importance, though it was incapable of being disassociated from the body. He got a practical proof of the fact, that both ought to be in good connexion; for, while he speculated, the door opened and he received a severe wrench. Faraday never afterwards disassociated his manipulative skill, great as it was, from his wise head and warm heart" (*On Primary and Technical Education*, 1870).

And in this connexion, it may be worth quoting some words of Lord Cherwell:

"The word 'technician' is used to mean a man who has been trained to carry out established techniques which may be complex, and require a high degree of skill, or to work in a prescribed field under instruction; whereas by a 'technologist' is meant a man who, as a result of broadly based studies and wide practical experience, has acquired a real understanding of scientific principles and can apply them to the development of industrial processes in diverse fields. It may sometimes be difficult to draw a sharp line of demarcation between the technician on one hand and the scientist or the technologist on the other, and nobody would like to see any barrier set up that might discourage the scientifically minded technician from becoming a scientist or technologist" (*Messel Lecture*, 1954).

Scientists and technologists and technicians should see themselves as bound together in a common effort to increase man's understanding of the natural world, and to further his constructive achievements.

Although I have recalled the sorry record of Britain in technological education, it is important to distinguish between the defects of the classical educationists and the merits of the humanities and the arts. In our enthusiasm for science and technology, let us not forget to develop our other interests. If there is a gap between the 'two cultures' it must be bridged; and it can best be bridged by men of science who are versed also in the humanities. The humanist who has not studied science in his youth has little hope of bridging the gap.

If this discussion has tended to take a national point of view, it is largely because most of us have to act inside the framework of the nation: and we must remember that despite the technological superiority of Germany, to which Playfair and Strange rightly directed so much attention, and the achievements of modern U.S.S.R., the British record in treatment of the individual is much better, thanks perhaps in some small part to a lack of obsession with science and technology.

Finally, neither science nor technology knows national frontiers; and in developing them we are sharing in a most exciting field of human endeavour—it is in the common basis of experience and of mutual respect between the scientists and technologists of one nation and of another that there lies one of the best hopes of international understanding.

TEST-TUBE FERTILIZATION IN A FLOWERING PLANT

By KUSUM KANTA, N. S. RANGA SWAMY and PROF. P. MAHESHWARI

Department of Botany, University of Delhi

IN animals artificial fertilization *in vitro* is easily accomplished with fishes and amphibians and recently some successful attempts have even been made with mammals including human beings[1]. However, in mammals the development of the embryo proceeds only up to a few-celled stage in culture. To our knowledge there has so far been no report of fertilization *in vitro* in any flowering plant.

With this aim in view a beginning was made in this laboratory by injecting pollen suspensions into ovaries. The technique involves: (*a*) determination of the time of anthesis, shedding of pollen, and pollination in Nature; (*b*) collection of pollen under reasonably aseptic conditions; and (*c*) insertion of pollen grains into the ovary. The experiments so far made on five species of poppies (*Argemone mexicana*, *A. ochroleuca*, *Eschscholtzia californica*, *Papaver rhoeas* and *P. somniferum*) have already been described[2–4]; but a brief summary may be given here.

In most cases a suspension of pollen was prepared in a solution of boric acid (100–200 p.p.m.) in sterile double-distilled water. After wiping the surface of the ovary with ethanol, two punctures were made on it (Fig. 1). With an all-glass 'insulin' syringe the pollen suspension was injected through one of the apertures until the ovarian cavity was full. The other hole served as an outlet for the air within. After the operation, the holes were sealed with petroleum jelly. Where suspensions proved less favourable, pollen grains were inserted direct into the ovary through a vertical incision made laterally.

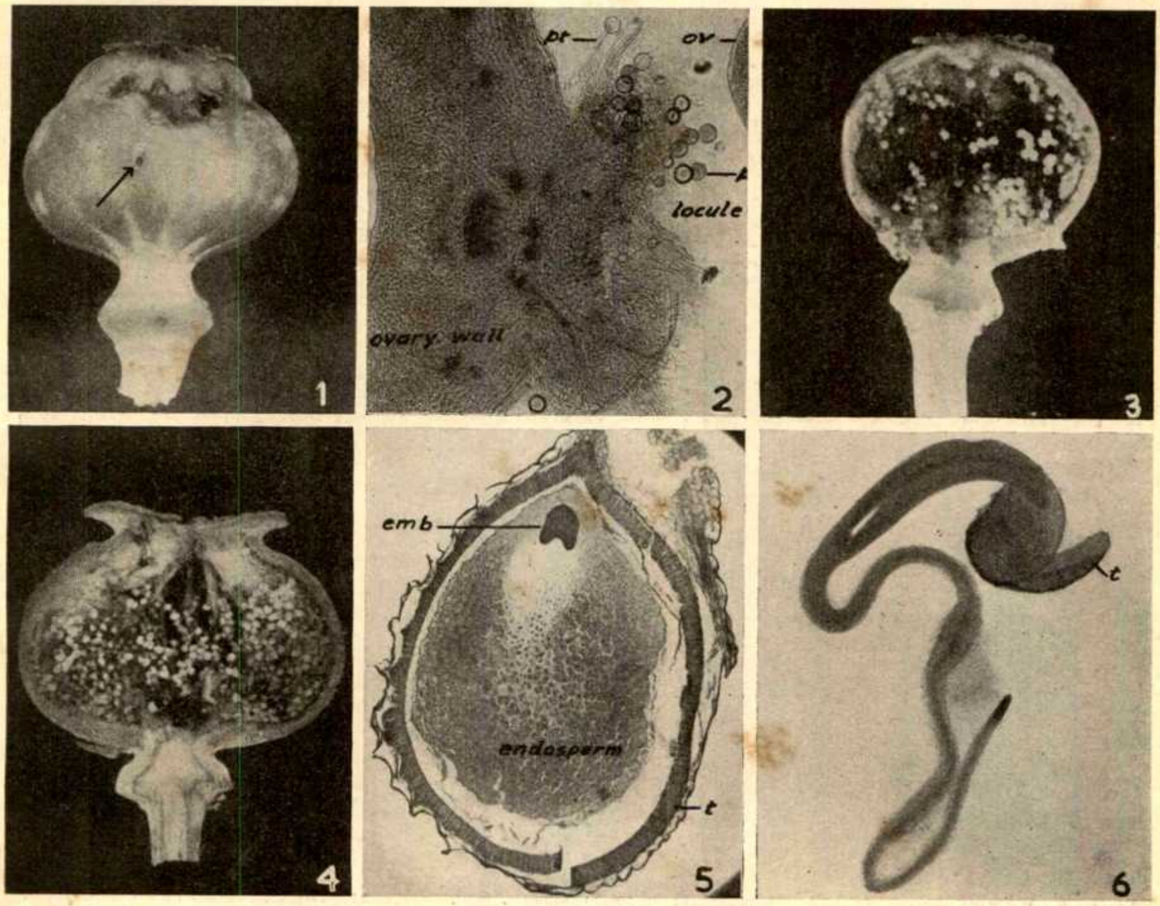
Except for minor variations in the growth period all the five species responded similarly and normal fruits were produced (Figs. 1, 3, 4). The pollen grains germinated in the ovarian cavity (Fig. 2), and both endosperm and embryo were formed normally in the developing seeds (Fig. 5). The seeds were fully viable. On germination (Fig. 6) they produced plants which flowered and fruited just like those obtained from seeds in Nature.

It was our success with intra-ovarian pollination and fertilization which led us to wonder whether a simultaneous culturing of excised ovules (taken before pollination) and pollen grains, on the same nutrient medium, could result in fertilization and eventually the formation of mature seeds *in vitro*. Encouraging results have been obtained with the opium poppy, *Papaver somniferum* L.

Under Delhi conditions the anthers of the opium poppy start dehiscing 12–24 hr. before anthesis. Dehiscence of all the anthers of a flower is complete by 10 a.m. on the day of anthesis in the peak period (January–February) or by 8.30 a.m. in the wane period (March). Pollination is effected almost immediately, and the germination of pollen grains begins within 15 min. afterwards. In about 9 hr. the pollen tubes reach the ovary and 24 hr. after anthesis several of them can be seen entering the ovules.

In devising a suitable medium for culturing the ovules we had the benefit of the observations of Maheshwari and Lal of this laboratory, who had already successfully cultured the fertilized ovules of the opium poppy containing either a zygote or a 2-celled pro-embryo (excised 6 days after pollination) and reared them to maturity on Nitsch's agar nutrient medium[5]. The same medium was used by us for the culture of unpollinated ovules and also pollen grains.

Since the anthers begin dehiscing even in the bud, flower-buds were bagged 24 hr. before anthesis. The following day the buds were brought to the sterile inoculation chamber, and the dehiscing anthers collected in a Petri dish and exposed to ultra-violet light for about 0·5 hr. just before inoculation.



Figs. 1–6. Intra-ovarian pollination. (*emb*, embryo; *ov*, ovule; *p*, pollen; *pt*, pollen tube; *t*, testa)

Fig. 1. *Papaver somniferum*. Capsule 12 days after injection of pollen suspension. Arrow shows one of the holes that had been made to inject the suspension. (× *c.* 1·35)

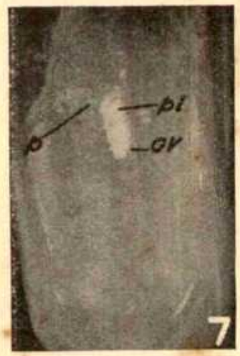
Fig. 2. *Eschscholtzia californica*. Transverse section of a portion of ovary 3 days after injection; note germination of pollen grains in locule. (× *c.* 60)

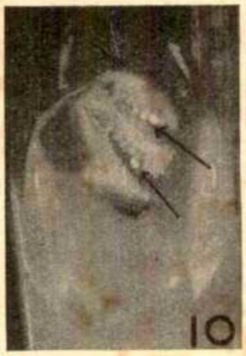
Fig. 3. *Papaver somniferum*. Vertical half of 24-day-old capsule; injection of pollen made on the day of anthesis. (× *c.* 0·9)

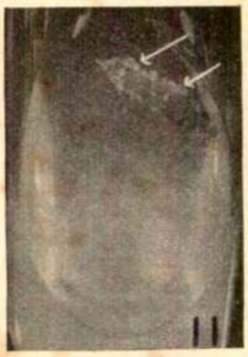
Fig. 4. *P. somniferum*. Vertical half of 28-day-old capsule; injection of pollen made 24 hr. after anthesis. (× *c.* 1·35)

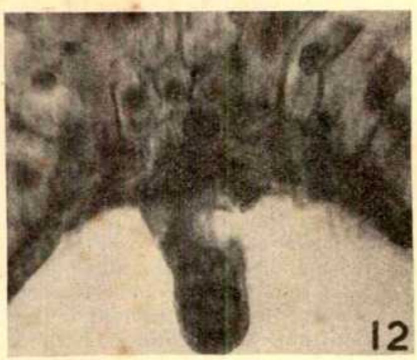
Fig. 5. *Argemone ochroleuca*. Longitudinal section of seed (from capsule 15 days after insertion of pollen into the ovary) showing normal endosperm and embryo. (× *c.* 33)

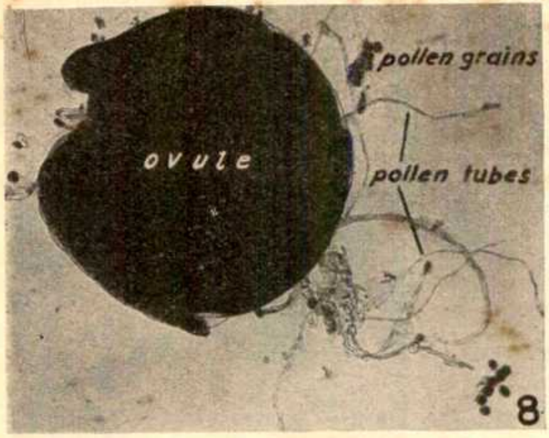
Fig. 6. *Papaver somniferum*. 3-day-old seedling raised from a seed obtained by intra-ovarian pollination. The testa is seen adhering to the tip of the cotyledons. (× *c.* 11)

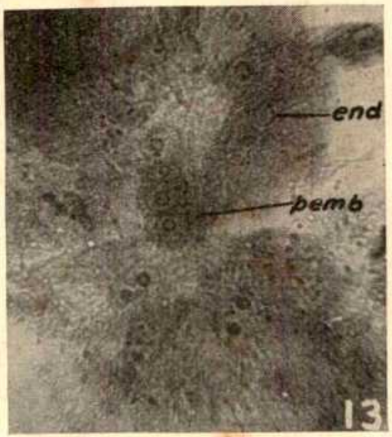


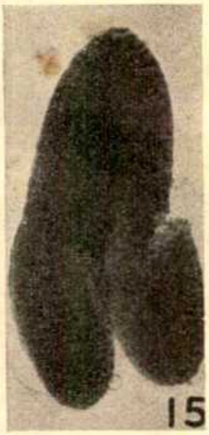


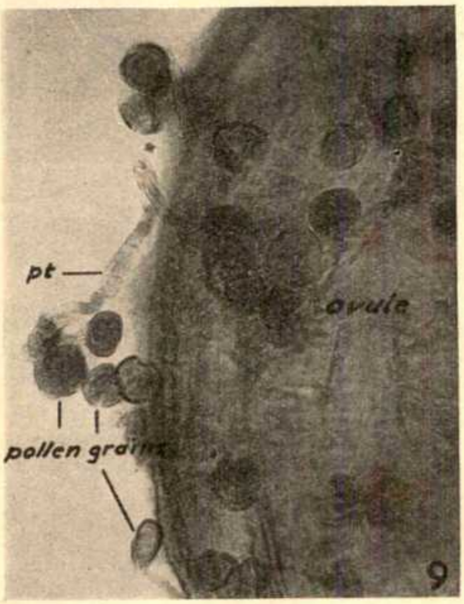


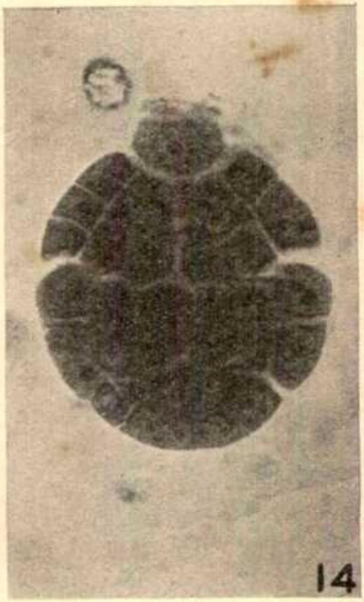


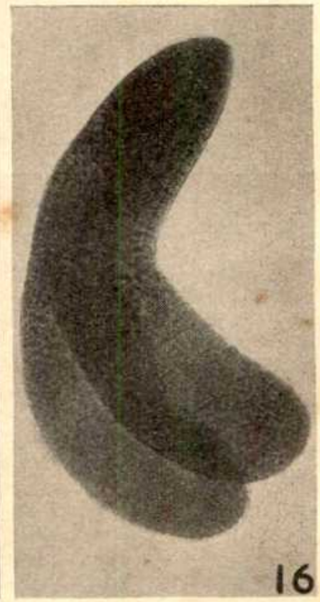












Figs. 7–16. *In vitro* fertilization and maturation of seeds in *Papaver somniferum*. (*end*, endosperm; *ov*, ovule; *p*, pollen; *pemb*, pro-embryo; *pl*, placenta; *pt*, pollen tube)

Fig. 7. Portion of placenta with ovules and pollen inoculated on Nitsch's agar medium. (× *c*. 1·2)

Fig. 8. Whole mount of ovule with pollen tubes from 3-day-old culture. (× *c*. 55)

Fig. 9. Portion of a similar ovule enlarged. (× *c*. 225)

Figs. 10, 11. Seven-day-old cultures. Arrows show the young seeds. Fig. 10 is a culture of pieces of 3 adjacent placentæ. (× *c*. 1·2)

Fig. 12. Longitudinal section of the micropylar portion of an ovule 3 days after inoculation, showing 2-celled pro-embryo. (× *c*. 600)

Fig. 13. Dissection of 5-day-old seed showing free nuclear endosperm and 4-celled pro-embryo. (× *c*. 220)

Fig. 14. Globular embryo dissected from 9-day-old seed. (× *c*. 515)

Figs. 15, 16. Older embryos from 15 and 22-day-old cultures. (Fig. 15, × *c*. 80; Fig. 16, × *c*. 70)

To obtain unpollinated ovules, the flower-buds were emasculated and bagged considerably in advance of shedding of pollen. The ovaries were gathered on the day of anthesis and dipped in ethanol. After allowing the superfluous alcohol to evaporate, they were flamed and then cut open by a sterile scalpel. The ovules were separated along with portions of the placentæ (1–1·5 cm. long × 0·5–0·75 cm. broad) and sown on the agar nutrient medium in test-tubes. With a sterile needle the pollen was scooped out of the anthers and dusted over the implanted ovules as well as the surface of the agar medium (Fig. 7). A few cultures were also made in Petri dishes to facilitate a direct observation under the stereoscopic microscope.

Nitsch's medium proved quite favourable for the germination of pollen as well as for the growth of pollen tubes. The pollen grains dusted over the ovules started germinating within 15 min. after inoculation while those lodged on the surface of the agar medium required 1 hr. or more. The pollen tubes grew rapidly and within 2 hr. the surface of the ovules was covered by an abundant growth of pollen tubes (Figs. 8, 9). In cultures containing bits of 2 or 3 adjacent placentæ (Fig. 10), the pollen tubes could be seen growing from one placenta to the other. A similar response had also been observed in our experiments on intra-ovarian pollination. Though the pollen grains germinated profusely, only a few of the ovules were fertilized *in vitro*. Presumably the ovules were still rather young and not quite receptive to the pollen tubes. Likewise, when intra-ovarian injections of pollen grains were made on the day of anthesis the seed set was lower than when pollen was injected 24 hr. later (cf. Figs. 3 and 4).

In further experiments the ovules were, therefore, excised 24 hr. after anthesis. A much larger number of ovules now remained healthy and grew in size, suggesting that they had been fertilized (Fig. 11; cf. with Fig. 10). That fertilization did occur *in vitro* was confirmed by dissections as well as microtome sections. During the first 4 days of culture the fertilized ovules enlarged, became turgid and opaque. In another 2 or 3 days they turned mealy white (Fig. 11), a feature natural to poppy ovules developing into normal seeds (see also Figs. 3 and 4). The other ovules shrank and finally collapsed. Sections of normally developing seeds from a 3-day-old culture showed a 2-celled pro-embryo (Fig. 12). Dissections of seeds from a 5-day-old culture revealed a healthy free nuclear endosperm and a 4-celled pro-embryo (Fig. 13), of a 9-day-old seed a globular pro-embryo (Fig. 14), of a 15-day-old seed a differentiated embryo (Fig. 15), and of a 22-day-old seed a fully formed normal dicotyledonous embryo (Fig. 16). In early stages, the rate of growth of the endosperm and embryo in test-tube seeds corresponded with that in the field controls[5] as well as with that in seeds obtained in intra-ovarian pollinations. Later, the growth in artificially fertilized ovules was quicker and better and even surpassed that in Nature[5].

Briefly then, 'pollination', germination of pollen, double fertilization, and the development of endosperm, embryo and seed all go through normally in test-tube cultures of pollen and ovules of the opium poppy. These results prompt us to conclude that with further refinements the technique of test-tube fertilization will prove useful to plant breeders, especially when the two parents are incompatible and the zone of incompatibility lies in the stigma, style or ovary.

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ZOOGEOGRAPHY OF EASTERN AFRICA: AN OUTLINE BASED ON ANURAN DISTRIBUTION

By Dr. J. C. POYNTON

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THAT the existence of an almost fully developed 'East African' association in northern Zululand should have been generally overlooked until very recently[1] is an indication of the rudimentary condition of zoogeographical studies in Africa. This condition is regrettable, not only because such work has its own inherent interest, but also because it appears to be a prerequisite for evolutionary studies on the nature and origin of the faunal complexes in Africa. Furthermore, an appreciation of the present general patterning of distribution and its causation gives a key to visualizing the overall patterning of distribution under different climatic régimes in the recent past, an insight which should be of value to studies in Quaternary palæontology.

A zoogeographical outline of the portion of Africa south of the Sahara and east of 30° E. is presented here in an attempt to give an overall picture of distribution in this area. This outline is based entirely on the distribution of the Anura, and its primary object is to invite comparisons from workers on the distribution of other groups. Despite the aquatic phase in the life-cycle of most of the Anura, this group shows an essentially terrestrial type of distribution, and as zoogeographical indicators, the Anura have some particular merits[2], not the least of which is the relatively insignificant extent to which human settlement and cultivation have affected their distribution. A zoogeographical account of an area based on the distribution of a single group obviously has its limitations, some of which are shown at the end of this article; but in the present state of knowledge, such inadequacies are inevitable.

Until recently, the only large-scale distributional survey of the Anura in eastern Africa was that of Loveridge[3]. On the basis of the herpetofauna, Loveridge divided East Africa into a number of "ecological life zones". The lists of the forms occurring in the different altitudinal zones are very interesting from a zoogeographical point of view. Species of *Hyperolius* were left out of account, but of the 24 anurans listed as occurring below 1,000 ft., all but

4 (17 per cent) are widely distributed; that is to say, have a range of more than 500 miles in at least one direction. On the other hand, of the 25 forms listed in the highest zone (5,000–12,000 ft.), 52 per cent are not widespread.

The East African lowlands appear to be almost entirely populated by forms that are widely distributed at least along the coastal belt. These forms also extend westwards to a greater or lesser extent up into the highlands, where they form a subtraction margin of the lowlands fauna. This subtraction margin becomes admixed with a variety of more localized upland forms, thereby giving the fauna of the highlands an essentially heterogeneous composition.

It would be an oversimplification to consider the fauna of the lowlands to be completely homogeneous: some altitudinal zonation is shown by a few very widespread forms (notably *Bufo carens* Smith, *Rana angolensis* Bocage). These forms do not occur below 1,000 ft. in the region of the equator, but they do occur below or near this altitude farther south within the tropical belt, and can thus be included in the tropical lowlands fauna. The composition of the lowlands fauna is sufficiently homogeneous to stand in marked contrast to the essentially heterogeneous nature of the fauna occurring in the higher areas. In these higher areas an impoverished fauna extending upwards from the warmer and climatically more equable lowlands becomes progressively replaced by a fauna composed of more restricted forms, which are evidently more adapted to local conditions, or may be unable to maintain themselves among the concentration of dominant forms in the lowlands.

Now this situation is exactly paralleled by the changes which occur to the south of the main tropical lowlands concentration on the east coast of South Africa[1]. North of Lake St. Lucia (28° S.), the amphibian fauna is composed almost entirely of widespread forms, since no less than 86 per cent of the total number of forms occur also in Tanganyika and Kenya. However, only 43 per cent occur to the south of Lake St. Lucia, and those forms which do pass southwards become joined by a 'South African' fauna composed of more restricted forms. South of Lake St. Lucia, therefore, the fauna changes from a homogeneous tropical assemblage to a heterogeneous assemblage.

The amphibian fauna of Nyasaland, lying between southern and East Africa, fits into this picture very well. This fauna has recently been investigated by Loveridge[4,5], and by me. A fully developed lowlands fauna enters the Zambesi–Shire basin, but subtraction commences as the land rises above about 1,200 ft. Here, characteristic lowland forms such as *Hylambates maculatus* Duméril, *Hyperolius argus* Peters and *H. pusillus* (Cope) disappear, and localized endemics make their appearance.

It has been pointed out[6] that in southern Africa the amphibian population is polarized into two main faunal groups, one tropical and the other temperate, and that between these there is an extensive area where the opposing subtraction margins overlap, and where an essentially intermediate or transitional fauna is developed. This tropical-transitional-temperate zonation in the south appears to be but a special case of the distribution of amphibians in eastern Africa as a whole. Because of there being a well-developed temperate fauna in South Africa, this zonation is here very clearly shown, particularly in Natal, where the highlands above 4,000 ft. carry a rich temperate fauna, while the fauna of the north-eastern coastal plain is tropical. To the north, however, this temperate fauna is extremely attenuated. In fact, to the north of the Limpopo River a typical relic pattern is shown, suggesting a former continuous distribution of temperate forms along the length of the highlands from the Cape to Kenya. For the Kenya highlands population of *Cacosternum boettgeri* (Boulenger) and the Nyasaland uplands population *Xenopus l. laevis* (Daudin) are not distinguishable from the South African populations of these forms, and the *Rana fasciata* of Nyasaland barely differs from the *R. fasciata* of eastern South Africa. *Xenopus l. laevis* and *Cacosternum boettgeri* still appear to have a tenuous connexion across the Limpopo; but the *Rana fasciata* and *R. grayi* Smith on the highlands of Southern Rhodesia are completely isolated from the South African populations of these forms. All these various separated populations must have been united during one or more periods of general cooling and/or greater rainfall, and the last period has not occurred sufficiently far back in time to have allowed much differentiation to have occurred between the isolated populations.

While this temperate fauna is now scarcely in evidence in most of eastern Africa, the localized faunal centres of endemism in the zone of tropical and temperate subtraction are a very prominent feature of the whole length of this zone from north to south. In South Africa and Southern Rhodesia there is a complex of these localized faunas[6]. In southern Nyasaland another pocket of endemism is found, which is not very well developed, but is distinct from a well-developed local centre in the northern montane fringe of Lake Nyasa[4,7]. More localized forms are scattered on the East African uplands, forming centres in the area from Lake Tanganyika to Lake Albert, and the Kenya mountains. A localized centre is also present on the Ethiopian highlands[8]. The isolated Uluguru and Usambara Mountains are also local centres of endemism, but they are more remarkable for the relic genera which they possess[3]. These relics have nothing to do with the southern temperate fauna, but show some relationship with the montane forms of the Cameroons[3]. This relationship appears to be due to the peculiar isolation of these mountains favouring the survival of primitive forms.

The great north-to-south expanse of the temperate fauna which must have existed at times in the past, and the more permanent north-to-south range of tropical forms, stand in contrast to the essentially discontinuous series of centres of endemism lying in the intermediate zone. Certainly there are some forms, such as *Leptopelis bocagii* (Günther), which, although confined to this intermediate zone, are widespread within it; but the general pattern is that of localized faunal centres. The localized faunas of this intermediate zone in southern Africa have been called 'transitional faunas'[6]. Now these faunas are zoogeographically no different from the other localized faunas occurring in the intermediate zone to the north. The more northern centres should therefore also be called transitional for the sake of uniformity, even if the 'transition' in East Africa is not through to a currently well-developed temperate fauna[9]. The present pattern clearly suggests the former existence of a more extensive 'south temperate' fauna in East Africa.

In the western region of the East African transition zone, the influence of the West African fauna is very apparent. However, a treatment of this fauna falls outside the scope of the present article.

The general pattern of anuran distribution in eastern Africa can therefore be visualized as having as its basis a largely homogeneous fauna on the lowlands stretching the whole way from Zululand to Somalia. From this assemblage there sweeps a great subtraction margin, covering the rising country to the west and the less-tropical lowlands to the south, becoming ever thinner as it extends outwards. Localized centres of endemism lie scattered throughout the length of this subtraction margin, from the Natal lowlands northwards along the eastern slopes and the crest of the great inland massif all the way to the eastern horn of Africa. Here arid conditions supervene, and the Ethiopian faunal complex eventually becomes halted by the northern desert. Overlying this pattern of predominantly westward subtraction is another pattern of northward subtraction shown by the temperate fauna of South Africa. This fauna occupies the south-western corner of the Cape Province, and has a well-developed subtraction margin on the eastern uplands of South Africa. At present it continues northwards only as isolated vestiges on the highlands north of the Limpopo River. Within the tropical and temperate subtraction zone occur the transitional faunal centres composed of forms with restricted ranges.

The overall pattern is thus one of a tropical-transitional-temperate zonation. This zonation appears to shift, probably as a whole, in accordance with the prevailing climatic régime. The close correlation between the thermal pattern and amphibian distribution indicates that, at least in the Anura, temperature is the main controlling factor of distribution over most of eastern Africa. The zones recognized here may in fact be considered basically to be 'thermal life zones'. In the north, however, the fauna is clearly being limited by arid conditions. At present the uppermost zone has been eliminated from most of eastern Africa, leaving only a scattering of relics to the north of the Limpopo River. This zonal stratification of the fauna is an altitudinal one in the equatorial region, but in the cooler southern latitudes it dips to lower levels. Thus, characteristically south temperate forms such as *Rana fuscigula* D. and B. (with which more northern forms are often confused) and *Kassina wealii* Boulenger occur at sea-level in the region of 34° S., but do not occur below 4,000 ft. north of 30° S.

During a warmer period than the present, the range of the temperate forms would presumably be even more restricted, although a reserve of south temperate forms is likely to be retained permanently in the southern Cape. This reserve could re-populate the northern uplands during a cooler period. The conjunction between the tropical and the transition zones at present strikes the coast in the region of Lake St. Lucia. It is obvious that the faunal composition of this coastal area must be subject to very considerable changes with changes in overall temperature. This instability must exist on the East African uplands as well, but the faunal pattern of the East African lowlands must remain very stable, not only because of the central position of the fauna, but also because temperature fluctuations should be less marked nearer sea-level than at higher altitudes, as well as being less marked near the equator than outside the tropics.

It is therefore probably in the East African lowlands that the fauna of eastern Africa has its main evolutionary potential. This probability is suggested both by Fischer's conclusion[10] that perennially constant tropical conditions are the most favourable for evolution, and by the principle of centrifugal speciation put forward by Brown[11], who points out that "The more important genetic changes tend to be incorporated in the central populations". With fluctuations in climate, the general shifting of the faunal zones in eastern Africa provides ideal conditions for centrifugal speciation to take place. The area which is now covered by the transition zone must in warmer periods have been populated by a richer tropical assemblage, and in cooler periods, populated by a greater number of temperate forms. Repeated expansion and recession over this intervening 'tidal zone' makes the formation of isolated populations within this zone very probable. These isolated pockets and the main faunal assemblage would in time be expected to diverge genetically, and further divergence in the direction of speciation would be likely on the return of the main fauna[11]. It is therefore understandable that the fauna endemic to the transition zone should be composed largely of restricted forms, whereas the fauna of the tropical lowlands is composed of widespread forms. A great number of species occurs around the tropical lowlands, but the pattern of distribution indicates that the major evolutionary activity takes place in the lowlands, and it is probably here that the major phylogenetic advances in the eastern African fauna have their origin.

The fauna of eastern Africa offers an endless and virtually untapped wealth of material for evolutionary investigations. It is hoped that this zoogeographical sketch will assist in ordering the complex patterning of distribution into a general system, which can be used as a background for investigations of centrifugal speciation, character displacement and the like. The pattern shown by the Anura is not comparable in every way with the distribution of the other terrestrial vertebrate groups. Birds and mammals tend to have more extensive ranges (for example, refs. 2 and 9), probably as a result of their homoiothermy. Consequently, at least in the case of birds, a relatively larger proportion of the transitional fauna is continuous from East Africa to South Africa[9]. However, such differences should not be made to obscure the common pattern that seems to exist (for example, refs. 1 and 2): rather the causes of the differences should be sought, and the common underlying factors thereby seen more clearly. A great amount of work by specialists in different groups is necessary before these factors can be understood. Although zoogeography has been in existence for a relatively long time, zoogeographical work in Africa has barely begun. But until this field has been properly worked, its methods standardized and its data published, an understanding of the evolution of the African fauna will remain an impossibility.

I thank Prof. S. F. Bush, Miss M. Beynon and Mr. B. Stuckenberg for criticism and discussion.

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OBITUARIES

Dr. D. D. Pratt, C.B.E.

All who at different stages of his scientific career knew Dr. D. D. Pratt will learn with regret of his death on May 5. As recently as 1959 he retired from the post of director of the National Chemical Laboratory after 34 years spent in the scientific service of the State.

David Doig Pratt, born at Anstruther in Fife in 1894, was educated at Waid Academy and the University of St. Andrews, which he entered in 1912. However, he joined the Army when war broke out in 1914, received a commission and served with the Highland Light Infantry. Wounded on the Somme, he was mentioned in dispatches and promoted to captain. After the First World War he returned to St. Andrews, graduated M.A., B.Sc. with honours in chemistry in 1921 and was awarded a Carnegie Scholarship. This enabled him to start research under Prof. (now Sir Robert) Robinson. He took his Ph.D., became a Carnegie Fellow and continued his work at the University of Manchester, still in association with Prof. Robinson. Pratt was one of the first of many in Robinson's school who found the naturally occurring flower pigments a rewarding study.

In 1925 Dr. Pratt made the move which was to determine the course of his future career. The Department of Scientific and Industrial Research was on the point of setting up at Teddington in a very small way a laboratory to be known as the Chemical Research Laboratory. The sponsors of this scheme were Sir Richard Threlfall and H. T. (later Sir Henry) Tizard; Prof. G. T. (later Sir Gilbert) Morgan was persuaded to take charge of what was avowedly an experiment. Pratt accepted a post in the unit when its staff numbered less than a dozen and was Morgan's stoutest supporter in the task of building the prestige of the laboratory and establishing its role as a national institution. In 1958 the embryo of 1925 officially became the National Chemical Laboratory with a staff of more than 200. Pratt had been its director for some seven years, and as officer-in-charge or superintendent he had been its effective head at other difficult periods during and after the Second World War when the post of director had been vacant.

The project which Morgan assigned to Pratt was an investigation of coal tars and liquors, very different in character from the synthetic organic chemistry in which he had made his mark, but equally one which gave scope to his ability as a chemist. Backed by Morgan and aided by a small group which he inspired with his own enthusiasm, Pratt set about nothing less than the complete revivification of coal tar research in Britain. Attention was first directed to tars from the low-temperature carbonization of coal. This method of making smokeless solid fuel was then undergoing development, and it was clear that no process could succeed unless the by-product tars could find profitable application. The extension of precise knowledge in regard to the constitution of these new materials must proceed *pari passu* with utilitarian aims. Aware of the very reactive nature of these tars, Pratt systematized the use of extraction by solvents and vacuum distillation in their rational analysis. But this was no more than a beginning.

The Teddington work, new in pattern, caught the attention of influential leaders in the industry and created a new climate of opinion as to what might be achieved by research well directed. When in 1934 the Association of Tar Distillers gave financial support to the Laboratory for work on one of the industry's most troublesome problems—the disastrously rapid corrosion of tar stills caused by certain types of tar—it had taken the first step on what turned out to be a long road. The industry formed its own Research Association in 1948, and in 1950 the ministerial head of the Department of Scientific and Industrial Research (Sir John Anderson), in the presence of its secretary (Sir Ben Lockspeiser), gave the blessing of the Department to the newly established Coal Tar Research Station at Gomersal. Pratt witnessed with amused satisfaction the lighting of a symbolical Bunsen burner by Sir John, although for him the ceremony was valedictory rather than inaugural.

After 1938 Pratt was fully engaged in the administration of the Laboratory's total research effort. His concern now was to maintain an environment in which good scientific work could be done by his colleagues.

One quality which fitted him for the responsibilities he held so long Pratt possessed in full measure. He had an understanding of human nature and was on easy terms with all members of his staff regardless of status or rank. He was shrewd in judging issues in which policy and personalities were mixed with matters purely scientific.

Dr. Pratt shared most of the simple pleasures of the ordinary man. He had an interest in every form of sport; he particularly liked to be among the crowd at a football match. He grew flowers in his garden; regularly sought relaxation at a variety theatre or cinema; and he could always be persuaded to attend a staff social and play cards, billiards or snooker, or join in a dance.

The effort to make the Laboratory the national institution he thought it should become was steadily pursued, and the distinctions bestowed on Dr. Pratt, C.B.E. and an honorary LL.D. of the University of St. Andrews, were recognition of the merit of his service to the Department and his success in securing for the Laboratory the esteem of the scientific world.

E. A. Coulson

Dr. G. I. Patel

Dr. Govindbhai Ishwerbhai Patel, who died on March 10, was born at Savli, District Baroda of the Gujarat State, on June 21, 1921. He was the second son of the late Ishwerbhai Kesurbhai Patel, an industrious farmer, having 5,000 acres of land under cultivation, who took great interest in the proper education of his sons. Young Govinddas, as he was addressed by his kinsmen, was different from the rest of his brothers and cousins, studying at Baroda. During his school days at Baroda, he showed average progress, but his zeal for knowledge was such that nothing was allowed to come in its way. Leaving aside the hardships of accommodation, food, etc., the main difficulty was the choice of his career. He matriculated in 1939 and joined Baroda College. The new atmosphere had a profound influence on him; the once meek and average student now began to shine as a scholar in the truest sense of the term.

His patriotism and eagerness for the uplift of the rural masses attracted him to the study of agriculture. He then went to the then only college of agriculture, claimed to be the best in South-east Asia, namely, that at Poona. Here his progress was remarkable and he secured first class with distinction in the B.Sc.(Agric.) examination in the University of Bombay in 1946.

While studying he decided to go to the United States in order to study agriculture at an advanced level. There he entered the Graduate School of the Iowa State College and the University of California at Davis. As he was much interested in horticulture, he preferred the University of California and received his M.S. degree in horticulture in 1948 and Ph.D. degree in plant genetics in 1951 working under Prof. H. P. Olmo on the genetics and cytogenetics of the grape vine. During his stay there he competed in two examinations and was awarded two fellowships of several thousand rupees in order to continue his studies. He returned to India in 1951.

He accepted a Class III post in a Government Vegetable Seed Multiplication Farm in the Kulu Valley. After serving there for a year he left and joined the Agricultural Research Institute, Sabour, Bhagalpore, in an honorary capacity to work under Dr. R. H. Richharia, the geneticist and plant breeder and director of the Institute, and he published some important papers. Later, he was appointed cytogeneticist in March 1953 by the Indian Central Jute Committee at its Jute Agricultural Research Institute, Barrackpore. From there he published several important papers on the cytogenetics of jute in several journals, which attracted world-wide attention. His papers on the grape vine were also outstanding. Later in 1956 he was appointed areca-nut specialist at Vittal. He worked there for some time and published a few papers. As the post carried more administration, he left and returned to his native home at Baroda and entered business for two years. Afterwards, he took up horticultural researches on joining the Scientists' Pool organized by the Council for Scientific and Industrial Research, and he was posted to the National Botanic Gardens, Lucknow. To continue his work it was necessary to go to the Banthra Farm, a few miles from Lucknow. On March 9, while cycling to the farm, he met with a fatal accident by colliding with a speeding car. Although he received prompt and adequate medical help, he succumbed to his injuries on March 10.

R. M. Datta

Mr. J. K. Starnecki

Mr. Jerzy Kazimierz Starnecki, who died on May 15, was born in Poland on August 26, 1908. He gained a diploma in engineering at the University of Warsaw and in 1935 was appointed head of the Inspection Branch of the Polish National Armament Factories, Radom. After the outbreak of hostilities in 1939 he served with distinction the Polish Armed Forces in France. In 1941 he was chosen for a special assignment as design engineer with the Research and Development Department of the Polish Army in the United Kingdom.

After the War, Mr. Starnecki spent a short time as a research engineer at Signals Research and Development Establishment; then, in 1947, he joined Pye, Ltd., where, among other projects, he played a leading part in the design of a new optical system used in the gunsight of the Conqueror tank and in the servo-controlled automatic stabilizing gear. After a successful stay of seven years with Pye, Ltd., Mr. Starnecki was appointed chief engineer and head of development of W. G. Pye and Co., Ltd., where he remained until his death. There his great foresight and sound engineering knowledge influenced the design of nearly all instruments. He was an active associate member of the Institution of Mechanical Engineers. He made a constant endeavour to keep abreast of latest scientific developments and several patents are to his credit. He has served on the council of the British Scientific Instrument Research Association and was a committee member of the Scientific Instrument Manufacturers' Association of Great Britain, Ltd.

Mr. Starnecki was a rather quiet little man but with a good sense of humour and a smile for everybody. In his limited amount of spare time he was a keen gardener. His sudden and untimely death came as a great shock. He will be missed by all who worked with him and remembered not only as an outstanding engineer but also for the great range of his human sympathies.

NEWS and VIEWS

International Meteorological Organization Prize for 1962: Dr. A. Ångström

Dr. Anders Ångström from Bromma, Sweden, has been awarded the International Meteorological Organization Prize for outstanding work in meteorology and international collaboration. Dr. A. Ångström studied at the Universities of Uppsala, New York and Jena. He obtained the degrees of Master of Science and 'Filosofie Doktor' and in 1916 became assistant professor of meteorology in Uppsala. After participating in several scientific expeditions and work at Cornell University as a Fellow in physics, Dr. Ångström became State Meteorologist and later First State Meteorologist at the Swedish Meteorological and Hydrological Institute in 1919. In 1940 he was appointed assistant director and chief of the Meteorological Bureau and in 1949 director of the Institute. He retired from this post in 1954. Dr. Ångström is a member of the Royal Swedish Academy of Science and of the Royal Swedish Academy of Forestry and Agriculture. His contribution to international collaboration has mainly been in meteorology, particularly in the field of solar and atmospheric radiation. He has participated in various meetings of the International Meteorological Organization and the World Meteorological Organization as well as of the International Union of Geodesy and Geophysics. The International Meteorological Organization Prize, which consists of a gold medal, was established in 1955 by the World Meteorological Organization in honour of the former non-Governmental Organization which had initiated international collaboration in meteorology in 1878, but which ceased in 1951 when the World Meteorological Organization took over.

Pure Mathematics at Birmingham :

Prof. P. J. Hilton

PROF. P. J. HILTON is vacating the Mason chair of pure mathematics in the University of Birmingham on being appointed to a professorship in pure mathematics in Cornell University. Prof. Hilton, who is thirty-nine years of age, was educated at St. Paul's School and Queen's College, Oxford, obtaining a first-class degree in mathematics while at the latter. After the War he returned to Oxford to work in algebraic topology under the late Prof. J. H. C. Whitehead and was one of the leading members of the brilliant band of topologists taught by Prof. Whitehead. After obtaining the Ph.D. degree he went to Manchester in 1948, where he remained until 1952. He then spent three years in Cambridge as lecturer and returned to Manchester in 1955, where he remained until 1958, when he was appointed to the Mason chair at Birmingham. Prof. Hilton has made outstanding contributions to algebraic topology, particularly in the field of homotopy. In recent years he has worked in close collaboration with Prof. Eckmann of Zurich, and their joint work is among the most interesting and important contributions being made to topology at the present time. In addition, Prof. Hilton has written a tract on homotopy theory and also, in collaboration with S. Wylie, a book on homology theory. Both are clear expositions of algebraic topology.

Prof. A. M. Macbeath

PROF. A. M. MACBEATH, who has been appointed to succeed Prof. Hilton, is thirty-nine years of age. He was educated at the Royal Belfast Academic Institution, The Queen's University of Belfast, where he obtained a first-class degree in mathematics, and Clare College, Cambridge. After obtaining first-class honours in the Mathematical Tripos, he commenced research in the geometry of numbers under Prof. L. J. Mordell. For his research in this subject he was awarded a Smith's Prize. From 1948 until 1950 he was at Princeton University with a Commonwealth Fellowship, where he obtained a Ph.D. degree. From this period dates his interest in measure theory and topological groups, in which fields he has carried out research in more recent times. After his return to England, he went to the University of North Staffordshire from 1951 until 1953 as a lecturer, and was then appointed to the chair of mathematics in University College, Dundee (University of St. Andrews), where he has remained until the present time. As already indicated, Prof. Macbeath's field of research has widened from his original interest in the geometry of numbers to embrace both measure theory and, more latterly, discontinuous groups. While at Dundee he has written a steady stream of papers on these subjects.

Applied Physics at Hull : Prof. J. Ring

DR. JAMES RING, who has been appointed to the new chair of applied physics in the University of Hull, was an undergraduate and research student in the University of Manchester. He later became lecturer, senior lecturer and reader in spectroscopy there. His first postgraduate work was on the improvement of spectroscopic techniques by applying the then new multiple dielectric layers to the Fabry–Perot interferometer. Dr. Ring has continued to play a prominent part in the modern revival of experimental spectroscopy which has resulted largely from recognition of the special qualities and the special optical requirements of such detectors as photomultipliers and infra-red sensitive cells. He has contributed especially by developing pressure-scanned interferometers and by applying them to astronomical and other purposes. He has also worked on the closely related interference filters, dealing with their production, the extension of their spectral range, and their application: astronomy has again received his special interest. In 1960 it was recognized by the Department of Scientific and Industrial Research that Dr. Ring's work, devoted to techniques used over a wide scientific field, occupied a special position, and he was made director of a specially financed project in which a team of research students and more senior assistants, with technical support, developed instruments and methods for use by chemists and astronomers as well as physicists. This team has worked successfully for a year or so and it is hoped that a considerable part of it will be transferred to Hull.

Computer Network for Australia

THE Australian Federal Cabinet has agreed to the establishment by the Commonwealth Scientific and Industrial Research Organization of a system of electronic computers for their own use, universities and Commonwealth Government departments and authorities. It is hoped that the first stage of the project, which will cost more than £1,500,000, will come into operation in about eighteen months time. The central unit of the system will be a large and powerful computer, located in Canberra. The Government is to set aside £100,000 for a laboratory to house it. 'Satellite' computers will be set up in Sydney, Melbourne, Canberra and Adelaide. All the units will be programmed in the same way and will make up an integrated network. About forty people, including twelve scientists, will be appointed to the project. C.S.I.R.O. will be the biggest single user of the network. Several other Government departments and agencies, including the Atomic Energy Commission, the Bureau of Meteorology, the Post-Master General's Department and the Aeronautical Research Laboratories, will make extensive use of the computers. In the future, the Atomic Energy Commission and some departments and universities will acquire their own 'satellite' computers which they will operate themselves as part of the system. It is estimated that by 1965 C.S.I.R.O. alone will need at least 5,000 hr. of 'satellite' computer time in a year, plus 1,500 hr. on the central unit. Other Government users will together need more than that. At the present time, Australia's computing needs are doubling every year.

The Commonwealth Agricultural Bureaux

IN reply to a question in the House of Lords on May 28, the Parliamentary Under-Secretary of State for Commonwealth Relations, the Duke of Devonshire, said that the Government had no reason to believe that the Commonwealth Agricultural Bureaux were unlikely to be able to continue successfully under existing arrangements for scientific co-operation in the Commonwealth. These arrangements for scientific research were a matter for agreement between all participating Governments, and if it became apparent that there might be advantage in reconsidering them, the Government would be ready to join in examining alternative possibilities. An interim conference of representatives of participating Governments was to be convened next year and a special committee appointed to review certain aspects of the working and financing of the Bureaux. This would provide an opportunity for discussion of possible changes in the existing arrangements, including any extension of existing collaboration with non-Commonwealth Governments. He thought that the terms of reference already recommended for the interim conference were wide enough to cover all points, including the cost of the abstract periodicals, the price of which was doubled on January 1, 1962. The Government was reluctant to believe that any of the participating Governments would be so short-sighted as to think of leaving the Bureaux.

The Royal College of Science and Technology, Glasgow

In a written answer in the House of Commons on May 29, the Chief Secretary to the Treasury, Mr. H. Brooke, stated that the University Grants Committee had informed him that, both on general grounds and in view of the need for more university places in Scotland, a decision to enable the Royal College of Science and Technology, Glasgow, to increase its number of degree students and to broaden the scope of its academic work should now be made. In particular, if the new Faculty of Industrial and Social Studies was to be developed adequately within the quinquennium, work on the necessary accommodation and recruitment of the necessary staff must be started this year. Although the work of the Robbins Committee is not yet complete, the University Grants Committee has felt justified to agreeing to the College's proposal to include accommodation for industrial and social studies in their building programme for 1962, and took into account the College's wish to develop these studies in their submission to the Chancellor of the Exchequer on the financial needs of British universities for the quinquennium 1962–67. The Committee accepted the case for the College being granted full university status, including the power to award its own degrees, but pending the report of the Robbins Committee they have deferred their final advice to the Committee and limited present action to that indicated.

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme

The eighth annual report of the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme covers the year ended December 31, 1961 (Pp. 8. London: Royal Society and the Nuffield Foundation, 1962). The report records 18 awards, of which five were for visits from the United Kingdom (three to Australia, one to Australia and New Zealand, and one to South Africa), nine for visits to the United Kingdom (two each from Australia, New Zealand and India, and one each from Malaya, Hong Kong and South Africa) and four for visits between countries of the Commonwealth other than the United Kingdom. At the end of 1961 applications were not accepted from persons wishing to visit South Africa or from South Africans wishing to work in the United Kingdom, but applications would be accepted from South Africans wishing to work in other Commonwealth countries.

University of London Observatory

A new laboratory at the University of London Observatory was opened by Sir Ifor Evans, provost of University College, London, on June 18. The laboratory is built around an Ebert-type plane grating spectrograph vertically mounted in a 12-ft. pit. There will be facilities for a wide variety of light sources including the Sun, fluid and solid rotatory arcs and flash tubes. Above the new laboratory, offices have been provided to relieve the overcrowding of the existing accommodation, allowing better accommodation of staff and postgraduate students. An undergraduate room having places for eight additional students attending practical courses at the Observatory has also been provided.

U.S. Research in Astronomy using High-Altitude Balloons

The Air Force Cambridge Research Laboratories are to conduct a series of research projects in astronomy using high-altitude balloons beginning later this year. Three separate projects, *Star Gazer*, *Sky Top* and *Balast*, are involved. The primary objective is to obtain more complete and accurate information about space environments with emphasis on the Moon, Mars and Venus. Only one of the three programmes, *Star Gazer*, will include a manned balloon flight. *Star Gazer*, under the scientific direction of Dr. J. A. Hynek, chairman of the Department of Astronomy, Northwestern University, Evanston, will measure the effect of turbulence of the atmosphere on image clarity at altitudes up to approximately 86,000 ft. A photoelectric experiment will investigate the variations in brightness of a star image caused by the atmosphere, while experiments will be conducted using infra-red techniques to determine how much effect water vapour has on different wave-lengths of light at different altitudes. The project *Sky Top*, under the direction of Dr. J. W. Salisbury, will carry its experimental payload to altitudes of 86,000–120,000 ft. It is hoped that temperature measurements will be obtained of the Moon at night that are accurate to within 5 degrees. An interferometer will be used to determine the amount of heat-energy released by the Moon at various wave-lengths of the infra-red spectrum. It is also hoped to determine the mean temperature of the reflecting surfaces of Mars and Venus; infra-red spectroscopy will be used to investigate the atmospheric composition of both these planets. The payload of *Balast* will weigh about 1,400 lb. and will be carried to an altitude of approximately 85,000 ft. In contrast to *Star Gazer*, the tracking system uses a completely passive (no moving parts) sensor. The system, under the direction of Dr. J. Strong of Johns Hopkins University, is expected to track Venus and other planets with an accuracy of ± 5 sec. of arc.

Education of Engineers

Prof. G. B. Warburton, giving his inaugural lecture as professor of applied mechanics at the University of Nottingham, spoke about "Some Effects of Recent Advances in Knowledge on the Education of Engineers" (Published in book form. Pp. 23. Nottingham: The University, 1962. 2*s*.). After surveying the present situation in Britain and the United States, he discussed possible changes in undergraduate courses, including regrouping of subject-matter, the provision of 'engineering science' and 'industrial engineering' options, and the provision of degree courses in general engineering with no specialization. He suggests that the new universities have scope for experiments in engineering education along these lines. Postgraduate courses may be of the 'follow up' or the 'new field' type. The former consists of further study in a field which forms part of a conventional honours course, with the intention of bringing the graduate to the boundary of knowledge in this field. The latter comprises subjects which do not follow directly from a major course in an engineering curriculum and are of interest usually to graduates from more than one discipline. Such courses enable the more advanced and specialized topics to be omitted from the first degree course and help to resolve the conflict between the rapid advances in knowledge and sophistication on one hand, and on the other the desire to preserve engineering as a single discipline at the undergraduate-level.

The Library Association

THE annual report of the Library Association for the year ended December 31, 1961, refers to the completion of a survey of library services in certain Commonwealth countries (Pp. 30. London: Library Association, 1962). A copy of the report was sent to Government Departments, members of Parliament and others, with a memorandum stressing the need for financial help in this field. On the recommendation of the Association, the Leverhulme Trust Fund agreed to award three scholarships in librarianship to students from the Commonwealth. There was a net increase in membership of 904, the total now standing at 13,937, including 1,254 institutional members. Reports of representatives on joint committees and other bodies appended to the Council's report include those from the British Standards Institution–Universal Decimal Classification Committee, which was concerned with the preparation of a guide to the Universal Decimal Classification (*BC*1000*C*) and protested to the editor of *Science Abstracts* on the abandonment of Universal Decimal Classification arrangement for *Part A* "*Physics*"; from the British Standards Institution Documentary Reproduction Committee, Photographic Aspects of Documentation Committee; from the National Committee on Regional Library Co-operation; and from representatives to the International Federation of Library Associations.

Schoolmaster's *Treasure Chest*

THE Schoolmaster Publishing Company has published an enlarged edition of *Treasure Chest for Teachers*. This booklet resulted from a series of articles with the same title, which appeared in *The Schoolmaster*, the journal of the National Union of Teachers. The articles showed teachers where they might find teaching aids either free or at low cost. The new edition has been thoroughly revised and, with thirty-two additional pages, is worth the increased price of 3*s*. 6*d*. The information is divided into four main sections—services available from societies and associations which have some bearing on the school curriculum; services available from embassies and legations; services available from industrial, commercial and transport concerns; and a general section giving information about associations which supply films and other visual aids

North Staffordshire Journal of Field Studies

THE North Staffordshire Field Club, founded in 1865, has published a journal for an unbroken period of ninety-three years. In recent years the rising cost of printing, together with other factors, has made it increasingly difficult to maintain the size of the journal. This has happened at a time when there is a growing interest in local studies and an increasing volume of research on the area, some of it initiated by members of the then University College of North Staffordshire. Rather than found a new journal to publish this work, the *North Staffordshire Journal of Field Studies* has been launched in which the *Transactions of the North Staffordshire Field Club* are incorporated (1; 1961. Pp. iii+140. Keele: University College of North Staffordshire, 1961. 25*s*.). The new journal will cover local history, archæology, architecture, geography, geology, and other local studies. While it is expected that these will be centred on North Staffordshire, the area will not be tightly drawn. The first volume of the new journal contains articles by E. A. Watkin on Staffordshire tokens and their place in the coinage of England; Prof. J. M. T. Charlton on excavations at the Roman site at Holditch, 1957–59; A. W. J. Houghton on the Roman road from Wroxeter to Whitchurch (Salop); and Mrs. Joan and Dr. Harold Taylor on the problem of dating pre-Conquest churches. Prof. A. E. Teale writes about the origin of the Keele experiment. Details are available from Prof. J. M. T. Charlton, Department of Classics, University of Keele, Staffordshire.

Scientific Glassworking Equipment

JENCONS' latest illustrated catalogue of scientific glassworking equipment can best be summed up as "everything for the glassworker and research worker using glass". It consists of items of interest in medical and scientific research and glassmaking generally, from small items like glass tubing to the largest glassworking lathes, and includes a comprehensive range of glassworking tools and glass-grinding equipment. The catalogue will save much time otherwise spent searching through numerous makers' catalogues and should be of special interest to physics departments in universities, technical and teachers' training colleges, and schools. Copies of the catalogue are available from Jencons (Scientific), Ltd., Mark Road, Hemel Hempstead, Herts.

Derwent Patents Manual

THE latest in the series of publications of the Derwent Information Service is the *Derwent Patents Manual* (Pp. ii+50. London: Derwent Information Service, 1962. 12*s*. 6*d*. or 2 dollars). It contains useful information for all with interest in the wide field of patents and contains articles on British, French, German, Russian, Belgian and Japanese patent laws and procedures. There is also an interesting analysis of the European patent concept; this describes the work of the European Economic Community's Committee which is at present drafting a new convention for creating an autonomous European patent system. Three new publications are to commence in July 1962, giving abstracts, in English, of Japanese, Belgian and Russian patents dealing with electricity, electronics, communications instruments, control and computation.

Optical Pumping and Masers

IT has been announced that a special supplement to *Applied Optics* will be published in December 1962, which will be devoted to optical pumping and masers. In the supplement all papers on the subject which have been published in *Applied Optics* during

1962, the first year of publication of the journal, will be gathered together and, in addition, there will be articles not previously published together with a 15,000-word review presenting a survey of the present state of the art and of the fundamental theories. The price of the supplement will be not more than five dollars. Orders should be sent to the Optical Society of America, 1155 Sixteenth Street, N.W., Washington 6, D.C.

U.K. Courses in Nuclear Energy, 1963–64

LIST No. 6 (May 1962) of *Nuclear Studies* has been compiled by the Science Department of the British Council. This lists courses in pure and applied sciences concerning the use and development of nuclear energy which may be undertaken in the United Kingdom during the year 1963–64. These courses are full-time and are generally of one week or more duration. The information provided by the catalogue includes notes on each of the courses and the institution at which it takes place; also the fee and qualifications required for participation. Further information can be obtained from the Science Department, The British Council, 59 New Oxford Street, London, W.C.1.

Clinical Research in the United States

THE Clinical Research Centers Program, established by the U.S. Public Health Service's National Institutes of Health on the recommendation of Congress, is now in its third year of operation. The programme seeks, through grants to private and public institutions, to enhance and increase the level of clinical research in the United States into the disease of man and into his fundamental biological conditions and problems. A recent report provides a review of the principal developments in the programme and an idea of the types of investigations undertaken by a few of the forty centres established under the programme of the Division of General Medical Sciences, National Institutes of Health, Bethesda, Maryland.

Fibrinolytic Activity of Arterio-Sclerotic Vessels

COMPARATIVE studies have been made by E. Perlick and A. Deutschinoff (*Hemostase*, 1, No. 3; 1961) on both normal and sclerotic aortic and carotid arteries, which show that tissue extracts made from intima and media of arterio-sclerotic vessels have a high tissue fibrinolytic activity when compared with similar extracts from normal vessels. The activators of fibrinolysis found in these vascular extracts exist in the form of a plasminogen activator or proactivator. On the other hand, an increased amount of plasminogen or a plasminogen-like material was also found. A marked increase in tissue fibrinolysis, resulting from arterio-sclerotic changes in the blood vessels, could be the cause of mural and intramural hæmorrhages in the arterio-sclerotic plaques.

Veterinary Research in Japan

UNDER the title "Deformation of the Vertebral Column in the 'Short-spine Dog' ", Toshihiko Ueshima (University of Tokyo, Japan) has described anatomical studies on four cases and discussed briefly cases in calves of the Oplandske breed in Norway, in turkeys and in a goat (*Jap. J. Vet. Res.*, 9, 155; 1961). Photographs of two dogs and X-ray photographs of the vertebral column of one of them give a clear picture of the condition. There was striking kyphosis in the anterior part of the thoracic segment and in the lumbar segment of the vertebral column, with scoliosis elsewhere. Excess of cartilaginous tissue in vertebræ and other abnormalities are described, with details of histological features and also secondary effects in other adjacent bones.

A new journal, *The National Institute of Animal Health Quarterly*, from Tokyo, completed its first volume in 1961. The director of the Institute is Susumu Ishii, and there are five branch laboratories of the Institute. In No. 4 of the volume there are eight original articles dealing with infective disease research and with irradiation studies.

Apprenticeship Schemes in the Cambridge Instrument Co.

A BROCHURE prepared by the Cambridge Instrument Co. for the information and guidance of young men contemplating a career in the instrument industry gives a brief summary of the role of instruments in the world to-day, sketches of the Company's history, describes and illustrates some of the instruments the Company makes, and provides information on the types of apprenticeships available. These include craft, technical and graduate apprenticeships. The brochure is attractively illustrated and is obtainable from the Personnel Officer, Cambridge Instrument Co., Ltd., Chesterton Road, Cambridge.

Safety in Laboratories

IN the article by Prof. R. A. Morton and Dr. J. Glover, entitled "Safety in Laboratories", which appeared on p. 809 of the June 2 issue of *Nature*, reference is made in the penultimate paragraph to a film, "Safety in the Laboratory", shown by courtesy of Imperial Chemical Industries (General Chemicals), Ltd., at the courses of lectures on "Safety in Laboratories" held at the University of Liverpool during the Lent Term. Imperial Chemical Industries (General Chemicals), Ltd., wish to direct attention to the fact that the title of the film is *Black Monday* and it was produced by the Imperial Chemical Industries Film Unit. While the film was produced primarily for the Company's own use, the needs of universities, technical colleges and schools were taken into consideration. The film is available on loan through the Imperial Chemical Industries Film Library, Imperial Chemical House, Millbank, London, S.W.1.

Mountbatten Research Studentships in Radio and Electronic Engineering

THE Council of the British Institution of Radio Engineers is to establish research studentships to encourage fundamental research in radio and electronic engineering. The award will be named after the Institution's charter president, Admiral of the Fleet The Earl Mountbatten of Burma. It is intended to award one studentship in October 1962, the second in October 1963, and thereafter as they become vacant. Each studentship will have an annual value of £500 plus university or college tuition fees, and will normally be tenable for two years, with a possible extension of one further year. Applications are invited from graduates (or those about to graduate), and the studentships are open to holders of degrees or diplomas in technology (Dip.Tech.) in appropriate subjects from any university or college in the United Kingdom, and can be held in any university or college of advanced technology in the United Kingdom which has established research in radio and electronic engineering. Further information can be obtained from the Secretary of the Institution of Radio Engineers, 9 Bedford Square, London, W.C.1.

Langley Memorial Prize, 1962

The Langley Memorial Prize was founded by the friends of the late Dr. W. H. Langley, principal medical officer, Southern Nigeria, to commemorate his work and the services he rendered both to Nigeria and to those who came under his care and skill. The Prize, value approximately £21, is open to competition among officers, past and present, of the West African Medical Departments; it is awarded periodically for the best paper on one of the following subjects: (*a*) tropical medicine or surgery; (*b*) tropical hygiene and sanitation; (*c*) tropical entomology and parasitology. Special consideration is given to original work. Joint applications are not accepted. No officer who has already been awarded the Prize is eligible to re-enter. Entries, which can consist of either published or unpublished work, should be sent to the Dean, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1, not later than October 1.

University News: Birmingham

The following appointments are announced; Prof. L. E. Davis, professor of industrial engineering in the University of California, to be Joseph Lucas visiting professor for the session 1962–63; *Research Fellows:* Dr. W. N. Cottingham (mathematical physics), Dr. G. P. McCauley (mathematical physics), Mr. J. A. R. Griffith (physics), Mr. H. C. Wilkie (pure mathematics); *Lecturer,* Dr. B. Musgrave (physics).

Bristol

The following appointments are announced: Dr. D. Russell Davis, reader in medical psychology and director of the Medical Psychology Laboratory in the University of Cambridge, to be professor of mental health and head of the Department of Mental Health; *Readerships,* Dr. D. T. Donovan (palæontology), Dr. W. D. Ollis (organic chemistry), Dr. B. F. Folkes (botany), Dr. P. H. Fowler (physics); *Senior Lectureships,* Dr. M. H. Rogers (mathematics, as director of the Computer Unit), Mr. T. V. Lawson (aeronautical engineering), Mr. R. C. Seymour (chemistry), Mr. S. R. Wragg (agricultural economics); *Research Associate,* Dr. J. G. Baker (physics); *Lecturer,* Dr. P. J. Bentley (pharmacology).

Cambridge

The following elections to fellowships at Churchill College have been announced: *Title A,* Dr. A. Howie, demonstrator-elect in the Department of Physics and College lecturer in physics; Dr. R. C. Campbell of Gonville and Caius College, University lecturer in agricultural statistics and College lecturer in mathematics for natural sciences; *Title B* (*Junior Research Fellowships*), B. W. Cherry of Trinity College, for research in metallurgy (the Goldsmiths' Fellowship in Metallurgy); M. A. A. Clyne of Trinity College, for research in physical chemistry; A. E. Dorey of Jesus College, for research in zoology; *Title C,* D. G. Kendall, professor-elect of mathematical statistics.

Oxford

Dr. M. D. Gwynne, Junior Research Fellow at Balliol College, has been elected to the Mount Endowment Senior Research Fellowship in tropical ecology (with effect from September 29) at that College.

Weizmann Institute of Science

The following appointments have been announced: Dr. J. H. Jaffe, to be professor in the Department of Infra-Red Spectroscopy; *Associate Professorships,* Dr. Yehuda Eisenberg (nuclear physics), Dr. David Danon (polymers), Dr. Carl A. Levinson (nuclear physics), Dr. Uriel Littauer (biochemistry); *Senior Scientists,* Dr. N. Haran-Gera (experimental biology), Dr. N. Trainin (experimental biology), Dr. A. Nir (isotopes), Dr. A. Patchornik (biophysics), Dr. A. Frankel (applied mathematics), Dr. A. Aharoni (electronics).

Announcements

Mr. D. G. Ryan has been appointed Rutherford Scholar by the Council of the Royal Society for three years beginning in October. Mr. Ryan will continue his work, in the University of Birmingham, on pion–pion resonances in the deuteron–deuteron interactions.

Prof. A. S. Parkes (Physiological Laboratory, Cambridge) has been elected chairman of the Scientific Publications Council in succession to Prof. G. W. Harris (Institute of Psychiatry, London). Mr. A. V. S. de Reuck (Ciba Foundation, London) has been elected honorary secretary, and the other members of the Steering Committee are Mr. A. L. Bacharach, Dr. R. K. Callow, Prof. G. W. Harris and Dr. D. Richter.

The sixteenth International Horticultural Congress, sponsored by the Government of Belgium, will be held in Brussels during August 31–September 8. Further information can be obtained from the Secretary, Sixteenth International Horticultural Congress, 233 Coupure Links, Ghent.

The Mullard Research Laboratories will be holding open days during July 4–6. During these days, each of the five divisions will be showing aspects of the research programmes in physics, electronics and allied fields that are being undertaken at the Laboratories. Further information can be obtained from Mullard, Ltd., Mullard House, Torrington Place, London, W.C.1.

An international symposium on "Environmental Control of Plant Growth", organized under the auspices of the International Union of Biological Sciences, will be held at the Academy of Science, Canberra, during August 25–31. Further information can be obtained from Dr. L. T. Evans, Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, P O. Box 109 City, Canberra, A.C.T.

An informal discussion, sponsored by the Faraday Society, on "Forces in Colloidal Systems", is to be held in the Department of Chemistry, University of Nottingham, during September 20–21. There will be two half-day sessions, which will include papers on: (*a*) stability of dispersions in aqueous and non-aqueous systems; (*b*) micellization. Further information can be obtained from Dr. G. D. Parfitt, Department of Chemistry, The University, University Park, Nottingham.

Erratum. In the article entitled "Isotope Effects in the Infra-red Spectrum of 1-^{13}C-Propene-1" by Dr. W. O. George, which appeared on p. 672 of the May 19 issue of *Nature*, the units on the abscissa of Fig. 1 should read 940, 920, 900, 880 and 860, and not 1,040, 1,020, 1,000, 980 and 960 as printed.

ATOMIC PHYSICS IN NORTHERN EUROPE

The Danish Atomic Energy Commission

THE fifth annual report of the Danish Atomic Energy Commission* covers the period April 1, 1960–March 31, 1961. It describes the building operations at the Risø Research Establishment and the work of the six sections of the Establishment; the reactors *DR*1, *DR*2 and *DR*3; international co-operation in the peaceful uses of atomic energy through the International Atomic Energy Agency; regional co-operation in Europe through the Organization for European Co-operation and Development and the European Atomic Energy Society; Nordic co-operation through the Nordic Liaison Committee for Atomic Energy; and various other activities including geological surveys in Greenland, educational and training courses, and general information services.

During the period under review there was no change in the membership of the Commission. Prof. Niels Bohr and Dr. H. P. Christensen continued as chairman and vice-chairman respectively and H. H. Koch, permanent under-secretary of State, as chairman of the executive committee. By the Royal decree of March 26, 1960, the jurisdiction of the Commission was transferred from the Minister of Finance to the Prime Minister. The total staff of the Commission on March 31, 1961, numbered 643, of which 420 were employed in the technical and scientific division and distributed over various sections—reactor, physics, electronics, radiation, agricultural research—and the three reactors. The secretariat consisted of a staff of twenty-two at the central office of the Commission at the Christiansborg Palace and of thirty at the subsidiary office at the Establishment.

After the completion of the third and biggest reactor *DR*3 in the beginning of 1960, building operations were concentrated on various supplementary installations required for the work at the Risø Research Establishment. These included a hot-cell facility, begun in August 1960, and the planning of expansions of certain laboratories, the construction of which commenced in March 1961. Research work, which was extended considerably when the big reactors came into operation, continued to develop, especially in connexion with a series of joint research projects on which the Commission has embarked in co-operation with other national and international atomic energy organizations. The Deuterium-moderated Organic-cooled Reactor study project has provided opportunity for study of the optimalization procedure and technical difficulties in the construction of an organic-cooled type of reactor, and participation in the two joint European projects, *Dragon* and *Halden*, has provided opportunity for following important aspects of development in the field of high-temperature gas-cooled and boiling-water types of reactor. Power production with breeder reactors, plutonium recycling and nuclear ship propulsion have not as yet been investigated at Risø. Neither has the Commission engaged much in fusion research, but both theoretical and experimental studies in plasma physics are being undertaken at Risø.

The physics department of the Establishment consists of the physics laboratory, the meteorology station, the reactor *DR*1 and the linear accelerator laboratory. During the year the linear accelerator was used for an extensive series of irradiation experiments for the Atomic Energy Commission and for industry. This has been reported in *Risø Report* No. 16. A group continued on the construction of a laboratory for the investigation of β- and γ-radioactivity, with the particular view of measuring the fundamental experimental constants of the β-radioactivity of the neutron. The solid-state group carried out intensive work on radiation damage, and some preliminary work on radiation damage to graphite at very low temperatures was commenced. The group working on problems of plasma physics and the utilization of the energy of fusion obtained data from a series of studies of electromagnetic shock waves using an instrument designed for this purpose. An international summer school course in plasma physics was held. This was arranged with the economic support of the Advanced Study Institute Program of the North Atlantic Treaty Organization, and of the Rask-Oersted Foundation. The course lasted 14 days and was attended by 130 delegates from 24 countries.

In the electronics department an experimental set-up was arranged in order to study the stability of photomultipliers and a recording single-channel pulse-height analyser mainly intended for use in γ-ray spectroscopy was developed. In addition to other work of a research and development character, work was carried out on nano-second photography using an image-converter tube, and a radio-altimeter was incorporated in the scintillometer used by the Greenland Geological Survey for uranium prospecting in Greenland. A new development within the electronics department was the introduction of a group for industrial instrumentation, the task of which was to advise on and plan experiments requiring conventional industrial control instrumentation. Since its establishment the group, consisting of two graduate engineers and two technical engineers, has worked on a testing cabinet for Wigner rigs; regulating equipment for the Wigner calorimeter; instrument panels for experiments in heat transmission in helium; and instrumentation for the *SAP*-2 experiments. In the annual report details also are given of the activities of the reactor engineering, chemistry and health physics departments.

A separate chapter of the Report deals with international co-operation in the peaceful use of atomic energy. This is discussed under the headings of the International Atomic Energy Agency; and regional co-operation in Europe, including the Organization for European Co-operation and Development, European Atomic Energy Society and Nordic co-operation. In addition to its direct educational and training activities in technical and scientific subjects the Commission tried to satisfy general public interest in nuclear energy and in the Risø Research Establishment by publications, radio and television broadcasts, and by conducted tours of the Establishment. During the period under review about ten thousand persons visited Risø. On March 31, 1961, the Library of the

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* Report on Activities of the Danish Atomic Energy Commission for the period from 1st April, 1960, to 31st March, 1961. Pp. 70. (Copenhagen: Danish Atomic Energy Commission, 1961.)

Establishment contained 74,000 bibliographical units, including 66,000 reports of which 25,000 were on microcards. On October 1, 1960, the Library engaged a librarian to act as adviser on Russian.

The report concludes with a list of publications by members of the staff of the Commission and with a detailed map of the layout at the Risø Research Establishment.

Nordic Institute for Theoretical Atomic Physics

The third annual report of the Nordisk Institut for Teoretisk Atomfysik (NORDITA)*, which contains both Danish and English versions of the text and covers the period July 1, 1959–June 30, 1960, reports the work continued on the same lines as previous years. The organization still operated on an interim basis, pending the working out by the committee, appointed by the Governments of the member countries (Denmark, Finland, Iceland, Norway and Sweden) and the Nordic Council, of the general principles for the administration of joint Nordic institutions. During the year the board consisted of four members from Denmark, Finland and Norway, respectively, three from Sweden and one from Iceland, with Prof. Niels Bohr as chairman. The staff comprised Prof. C. Møller as director and four scientific and four administrative staff. There were fourteen research fellows, six of whom had had their fellowships extended for a second year. Eleven research fellows and a member of the staff of Nordita, together with other Danish and Swedish physicists, undertook a week's study trip to Geneva for the purpose of a visit to CERN. The report concludes with nine appendixes in which are listed the various publications and lecture notes issued by the Institute during the year; the colloquia and courses of lectures held; the lectures given by visiting foreign physicists; the visits paid by members of the staff of the Institute to other institutions and the attendance of staff and research fellows at congresses and meetings; and the visits paid by the research fellows to their home institutions.

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* NORDITA: Nordisk Institut for Teoretisk Atomfysik Arsberetning 1959/60 (Annual Report). Pp. 45. (Copenhagen: NORDITA Blegdamsvej 17, 1962.)

LONG-TERM PLANNING OF RESEARCH IN JAPAN

IN 1954 the Science Council of Japan, a body consisting of 210 members, formed a committee for long-term planning of research. This committee did its main work between 1954 and 1956, but is still continuing its investigations; among its original members were the chairman (Prof. Arisawa), who is an economist, and three secretaries—one an economist (Prof. Tsuru), the second an agricultural scientist (Dr. Fukushima) and the third a mathematical statistician (Prof. Kitagawa). The Committee was concerned with recommendations on population, food, energy, industrial techniques and fundamental sciences.

Prof. Kitagawa has summarized, in the January issue of the *Australian Journal of Science*, the White Paper on Research, published in 1956 by the Science Council of Japan, which deals with fundamental science.

The method of investigation was that of questionnaires to individuals and institutions, issued with the co-operation of national committees in the various sciences. These consisted of two overlapping sections, the first endeavouring to obtain objective information about the departments in which the individuals concerned were working, and the second part asking for suggestions for the improvement of the departments and of Japanese science in general. The results were then submitted to leading experts in each field investigated, and, after this check, proposals were made to meet the problems outlined.

The recommendations were: (1) To raise the general standard of training and research in universities and research institutes by increasing their budgets, and improving their staff position and equipment. (2) To encourage co-operation among university research workers and promote the common uses of apparatus and facilities, partly in order to avoid unnecessary duplication of expensive equipment. (3) 'The liquid scheme'—a proposal for the establishment of central institutes in specialized topics, which would allow the easy exchange of staff between universities and the institutes. The advantages of this scheme would be improved communication between scientists, and considerable savings on equipment which could be located at the central institutes. (4) The uses of National Committees in respective sciences for discussion of important problems in their fields, and the planning of future developments. (5) Availability to the public of all Governmental data and scientific publications.

The Japanese Government has already taken some steps to meet the first recommendation, the second has been partly realized, the third has been established though with a more moderate budget than had been proposed; recommendations 4 and 5 still await full implementation.

In 1959 a report entitled "The White Paper on Fundamental Science, No. 1" was brought out; this pointed out that the development of fundamental science was essential to technical innovation. Japan was lagging badly in the sciences and, unless strong steps were taken, there was a danger that the country would fall farther below international levels. The White Paper contains a discussion of budgets for the pure and applied sciences, including a comparison of total expenditure for these sciences in Japan with the equivalent expenditures in the United States, the U.S.S.R., the United Kingdom and Western Germany. A comparison with these countries of the ratios: (*a*) budget for sciences/total Government budget, and (*b*) budget for sciences/total national income, showed that Japan's relative expenditure was lagging. An example was also quoted of a particular department showing that only about one-third of the total budget set aside for the sciences, less administrative and other costs, finally found its way to the scientists themselves. A study of the numbers of scientific staff showed that, in 1957, in the Faculty of Engineering of the University of Tokyo, 72 per cent of the scientific equipment was antiquated (dating before 1925). Examination of the research system in universities showed that the yearly system of funds and accounts was unsuitable for long-term planning of research. Moreover, the

Japanese 'chair system', whereby the establishment in any particular subject consists of one professor, one assistant professor and two assistants, is not entirely favourable to the pursuit of research particularly in view of the wide range of modern research work.

On postgraduate training and the numerous newly established universities, the White Paper points out that the number of staff, the budget of departments and the scientific apparatus were insufficient for postgraduate training. It was desirable to achieve high academic standards in the large number of new universities created in Japan since the end of the Second World War: these had made an effective contribution to local culture and technical education, and had produced many scientists.

Finally, recommendations were made for the increase of university salaries including the salaries of technicians and assistants, for the defraying of travel costs to conferences, for book discounts to academies, for tax deductions on donations to universities, for study abroad of Japanese scholars and their attendance at international conferences, as well as the invitation of foreign scholars to Japan, and, finally, the encouragement of universities not subsidized directly by the Government.

Some points on scientific education were discussed. In universities the separation into faculties, which is fairly rigid in Japan, was a bad principle, and departmental groupings would be preferable. Broad groupings under mathematical sciences, physical sciences, chemistry, geology and biological sciences would be better than specialized subdivisions in any of these branches. It was suggested that faculties be abolished so that a student could, for example, graduate in both engineering and mathematics if his specialized field were electronics. This is not possible at present.

The final section of the White Paper consists of comparisons, by experts in various fields, of Japanese science with science in foreign countries.

WOMEN IN SCIENCE AND TECHNOLOGY

THE first edition of *Opportunities for Girls and Women in Science and Technology* was published five years ago and was in great demand, indicating that many schoolgirls, teachers, parents and employers were interested in careers for girls in the scientific and technical field. During the past five years more physics and chemistry laboratories have been built and equipped in girls' schools so that a larger proportion of girls who are interested in science have the opportunity to study it basically and prepare themselves for further specialized training. There has been a substantial increase in the number of girls taking mathematics, physics and chemistry in the General Certificate of Education at Ordinary- and Advanced-level, but still the number of young women scientists entering industry and the professions is disappointingly low. At the same time, there are women of real ability in all branches of pure science. There are now fifteen women who are Fellows of the Royal Society.

When looking for reasons for the reluctance of girls to work in the scientific field, it would still seem that there is some fear on the part of girls, and possibly also of parents, of work which is new and a little unusual. They do not realize the satisfaction to be obtained from practical scientific work at all levels. The reason why some schools have a steady flow of girls into technical positions may be because one or two older ones come back to school and talk with enthusiasm about the work they are doing, and make the younger ones interested.

The present demand for scientific manpower means that there are many opportunities for girls and women who have qualifications or who are willing to study while working. A new edition of *Opportunities for Girls and Women in Science and Technology* * describes the kind of professions which are now offering openings. Mathematicians are in great demand, as are physicists and chemists in laboratories. There are many special branches of science such as metallurgy, biochemistry, biophysics and physiology, which are not school subjects but which depend in the first place on the basic scientific subjects learnt at school. An introduction to these branches of science may only come at university-level, but it is the object of the booklet to indicate that such subjects exist and to point to the sources of further information about them. Engineering also falls into this category, and even now few girls take up this work. It is significant that those who enter this field do well, probably because they have to fight against a certain amount of opposition before they get established, so that only the best survive.

The booklet contains details of the many openings in different careers.

* *Opportunities for Girls and Women in Science and Technology.* Pp. 42. (London: British Federation of University Women, Ltd., 1961.) 3s. 6d.

ORAL POLIOVIRUS VACCINATION IN COSTA RICA

AN investigation, planned and carried out jointly by the Yale Poliomyelitis Study Unit and the World Health Organization Regional Poliomyelitis Laboratory of the Americas, during the course of a nation-wide vaccination campaign in Costa Rica*, sought to determine the effectiveness of a trivalent oral poliovirus vaccine in immunizing young children living in a tropical environment. More specifically, its aims were to measure the rate of antibody conversion and antibody increase induced by two doses of vaccine given at a month's interval; to determine the prevalence of non-poliovirus enteroviruses in the children, and their possible inhibitory effect on the efficiency of vaccination; and to estimate the extent to which attenuated strains that had been administered spread within the families of vaccinees, and the duration of their excretion by vaccinees and

* *WHO Chronicle*, 16, No. 4; April, 1962.

other children in their families. At the same time, a study was made of polioviruses recovered from flies caught in the area of the trial to see if they were related to those used in vaccination.

The investigation centred on a group of families living in the same village. In each a single child under two years of age, the index child, received the trivalent vaccine on two occasions. In most of the families there were several children under five years of age. The whole group of vaccinees and sibling contacts was tested for enteroviruses and antibody-levels by means of rectal swabs and blood sampling before, during and after the trial.

The results showed that, in general, the immunizing capacity of this particular vaccine, though moderately good, was not entirely satisfactory. Its effectiveness varied considerably for each of the three strains of poliovirus included. The post-vaccinal response to the Type 3 component was excellent; the first dose produced antibody conversion in 79 per cent of susceptible vaccinees, the second in 91 per cent. For Type 1 the conversion-rate was only 35 per cent after the first dose and 71 per cent after the second dose, while the Type 2 strain had little effect—only 35 per cent being converted even after the second dose. There was some evidence that heavy non-poliomyelitis enterovirus infection preceding the trial reduced the response to the Type 1 component to some extent, and that such inhibition was possibly due to *ECHO* virus 12. This interference did not seem to affect Type 3 responses, and the situation with regard to Type 2 was difficult to evaluate. On the whole, interference by enteroviruses was not of great importance; but interference between the vaccine strains themselves was of some significance—the highly infectious Type 3 seemed to some extent to crowd out the competing polioviruses, as well as other enteroviruses. It might well be desirable to enhance the future efficiency of this particular trivalent vaccine by reducing its content of Type 3 poliovirus and increasing the dose of the other types, particularly Type 2.

The degree of spread of attenuated vaccine strains within families, from the index child to other young children, proved to be considerable, the conversion-rates among susceptible siblings for Types 1 and 3 being 85 per cent and 77 per cent respectively. Type 2 had little opportunity to spread as it infected so few vaccinees. The speed of spread was impressive. Vaccinees could be shown to be excreting virus within 18 hr. of administration, and at least one sibling contact had picked it up within five days of the original infection. This is consistent with the familiar clinical experience that, in family outbreaks caused by wild virulent strains, several cases often appear within a day or two of each other, giving the false impression that a single extra-familial source has been responsible for a number of separate infections.

It was noted that susceptible children, whether vaccinees or only contacts, excreted Type 3 virus after infection for periods of up to 40 days. Half were infectious for at least three weeks. This did not happen with children who had some level of pre-vaccinal antibodies; most of these failed to become infected at all and those who were infected excreted virus for only 2–4 days on the average.

Although it proved surprisingly easy to recover polioviruses and other enteroviruses from flies trapped in the village during the trial, and although the kinds of virus recovered roughly paralleled in incidence those found in rectal swabs collected from children studies, the evidence provided by genetic markers was that many of the Type 3 insect-borne strains were, in fact, wild ones. But not all could be so identified, and it seems quite possible that flies are capable of picking up and disseminating the attenuated strains used in oral poliovaccine campaigns.

INSECTS OF THE PACIFIC AND ANTARCTIC AREA

The first report on the zoogeography and evolution of Pacific insects and studies of airborne insects in the antarctic area, which was supported mainly by grants from the U.S. National Science Foundation, the U.S. Office of Naval Research, U.S. Antarctic Research Program, the J. S. Guggenheim Memorial Foundation, and the U.S. Educational Foundation (Fulbright fellowship), has recently been published*.

Dr. Gressitt is to be complimented on the energy and thoroughness with which he has carried out these projects, and considerable progress has been made in an investigation which will take many years to complete.

In the introduction, Gressitt discusses the history of the zoogeography of the area and the various problems involved. Then follow sections on environment, dispersal, establishment, evolution, nature of island populations and faunal origins and affinities. Next comes a section on the Island Groups including Micronesia. This section is followed by an account of the Papuan Sub-region, Australia, New Zealand, the Sub-antarctic Islands, and Antarctica. The latter two sections are illustrated by excellent colour photographs and the remainder by good photographic figures. Experiments were made in the sub-antarctic in trapping flying insects by means of many large nets flown from the rigging of a ship. Although insect dispersal by air was demonstrated, most insects arriving on the Antarctic continent by this means would fail to establish themselves, because of lack of appropriate food and environment. However, this would not be the case in many of the Pacific Islands, but the impoverished insect fauna of most islands indicates that this method of dispersal is rarely effective.

The rest of the report, illustrated by excellent distributional maps, consists of sections on the detailed zoogeography in the Pacific area of the insect groups, Cerambycidae and Chrysomelidae, by Dr. Gressitt and, in the appendixes, on the Machaerotidae (Hemiptera Homoptera: Cercopoidea) by Dr. T. C. Maa, Tabanidae (Diptera) by Dr. I. M. Mackerras, Phasmatodae by Dr. Setsuko Nakata, and Psychodidae (Diptera) by L. W. Quate.

W. E. China

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* *Problems in the Zoogeography of Pacific and Antarctic Insects.* By J. Linsley Gressitt, with appendixes by T. C. Maa, I. M. Mackerras, S. Nakata and L. W. Quate. (Pacific Insects Monograph, No. 2.) Pp. 127+24 plates. (Honolulu, Hawaii: Bernice P. Bishop Museum, 1961.)

MESOSPHERIC DENSITY AND WINDS DETERMINED BY THE FALLING SPHERE METHOD AT WOOMERA

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KNOWLEDGE of density and wind profiles is important in the design and in the performance assessment of guided weapons and of vertically rising rocket-vehicles. Standard meteorological flights using radar-tracked balloons carrying radiosondes may, under favourable conditions, cover conditions to 120 kft. (1,000 ft.). The space between 120 kft. and satellite height at 500 kft. can only be probed by rocket-borne sensors. The Weapons Research Establishment has designed rocket-vehicles to cover soundings to a height of 300 kft. Mesospheric winds at Woomera have been previously obtained by the radar tracking of 'window' clouds and by the optical tracking of glowing clouds formed by grenade explosions. Temperature and wind profiles have also been determined in the Royal Society programme of *Skylark* rocket firings ejecting grenades and 'window'. The University of Adelaide[1], using the Doppler effect on radio waves reflected from ionized meteor trails, has determined wind profiles in the 260–328 kft. height band. Using all results, the seasonal pattern over southern Australia, particularly of wind profiles, is becoming clearer.

The falling-sphere experiment, where an inflatable sphere is ejected from a high-altitude rocket and, as it falls, is tracked optically or by radar, allows a density and a wind profile to be obtained with a minimum of ground instrumentation. The results are readily analysable using the IBM 7090 computer available at the Weapons Research Establishment, and from the simple relationship which exists to 300 kft. between pressure, temperature, density and height, pressure and temperature profiles may be obtained from the density profile. The simplicity of the experiment indicates that it may be suitable for firings on a synoptic basis.

Table 1. ACCURACY OF TRACKING INSTRUMENTATION

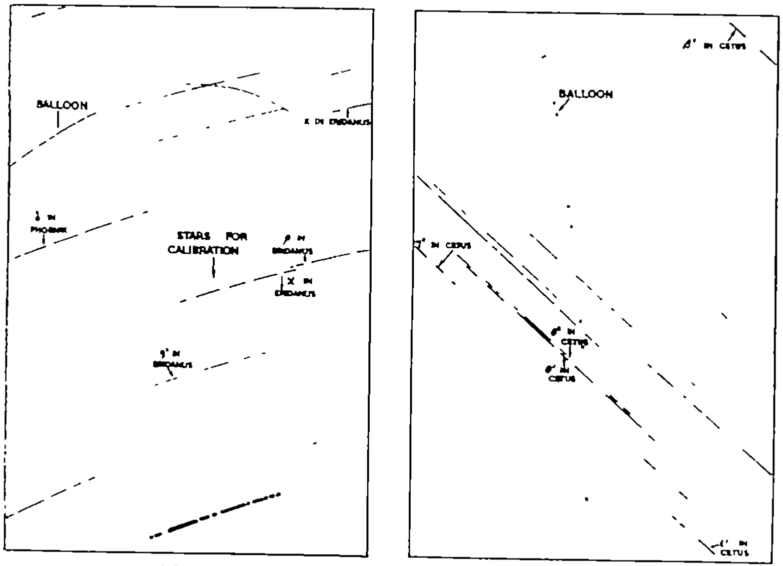
Instrument	Performance and error
*FPS*16 radar Power output of 1 MW. at 6 cm.	A 10-sq.m. target can be tracked to a range of 180 miles with a range error of 5 yd. and angular error of 0·1 mil.
Ballistic cameras on a 50-mile baseline	Can record seventh magnitude stars at stellar rates. The angular accuracy of the instrument is 8 sec. of arc.
Baker-Nunn camera *f*1·0 with 30° × 5° rectangular view	Objects equivalent in brightness to thirteenth magnitude stars and moving at satellite rates can be recorded with an accuracy of 2 sec. of arc.

On December 14, 1961, a sphere was ejected at 384 kft. at Woomera from a two-stage sounding rocket. After inflation, the sphere continued to an apogee of 423 kft., and from the rates of fall during its descent and from the ground projection of the trajectory, density and wind profiles have been determined.

The instrumentation covering the firing was two type *FPS*16 radars, two ballistic cameras and the Baker–Nunn satellite camera. One radar was situated 11 miles behind and the other 105 miles in front of the launch point. Taking the pictures shown in Fig. 1, the ballistic cameras on each flank of the trajectory were using rotating and sequenced shutters to give an analysable time-history of the sphere. Situated some 19 miles behind the launch point, the Baker–Nunn camera, at a slant range of 90 miles, took pictures of the sphere as shown in Fig. 2 at 2-sec. intervals and 1/5th sec. exposure. The performance capabilities of the instrumentation used are given in Table 1.

The sphere was constructed with 20 gores of 0·0005 in. metallized 'Melinex' and inflated to a superpressure of 9 m.bars by isopentane. The diameter of the sphere had been tested prior to the trial, and over randomly selected diameters, the cross-sectional areas varied from those of a 2-metre sphere by less than 0·25 per cent. Previous work at the University of Michigan[2] had determined the drag coefficients of a sphere at various Reynolds and Mach numbers, and these were used in the determination of the density profile.

The firing took place at 1959 C.S.T., 36 min. after sunset when the Earth's shadow extended to 190 kft. The nose cone separated from the body at 270 kft. and some 40 sec. later the balloon was ejected at 384 kft. Sequences as seen by the Baker–Nunn camera are given in Fig. 2. After ejection the sphere rapidly inflated, and was easily seen in the sunlight



(*a*) (*b*)

Fig. 1. *a*, Camera with a rotating shutter; *b*, camera with a sequenced shutter

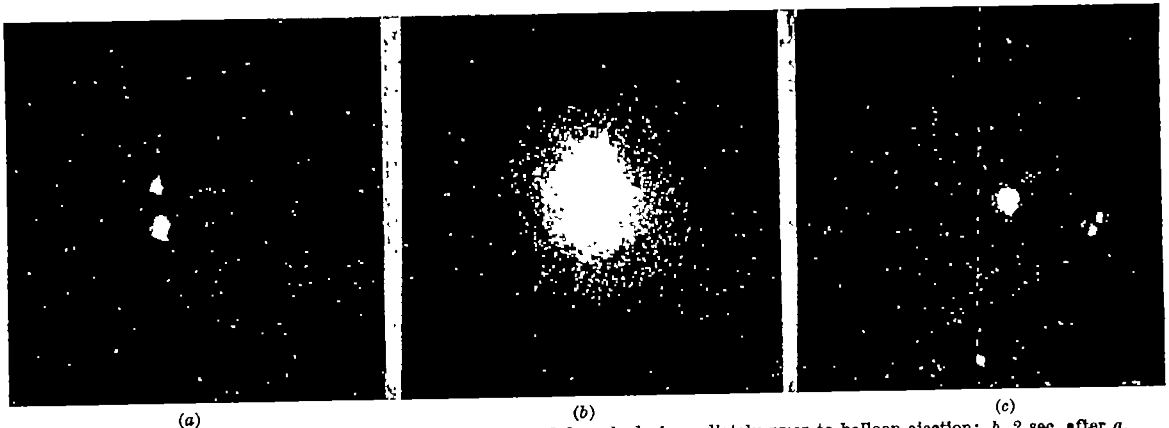


Fig. 2. Baker–Nunn exposures (*c.* × 16) (*a*) head separated from body immediately prior to balloon ejection; *b*, 2 sec. after *a*, indicating reflexion from cloud of French chalk; *c*, 14 sec. after *b*, showing chalk particles travelling with the balloon and images of rocket parts

having an intensity of a $+\frac{1}{2}$ visual magnitude star at apogee, ensuring optical coverage and allowing the radars to acquire the sphere by optical aids.

An initial check on the density at 226 kft., using unsmoothed radar data and the mean of values from 217 to 237 kft., indicates a density 6 per cent lower than the mean *ARDC* 1959 value. Vertical winds in the mesosphere of the order of 50 ft. sec.$^{-1}$ have been previously measured at Woomera[3], and assuming such vertical winds, an analysis has shown that this falling sphere of $\frac{\text{area}}{\text{mass}}$ ratio = 25 will give density with an accuracy of 5–10 per cent for the height-range 240–300 kft. Final figures will be obtained using a smoothing technique for the range elevation and azimuth values of the radar.

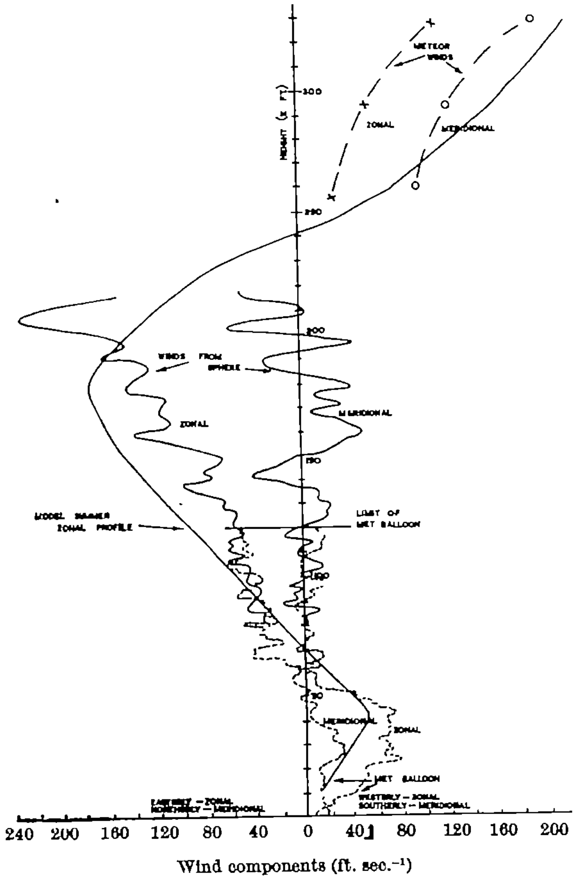


Fig. 3. Composite wind profile, December 14 1961

The rapid rate of fall of the sphere, a maximum of 2,700 ft. sec.$^{-1}$ at 285 kft., did not allow reasonable wind sensing to commence until the sphere had fallen to 220 kft., where the rate of fall was 690 ft. sec.$^{-1}$. The wind profile obtained by the two *FPS*16 radars, although separated by 116 miles, showed reasonable agreement indicating the reality of the fine structure. For the 80–120 kft. region, winds obtained from the falling sphere were compared with winds obtained from a meteorological flight which was about $2\frac{1}{2}$ hr. later at these heights. The differences which are shown in the composite wind profile of Fig. 3 may be due to time variation or a height error of up to 3,000 ft. in the determination of the meteorological balloon height in this region. Winds in the 250–350 kft. region were determined at the University of Adelaide using readings extending over the period December 4–14, 1961, and while the limitations in applying winds taken at Adelaide (lat. 35° S.) to Woomera (lat. 31° S.) and in using mean winds over 10 km. height bands are appreciated, Elford's results[1] indicate the errors are not extreme.

The composite wind profile is compared in Fig. 3 with a model summer zonal profile which had been previously prepared. Although this firing occurred in the first third of the summer, the strongly established easterlies at 150–200 kft. confirm what has been previously noted by Keegan[4] and Rofe[5] that the seasonal transition is often more advanced in the higher levels.

The Baker–Nunn camera, although some 90 miles from the point of ejection of the sphere, gave evidence of an interesting phenomenon. The sphere had been packed with approximately 20 gm. of French chalk for lubrication from the chamber at ejection, and to prevent adhesion between touching gores when packaging. Fig. 2*a* shows the 18-in. nose cone some time after separation from the main body with both portions being seen in the sunlight. 2 sec. later the sphere and the chalk had been ejected as seen in Fig. 2*b*. The image is assumed to be formed from the chalk by the diffuse reflexion of sunlight rather than by excitation of particles in a shock front or by photoluminescence. Assuming an albedo of 0·8 and the mean diameter of chalk particles of 2μ, the luminance is approximately 6,000 candles/gm. Further experiments will be performed using more chalk, and spectra, if possible, will be obtained.

The results reported here are an initial assessment only, and no attempt has been made to analyse errors. Continued experiments with the falling sphere will no doubt reveal differences in the mesospheric structure between the northern and southern hemispheres.

[1] Elford, W. G., *Planetary and Space Sci.*, 1, No. 2 (April 1959).
[2] May, Albert, and Witt, W. R., jun., *Aeroballistic Res. Rep.* 80, *NAVORD Rep. 2362*, U.S. Naval Ordnance Laboratory (1952).
[3] Rofe, Bryan, *W.R.E. Tech. Mem. SAD125* (August 1961).
[4] Keegan, Thomas J., *G R.D. Res. Note, No. 52, A.R.D.C.* (Feb 1961).

TRANSFER COEFFICIENTS AND THE ACTIVITY OF ELECTRODE SURFACES

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A GENERAL problem in the investigation of heterogeneous processes at solid/fluid interfaces is the difficulty of accounting quantitatively for variations in the activity of the solid surface from sample to sample, or from point to point on the surface of a given sample. In the case of electrode processes, an alteration in the activity of the electrode surface is reflected by a change in the observed exchange current density, i_0. Similarly, a heterogeneous surface is characterized by different values of i_0 at different points on the surface[1-3]. Presumably, therefore, it should be possible to account for the variation in activity in terms of the factors which influence i_0.

For an electrode process with a single rate-determining step, i_0 is a measure of the velocity, at equilibrium, of the anodic and cathodic partial reactions which constitute this step. Thus, it is essentially the product of a characteristic rate constant and a mass action term, which expresses the influence of the activities of the reactants and products. On this kinetic view, a variation in the activity of the electrode surface can be assessed through a change in the characteristic rate constant.

Often, the rate-determining step involves electron transfer between the electrode and a species adsorbed on its surface[4]. In this case, the rate constant is potential dependent and may be regarded formally as the product of three terms, a pre-exponential factor, an exponential term involving the height of the energy barrier at some arbitrarily chosen potential, and an exponential term which, through the transfer coefficient, expresses the way in which the height of this energy barrier is modified as the potential of the electrode is altered. There is much to be said in favour of choosing, as the reference potential, that value at which the height of the energy barrier is the same for both the anodic and cathodic partial reactions[5]. This has been termed the characteristic potential[6].

It is pertinent to inquire how these three terms alter when the activity of the electrode surface changes, a point which does not appear to have been the subject of explicit discussion. In so far as the question has been considered at all, it may be inferred from the literature that most workers would agree that it is the height of the energy barrier at the arbitrary reference potential which varies, and that the transfer coefficient is invariant. We support this contention by reference to the work of Parsons[7], Gerischer[8] and Thomas[9], in which they consider the variation in i_0 for the hydrogen electrode process on different metals.

On this view, heterogeneity of the solid surface is to be interpreted as a variation in the height of the energy barrier from point to point, reflecting a variation in the adsorption energy, a view which finds its counterpart in the field of heterogeneous catalysis. A refinement leads to the 'differentiated site theory', which was developed for the hydrogen electrode[1,10] from a suggestion by Audubert[11] that charge and discharge do not necessarily occur at the same sites and are not necessarily strictly the reverse of each other.

Audubert's work merits more consideration than it has received, since it indicates that a change in the adsorption energy from site to site on a heterogeneous surface may be accompanied by a change in the transfer coefficient, that is, by a change in the shape of the energy barrier. Certain cases of transport-controlled processes, in which the slope of the plot of the Nernst equation is greater than that to be expected from the stoicheiometric equation, have recently been interpreted from this point of view[4].

We now wish to extend these considerations by observing that, in certain cases at least, a variation in activity can be expressed completely in terms of a change in the transfer coefficient. For the iodine, iodide[6] and ferricyanide/ferrocyanide[12] redox processes at platinum disk electrodes, the apparent transfer coefficient varies with the bulk concentrations of the participating species, and with the angular velocity of the disk. At first sight surprising, this can be attributed to two factors. The method adopted for determining the transfer coefficient from the observed current/potential data considered both the anodic and cathodic partial currents, and was based on the assumption that the electrode surface was uniform, that is, that the anodic and cathodic transfer coefficients summed to unity. This assumption is clearly false for most solid electrodes, but it can be removed by introducing a potential[6] $\varphi_{Z=i}$ defined by:

$$\varphi_{Z=i} = \varphi_0 + \frac{RT}{(1-\alpha)F}\ln i_0$$

where φ_0 is the reversible electrode potential, and $1-\alpha$ is the cathodic transfer coefficient. For a uniform surface, $\varphi_{Z=i}$ is a function of the composition of the solution, but should be independent of the angular velocity of the electrode. Since, however, $1-\alpha$ varied with the angular velocity, $\varphi_{Z=i}$ also varied; but plotting this potential against $1/(1-\alpha)$ gave a linear plot for each different solution. Correcting the slopes to unit concentrations of the participating species gave a constant value for the rate constant at the characteristic potential φ^*, indicating that there was no change in the height of the energy barrier at this potential. Each plot gave

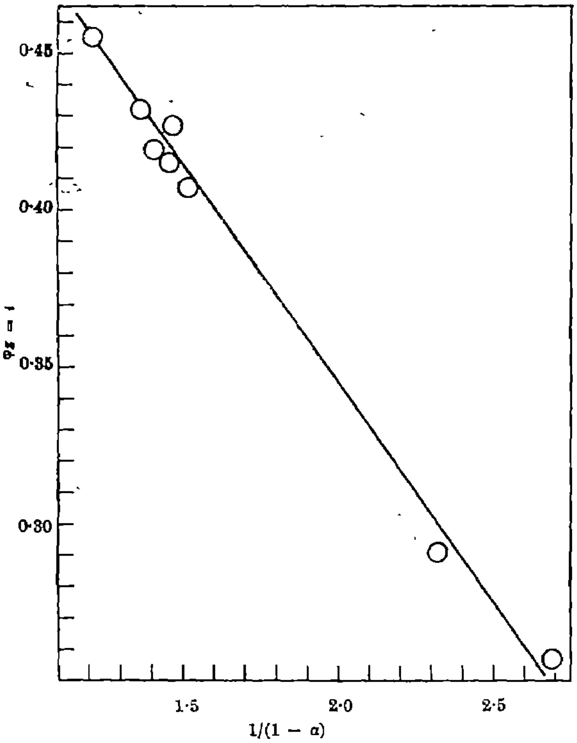

Fig. 1. $\varphi_{E=i}$ (V., on decinormal silver/silver iodide scale) against the reciprocal of the apparent cathodic transfer coefficient. In each case the solution was 1·05 × 10^{-4} *M* in I_3^- and 0·1 *M* in I^-. The disk was rotated at 60 r.p.m.

the same intercept, φ^*, when extrapolated to $1/(1-\alpha) = 0$, and, for both electrode processes, φ^* was close to the standard potential.

In these experiments, no particular care was taken to ensure that the electrode was in the same state of activity each time, and it was noted that duplicate experiments sometimes gave markedly different values for $1-\alpha$ and $\varphi_{E=i}$. Nevertheless, these fitted the linear plot appropriate to the particular solution composition. This suggested that it would be of interest to examine the effect of deliberately altering the activity of the electrode surface to see if the same correlation holds.

Preliminary results on the iodine redox process at platinum disk electrodes indicate that the correlation does hold. In Fig. 1, the points correspond to a series of experiments in which the disk was subjected to different pre-treatments. These included cleaning the disk with a chromic/sulphuric acid mixture, anodically polarizing it in this mixture, and anodically polarizing it in dilute hydrochloric acid for different periods. Between experiments, it was left exposed to a laboratory atmosphere which contained alkyl phenols.

The slope, corrected to unit concentration and unit apparent area, corresponds to a rate constant of 5·6 × 10^{-8} mole.cm.$^{-2}$sec.$^{-1}$, and the intercept gives φ^* = 0·623 V. on the decinormal silver/silver iodide scale. These may be compared with the values 3·4 × 10^{-8} mole.cm.$^{-2}$sec.$^{-1}$ and 0·626 V. found previously in a different laboratory atmosphere[6]. The agreement is satisfactory, particularly in view of the fact that the slope is a function of the actual, rather than the apparent, surface area.

The correlation is striking, although the actual effect of these different treatments on the surface is as yet unknown.

We thank the Chemical Society for a grant to one of us (W. A. L.), and Messrs. Burts and Harvey, Ltd., for providing laboratory facilities.

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OSMOLARITY

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THE clinical importance of the measurement of osmotic pressure of body fluids led to the definition of a new system of units which are used in biology and medicine, osmoles and milliosmoles. By analogy with molality and molarity, the expressions of osmolarity and osmolality are being used (although physical chemistry ignores them) and represent the molar concentration of all the osmotically active particles which are in a solution; osmolality is expressed per unit of weight and osmolarity per unit of volume.

If the solution is an ideal non-electrolyte, which means that it does not dissociate, the osmolality equals the concentration of the substance. If the substance dissociates and behaves like an ideal electrolyte, that is, an electrolyte which dissociates completely, the expression looks like: osmolality = nC, where n equals number of ions or particles in which the substance dissociates, and C equals concentration of the substance.

Real solutions require a more elaborate equation. The dissociation of a salt depends not only on its concentration but also on its activity, which itself may depend on the *p*H, the temperature, the pressure and even the concentration of the salt. Moreover, splitting enzymes are present in body fluids, which can break larger molecules into smaller fragments, increasing the osmolarity without modifying the solute content. Finally, the presence of ion-complexing substances in body fluids introduces another factor of error.

Differences between calculated and measured values of urine osmolarity can be more than 30 milliosmoles per litre, and percentage differences more than 20 per cent[1]. The same discrepancies between calculated and measured values have been observed in the plasma of patients with abnormal osmotic pressure[2].

When reference is made to osmolarity in this article, it concerns measured osmolarity. There will be no mention of the so-called 'ideal' osmolarity which represents the expected osmolarity, assuming a complete dissociation.

"Measurement," writes Nagel, "has been defined as the correlation with numbers of entities which are not numbers." Measurement leads to define units, and there are two kinds of units. First of all, units measuring additive or extensive qualities, units of weight, for example. On the other hand, densities, for example, are intensive or non-additive units, since mixing two liquids of known density does not result in a mixture the final density of which equals the sum of the two initial ones. The same holds for osmotic pressure.

E. Nagel[3] mentions a series of axioms which are the formal conditions of measurement. Assuming that a, b and c are three entities to be measured, then: (1) either $a > b$, or $a < b$, or $a = b$; (2) if $a > b$, and $b > c$, then $a > c$; (3) for every a there is an a', such that $a = a'$; (4) if $a > b$, and $b = b'$, then $a > b'$; (5) if $a = b$, then $b = a$; (6) for every a there is a b, such that $a > b$ (within limits); (7) for every a and b there is a c, such that $c = a + b$; (8) $a + b > a'$; (9) $a + b = a' + b'$; (10) $a + b = b + a$; (11) $(a + b) + c = a + (b + c)$; (12) if $a < b$, there is a number n, such that $na > b$ (also within limits).

The last six axioms are to be fulfilled if the measured quantities are to be additive. It is clear that, in the case of osmoles, axiom (9), for example, is not necessarily fulfilled. If two solutions of salts are mixed and if these salts are totally dissociated, and have no common ion, the final number of osmoles might equal the sum of the osmoles measured in the two initial solutions. However, if the same operation is made with two salt solutions having a common ion (in the presence of a common ion, the dissociation diminishes), or if their dissociation is dependent on temperature or pH, and if one of these parameters changes, the final number of osmoles may not be equal to the sum of the initial ones. So it appears that a major shortcoming of the concept of osmole is that this unit is not additive, although it was intended to be so, as shown by the analogy of nomenclature with moles, which are additive units.

Gamble[4], for example, evaluates the number of osmoles excreted per 24 hr. as a function of the diet with the implicit assumption that a patient must be in osmolar balance and must excrete as many osmoles as his diet brings him.

Another restriction imposed on the osmolarity comes from such metabolites as urea and glucose, for example, which can diffuse in the cellular department. Urea diffuses so freely through the body membranes that, physiologically, it exerts no osmotic effect, except at the level of the renal tubule, which is not permeable to urea. It is therefore evident that urea should not be accounted for in the measurement of the blood osmolarity, and some authors indeed subtract it from the measured values and express their units in terms of 'efficient osmolarity'. The reverse is true for the urine where such a subtraction should not be done, since urea exerts an osmotic pressure during the elaboration of urine in the kidney.

Examination of water excretion led some authors to consider two fractions or clearances, the osmolar clearance and the free water clearance. C_{osm}, the osmolar clearance, is that water which would contain a concentration of osmoles equal to that of the parent filtrate. C_{H_2O} or the free water clearance is positive or negative according to whether the urine osmolarity is smaller or higher than that of the glomerular filtrate[5].

$$C_{\text{osm}} = \frac{U_{\text{osm}}V}{P_{\text{osm}}}.$$

This equation might be very difficult to apply, since U_{osm} is a partial function of V; that means that the total number of osmoles depends on the urinary volume, which is another example of the non-additivity of osmoles. (Multiplication is repeated addition: U times V corresponds to adding U, V times to itself.) Moreover, what is the clearance of osmoles in a uræmic patient ? Should the osmolarity of the blood urea be subtracted from the measured value ? What then about the urine urea ?

In summary, the purpose of this article is to point out some of the inadequacies of the concept of osmolarity, and its fallacies: (1) Osmolarity is not necessarily a linear function of the amount of solutes. (2) Osmoles are non-additive units. (3) 'Efficient osmolarity' adds more imprecision to the evaluation and the comparison of biological data.

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USE OF SURFACE ACTIVE AGENTS FOR ESTIMATING THE CHARGES IN GELATIN AT THE ISOELECTRIC POINT

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THE titration of gelatin with acids and alkalis gives information on the net combining power of the basic and acidic groups on the protein molecules. This method has been used[1,2] to estimate the position of the isoelectric point, at which the number of positively and negatively charged groups are equal. thus, Hitchcock[2] measured the pH of sols of gelatin in dilute hydrochloric acid and sodium hydroxide at various concentrations. From the measured pH, he calculated the free H^+ and OH^- concentrations in the sols. He considered that the difference between these values and the molarity of the acid and the alkali used would represent the amount of H^+ or OH^- which had combined with the gelatin. These reactions may be schematically represented thus:

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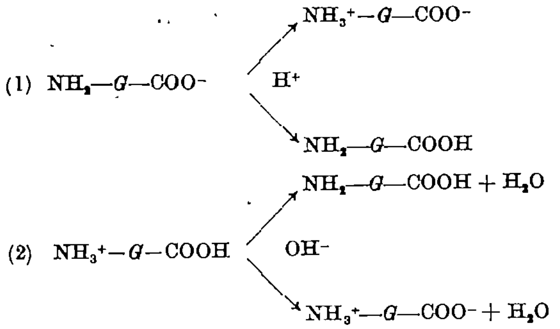

$$(1)\ NH_2{-}G{-}COO^- \xrightarrow{H^+} \begin{cases} NH_3^+{-}G{-}COO^- \\ NH_2{-}G{-}COOH \end{cases}$$

$$(2)\ NH_3^+{-}G{-}COOH \xrightarrow{OH^-} \begin{cases} NH_2{-}G{-}COOH + H_2O \\ NH_3^+{-}G{-}COO^- + H_2O \end{cases}$$

In these equations, G represents the protein residue, and NH_3^+, COO^-, refer respectively to all the positively and negatively charged groups attached to the protein residue. At pH 4·9, Hitchcock showed that there was no difference between the H^+ concentration of the sol and the molarity of the hydrochloric acid solution used, thus characterizing the isoelectric point.

Titration methods involving acids and alkalis give no indication of the actual number of positive and negative charges at the isoelectric point. It was thought that a study of the uptake of surface active agents could lead to a value for this quantity, which does not appear to have been reported in the literature. Pankhurst and Smith[3–5] have shown that gelatin forms complexes with anionic and cationic surface active agents (for example, sodium alkyl sulphates and cetyl pyridinium bromide respectively). They conclude that under the following conditions the polar groups of the surface active agents unite stoichiometrically with the charged nitrogenous and carboxyl (or phenolic) groups: (1) absence of concentrated electrolyte solutions; (2) relatively low pH values (that is, less than about pH 7).

In the presence of molar potassium chloride, and at high pH values, anionic surface active agents can be taken up in far greater quantity and appear to unite with the peptide groups of the protein chains. This effect is less pronounced for the secondary alkyl sulphates (for example, 'Teepol'[6]) than for the primary compounds.

Cetyl pyridinium bromide (CPB) solutions are easily standardized with potassium dichromate using the method of Epton[7], who also describes a technique for standardizing sodium alkyl sulphates against CPB. Solutions of 0·00912 N CPB and 0·00507 N 'Teepol' were prepared in this manner. 0·1 gm. powdered bone gelatin, purified by the method of Northrop and Kunitz[8], was added to 20-ml. portions of standard CPB or 'Teepol' solution in a series of test-tubes. To each of these were added 20 ml. dilute hydrochloric acid, water or dilute potassium hydroxide in order to vary the pH of the aqueous phase. The tubes were corked and the contents equilibrated on a rotary shaker for two days at room temperature, at which the solubility of the gelatin was found to be not more than 0·2 per cent. The gelatin was allowed to settle, and aliquots of the aqueous layers of the CPB series were titrated with standard 'Teepol'. Similarly, the 'Teepol' series was titrated with standard CPB. The quantities of surface active agents taken up by the gelatin were given by the difference between the quantities present in the original standard solution and those in the final aqueous phase, calculated from the titres.

Enough solid potassium chloride was then added to each tube to produce saturated solutions. The pH of these was determined by means of a Cambridge pH meter. The potassium chloride was introduced in order to suppress H^+ concentration gradients in the gel phase, so that the measured pH of the aqueous phase should more nearly approximate to that in the gelatin[9]. A sample of the original gelatin was dried to constant weight in an air oven at 100° C. so that the results could be expressed as uptake of 'Teepol' or CPB per gm. oven-dry gelatin. These results are plotted against the measured pH in Fig. 1. The point at pH 2 (0·87 m.equiv./gm.) for the anionic surface active agent is taken from the results of Pankhurst[5], who used sodium dodecyl sulphate.

The pH at which the 'Teepol' and CPB curves intersect should represent the isoelectric point. The value of 4·9 is in good agreement with that derived from titration curves and from electrophoresis experiments. Fig. 1 also shows that there are still about 0·2 m.equiv. of positive and negative charge per gm. gelatin at this point.

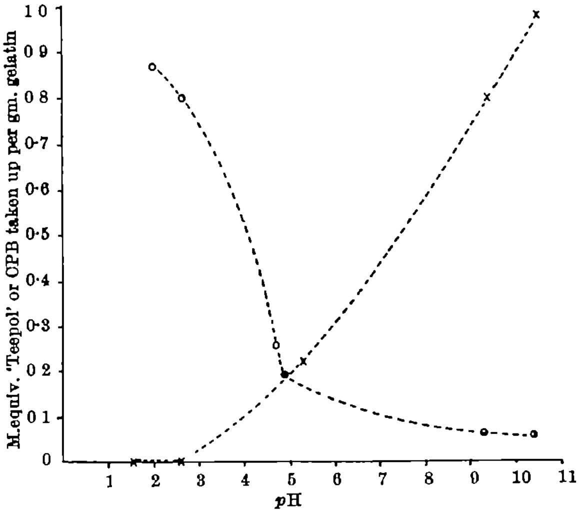

Fig. 1. Uptake of 'Teepol' (○) and cetyl pyridinium bromide (CPB) (×) with varying pH

Published titration curves[1,2] indicate that at pH 2, at which the carboxyl groups are almost entirely undissociated, there are about 0·9 m.equiv. of positive charge per gm. gelatin, which agrees reasonably well with Pankhurst's figure of 0·87. The results obtained at the alkaline end must be treated with caution; the general form of the 'Teepol' curve is, however, consistent with the progressive discharging of the imidazolium (pK 5·6–7·0), α-ammonium (pK 7·5–8·4), ε-ammonium (pK 9·4–10·6) and guanidinium (pK 12·0–13·0) groups in gelatin. Possible hydrolysis of —CO—NH— linkages at high pH values introduces uncertainties in the value of the total negative charge developed at the far alkaline end; Pankhurst's results indicate that at pH 12·5 this may have a value of from 1·3 to 1·7 m.equiv. depending on the chemical nature of the cationic surface active agent.

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MODEL COMPOUNDS FOR METAL–PROTEIN INTERACTION: CRYSTAL STRUCTURES OF TWO GLYCYLGLYCYLGLYCINE–COPPER COMPLEXES

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IN an effort to gain a detailed understanding of the steric relationships involved in metal–protein interaction, we have embarked on a programme of crystallographic studies of metal–peptide complexes[1]. In this article we wish to describe the first two determinations of the crystal structures of complexes between a metal and a tripeptide.

When a concentrated aqueous solution of equimolar proportions of cupric chloride and glycylglycylglycine is allowed to stand for some days, stable blue-green crystals of glycylglycylglycino copper(II) chloride 1·5-hydrate (compound *a*) are formed. The crystals have the shape of a distorted boat: two parallel faces are planar, while the two other faces are distinctly curved and meet at the 'bow' and 'stern' of the boat. The sides of the boat are not perpendicular to the top and bottom faces. The crystals are monoclinic, space-group $C2/c$, with $a = 21{\cdot}36$ Å., $b = 6{\cdot}72$ Å., $c = 15{\cdot}64$ Å. and $\beta = 98°\ 15'$. With eight formula units in the unit cell, $D_x = 1{\cdot}88$ gm./cm.^{3}.

Under alkaline conditions, cupric ion and glycylglycylglycine yield a completely different complex. Violet crystals of sodium glycylglycylglycino cuprate(II) monohydrate (compound *b*) may be obtained by a method described by Rising, Parker and Gaston[2]. Freshly precipitated cupric hydroxide dissolves in a solution of glycylglycylglycine containing an excess of alkali. When the resulting violet solution is added to a large excess of an alcohol–ether mixture and is allowed to stand for some days, violet crystals of the glycylglycylglycine–copper(II) complex are formed. These crystals are flat hexagonal prisms, monoclinic, with space-group $I2/c$. There are eight formula units in a unit cell with dimensions $a = 14{\cdot}33$ Å., $b = 10{\cdot}56$ Å., $c = 13{\cdot}18$ Å., $\beta = 92°\ 58'$, $D_m = 1{\cdot}92$ and $D_x = 1{\cdot}94$ gm./cm.^{3}.

The structure of compound *a* is shown in Fig. 1. It was determined by three-dimensional Patterson and Fourier methods from the complete data observable with copper K_α-radiation. In addition to the water molecule included in the formula of the substance written as a monohydrate[2], an additional water molecule was discovered on a two-fold axis, so that the correct molecular formula is that of a 1·5-hydrate. After preliminary least-squares refinement with a single isotropic temperature factor and two cycles of refinement including individual anisotropic atomic thermal parameters, the residual $R = 14{\cdot}1$ per cent.

For compound *b*, the structure was similarly solved by using the three-dimensional copper K_α-data. After least-squares refinement with anisotropic thermal parameters, the residual $R = 13{\cdot}2$ per cent. The structure is shown in Fig. 2.

The details of the structure analyses and refinements will be reported in subsequent communications. This article is confined to a general description of the two structures and to some comments on their relationship to some other recently determined structures of metal complexes.

In compound *a*, the peptide chain is attached to two copper atoms, and each copper atom is bonded to two different peptide molecules. Five-fold co-ordination about each copper atom results from a square-pyramidal arrangement of nearest neighbours. The base of the pyramid consists of the nitrogen and peptide oxygen atoms of the first glycine residue of one peptide molecule, the negatively charged oxygen of the terminal carboxyl group of another peptide molecule, and a chloride ion. The three peptide ligand atoms are all about 2 Å. from the copper atom, and the chlorine is located 2·2 Å. from it. At the apex of the pyramid, 2·3 Å. from the copper, lies the oxygen atom of a water molecule. The copper atom itself lies slightly 'above' the base of the pyramid, that is, displaced in the direction of the water molecule.

An additional water molecule (the .5-H_2O in the formula) lies on a two-fold symmetry axis, neatly hydrogen-bonded between four peptide chains.

The copper atom in complex *b* is also 5-co-ordinated. Its nearest neighbours are a square of three nitrogen atoms and one oxygen, all about 2 Å. away, plus a fourth nitrogen lying along the normal to the square and about 2·6 Å. above the copper. This complex has a dimeric character, the two halves of the dimer being related by a two-fold axis. In each half of the dimer the peptide molecule lies close to the plane of the three nitrogen atoms which are bonded to the copper. Only the terminal carboxyl group leaves this plane so that one oxygen atom occupies the fourth position in the co-ordination square of the adjacent copper atom. The third nitrogen atom of the peptide is not only bonded to one copper atom but is also at the apex of the square pyramid about the other copper atom of the dimer. The Cu–Cu distance is about 3·1 Å., and the copper atoms are not quite co-planar with the four closest ligand atoms.

The sodium ion and the water molecule occupy interstitial sites in a way which suggests that they make links between the dimers. The water molecule is hydrogen-bonded to a free carboxyl oxygen of one dimeric complex and to the first peptide oxygen of another. A pair of hydrogen bonds links the terminal amino nitrogen to the free carboxyl oxygen of one neighbouring complex and to the second peptide oxygen of another.

The glycylglycylglycine complexes *a* and *b* extend a series of structure determinations beginning logically with those of *bis*-glycine copper(II) monohydrate (compound *c*) and glycylglycino copper(II) trihydrate (compound *d*). The structure of compound *c* was recently determined by Tomita and Nitta[3] (and confirmed by M. R. Snow in our Laboratory), and that of compound *d* by Lindqvist, Strandberg and Rosenstein (private communication).

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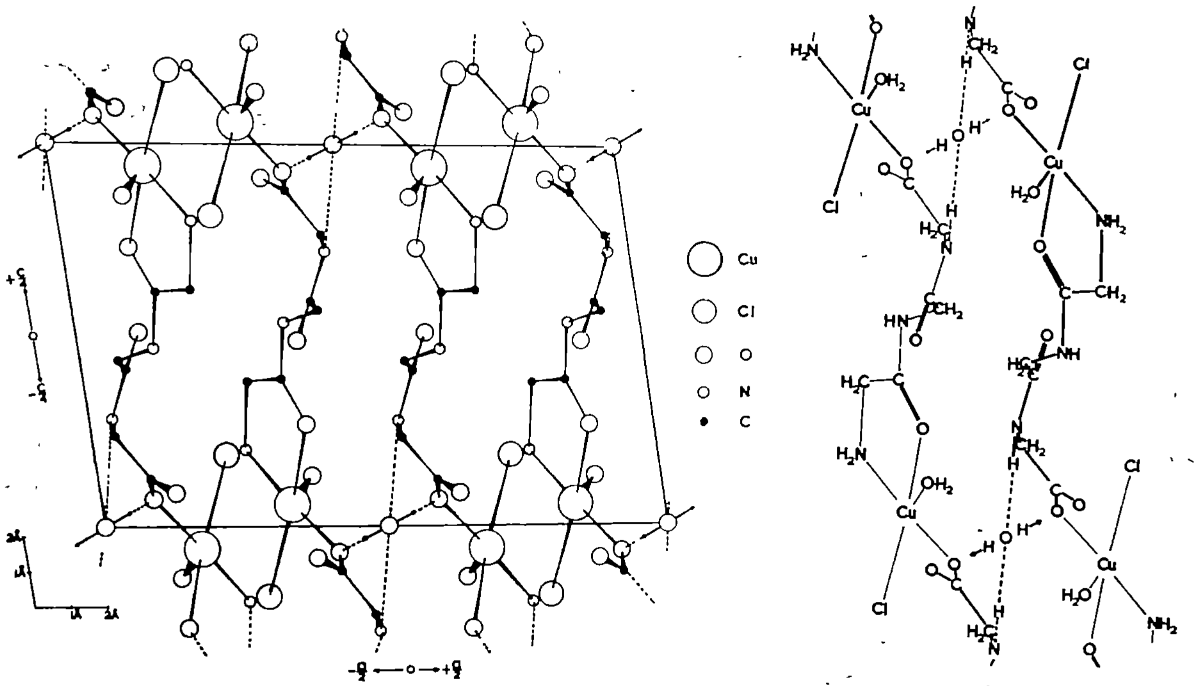

Fig. 1. (*a*) Projection of unit-cell of [Cu(glyglygly)]Cl.1½H₂O (compound *a*) along *b*-axis; (*b*) explanation of molecular arrangement in (*a*)

A significant structural feature which compounds *a*, *b* and *d* have in common is the planarity of their peptide groups. The fact that this characteristic feature of the un-complexed peptides is retained (as it is in two biuret–copper complexes[1]) implies that the complexes tend to form without sacrificing the resonance energy of their peptide groups. On the other hand, none of the chelated peptide residues in compounds *a* and *b* or in the biuret–copper complexes is quite co-planar with the copper atom to which it is joined. This statement applies also to compound *d*, in which the central copper atom does not lie in the plane of the reportedly flat dipeptide molecule (Lindqvist *et al.*, private communication). Accordingly, the orbitals of the peptide ligand atoms are not pure sp^2.

A second property shared by compounds *a*, *b* and *d* is the 5-co-ordination of the copper atoms. It remains to be seen whether this is characteristic of copper–peptide complexes or not. It is possible that in complex *a* the 5-co-ordination may be a result of steric hindrance: the orientation of the carboxyl group which is linked to the copper through one of its oxygen atoms is such that its second oxygen atom obstructs the approach of a sixth ligand to the copper. In compounds *b* and *d*, on the other hand,

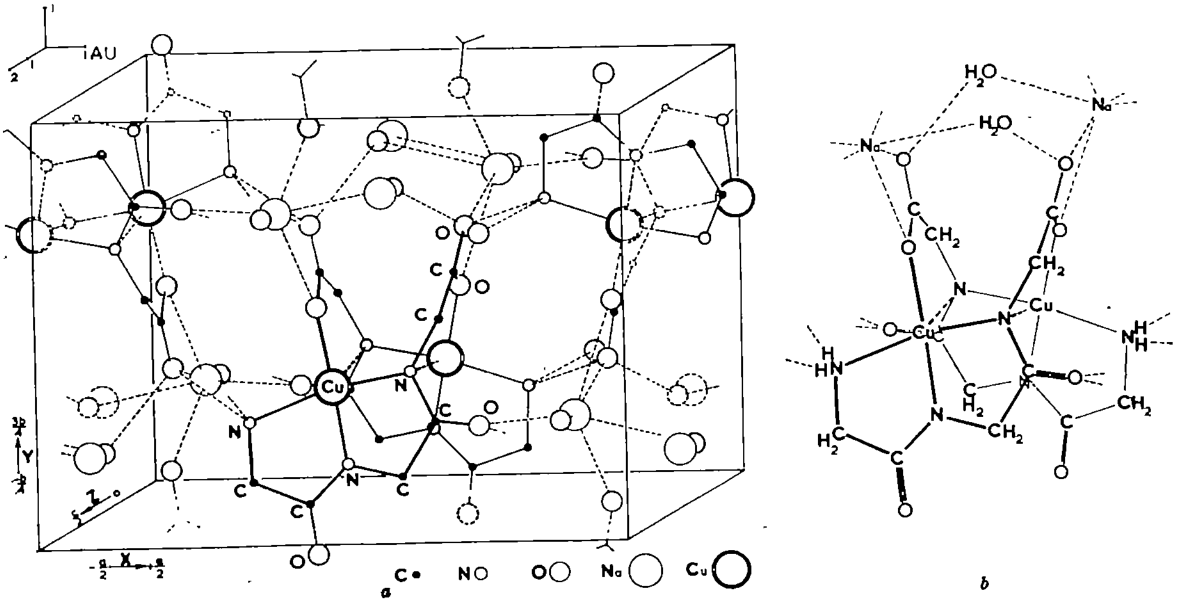

Fig. 2. (*a*) Unit-cell of Na[Cu(glyglygly)].H₂O (compound *b*); (*b*) explanation of molecular arrangement in (*a*)

no such steric factors hinder copper from adopting its more common 6-co-ordinated, distorted octahedral configuration.

A number of structures in which 5-co-ordinated copper(II) occurs have recently been described[4]. Whether 5-co-ordination of a metal is related to catalytic activity is another speculative matter: there has recently been a suggestion that the catalytic activity of metal chelates depends on the existence of vacant valences ('*Koordinationslücken*')[5].

Glycylglycylglycine is the shortest peptide which gives the characteristic 'biuret' colour-change in the presence of an alkaline solution containing cupric ions. The participation of the peptide oxygen atoms of triglycine in chelation in neutral solution parallels the use of the amide oxygen atoms as donors in the blue-green complex, *bis*-biuret copper(II) chloride[1]. In alkaline solutions, both biuret and triglycine use the maximum numbers of nitrogens as ligand atoms and form violet copper complexes. Under the same alkaline conditions, glycine and glycylglycine yield only blue solutions from which the blue crystalline substances *c* and *d* have been isolated.

The two structures *a* and *b* thus illustrate the extremes of the change which occurs when a neutral solution of copper ions and a peptide is made strongly alkaline. We are attempting to produce suitable crystal specimens of the blue intermediate complex Cu(glyglygly).$3H_2O$ which was reported by Manyak, Murphy and Martell[6]. This neutral complex would represent the mid-point of the transition between the blue-green cation $[Cu(glyglygly)]^+$ and the violet anion $[Cu(glyglygly)]^-$.

On the other hand, a crystal structure analysis does not provide proof that a complex has a particular structure in solution. For example, compound *b* may not retain its dimeric form when dissolved; the fourth position in the plane of the three peptide nitrogen ligand atoms may be taken up by a water molecule, with two other water molecules—less strongly bonded—completing an octahedron about the copper atom. Similarly, in the parent copper–biuret complex, potassium *bis*-biuret cuprate(II) tetrahydrate[1], two nitrogen atoms of adjacent complexes occupy the 'polar' positions in the co-ordination octahedron about each copper in the solid state. These more distant nitrogen atoms are apparently replaced in solution by loosely bonded water molecules, without any change in the violet colour of the complex. (*Note added in proof.* Recent unpublished potentiometric, spectral and kinetic measurements (Koltun, W. L., Roth, Reta H., and Gurd, F. R. N., private communication) suggest that the dimeric form of compound *b* does not persist in solution. Compounds *a* and *b* correspond to species in solution represented by the above authors as $CuGGG^+$ and $CuGGG^-$, respectively.)

With this general reservation, the four structures *c*, *d*, *a* and *b* support the scheme for metal–peptide interaction proposed by Rabin[7] on the basis of potentiometric and spectroscopic measurements. In particular, in neutral solution the tripeptide binds the copper atom *via* the nitrogen and oxygen atoms of the *N*-terminal peptide residue as predicted, while in the complex formed in alkaline solution the bonding is *via* the nitrogen atoms of three adjacent amino-acid residues. The structures of *b* and *d* were also predicted correctly by Manyak, Murphy and Martell[6].

Although Rabin considered that (in dipeptides) copper–carboxyl bonding is unlikely at pH's where the first peptide protons are not dissociated[7], the terminal carboxyl group in complex *a* as well as that in complex *b* is attached to a copper atom, albeit not the same one as is bonded to the rest of the same peptide molecule. In the cationic complex *a*, this quasi 'side-chain' behaviour of the carboxyl group is reminiscent of the otherwise incorrect extended chain structures proposed by Klotz *et al.*[8].

Finally, the dimerism of complex *b* suggests a way in which metal atoms in proteins may help to stabilize the tertiary protein structure. In crystal *b* we have a potential model of a set of geometrical conditions for the metal-linking of two polypeptide chains.

In future communications we hope to be able to report our crystal structure analyses of complexes between copper and glycylglycylglycylglycine, alanylglycylglycine and other short peptides with acidic and basic side-chains. Endeavours to extend this work to the peptide complexes of other transition metals have hitherto been limited to those of cobalt by our inability to produce suitable crystalline specimens of complexes from other metals. Even with the copper and cobalt complexes which we have at present under investigation we are working with compounds the compositions of which we do not know: our preparations have been carried out on such a small scale and our crystal yields have been so low that chemical analyses have not been possible.

This investigation was supported by a research grant (*A*-3460 *B.B.C.*) from the Institute for Arthritis and Metabolic Diseases, U.S. Public Health Service; and by the award of an Alexander Boden Fellowship at the University of Sydney to one of us (J. C. S.).

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CONVERSION OF SHIKIMIC ACID TO AROMATIC COMPOUNDS

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THE synthesis of aromatic amino-acids is now known to follow a common pathway at least so far as shikimic acid. The number and identity of later common intermediates is not yet established. The experiments outlined here concern the conversion of shikimic acid to anthranilic acid and other aromatic compounds by cell-free extracts of various strains of *Aerobacter aerogenes* and *Escherichia coli*. The cells were grown in limiting indole medium as described by Gibson and Yanofsky[1] and the cells disrupted using an M.S.E. Mullard 500-W. ultrasonic disintegrator. The substrate mixture used with crude

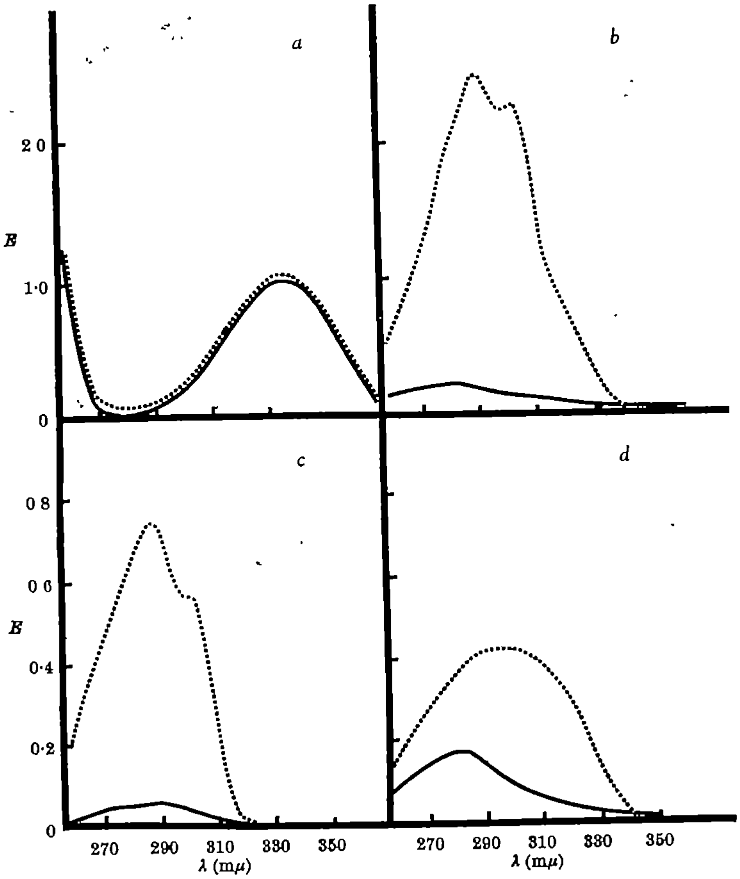

Fig. 1. Formation of anthranilic acid and phenylpyruvic acids in the presence and absence of glutamine respectively. *a*, Spectra of anthranilic acid in ethyl acetate after extraction from acidified reaction mixture. Test consisted of crude extract of *A. aerogenes T*17 (1·7 mgm. protein), shikimic acid (1 μmol.), diphosphopyridine nucleotide (1 μmol.), adenosine triphosphate (1 μmol.), magnesium chloride (5 μmol.), ribose-5-phosphate (2 5 μmol.), L-glutamine (5 μmol.), *tris* buffer *p*H 8·2 (50 μmol.) in a final volume of 1 ml. Incubated 45 min. at 37°. After acidification with hydrochloric acid the experimental samples were extracted with 4 ml. ethyl acetate, the spectra measured at 5 mμ intervals or less immediately and after storage of the ethyl acetate extract overnight at 30°. The spectra are identical with those given by synthetic anthranilic acid. *b*, Substrates and conditions as above except glutamine omitted from reaction mixture. *c*, Spectra of an ethyl acetate solution of phenylpyruvic acid obtained by acidifying 1 ml. of an aqueous solution (0·25 μmol. in 0·05 *M tris* buffer *p*H 8 2) and extracting with 4 ml. of ethyl acetate. *d*, Spectra of 4-hydroxyphenylpyruvic acid obtained as for (*c*). ———, Immediate spectrum. . . . , Spectrum after storage of ethyl acetate extracts at 30° C.

extracts consisted of shikimic acid, adenosine triphosphate, magnesium chloride, diphosphopyridine nucleotide, ribose-5-phosphate and L-glutamine in *tris* buffer. The substrates differed from those originally described by Srinivasan[2] for anthranilic acid synthesis by crude extracts of *E. coli*. We find that shikimic acid is an effective substrate and that ribose-5-phosphate or glucose-6-phosphate or fructose-6-phosphate stimulate up to ten-fold the amount of anthranilic acid formed. Adenosine triphosphate was usually included, although it was not always found to stimulate anthranilic acid formation.

When glutamine was omitted from the mixture already described it was found that anthranilic acid formation was greatly reduced but other compounds were formed. These compounds were detected after experimental samples had been acidified and extracted with ethyl acetate and the ultra-violet absorption spectrum of the solvent layer examined (Fig. 1). It was found that after incubation of the ethyl acetate layer at 30° overnight, peaks developed with maxima at 288 mμ and 302 mμ (Fig. 1*a*, *b*). These peaks appear to be due to a mixture of phenylpyruvic acid and 4-hydroxyphenylpyruvic acid, these compounds also giving spectra showing increasing optical density following ethyl acetate extraction from acidified aqueous solution in *tris* buffer (Fig. 1*c*, *d*).

Concentrates of the ethyl acetate solutions obtained as already indicated from the reaction mixture without glutamine were chromatographed in three solvents and sprayed with several detecting reagents. By this means the presence of phenylpyruvic acid and 4-hydroxyphenylpyruvic acid was confirmed. 4-Hydroxybenzoic acid and other unidentified compounds reacting as phenols were also found to be present. Phenylpyruvic acid and 4-hydroxyphenylpyruvic acid are established as intermediates in the formation of phenylalanine and tyrosine respectively. The immediate precursor of the phenylpyruvic acids, prephenic acid, if present in experimental samples would have been converted to phenylpyruvic acid following acidification for extraction with ethyl acetate. The mode of formation of 4-hydroxybenzoic acid is not understood and it is hoped to gain some information about it using the system described above.

In preliminary purification procedures the nucleic acids were precipitated from crude extracts using manganous chloride, the proteins precipitated by ammonium sulphate, re-dissolved in *tris* buffer and dialysed. The dialysates were examined for their ability to convert shikimic acid-5-phosphate to anthranilic acid and the phenylpyruvic acids. Reduced diphosphopyridine nucleotide was regularly required and phosphoenolpyruvate stimulated either conversion. One experiment showing these requirements for anthranilic acid formation is illustrated in Table 1. It was not necessary to add diphosphopyridine nucleotide in the presence of reduced diphosphopyridine nucleotide. Boiled crude extracts were found to contain an unidentified factor or factors, which restore activity lost in dialysates stored at −20°.

The stimulation of both anthranilic acid synthesis and the formation of the phenylpyruvic acids by phosphoenolpyruvic acid would suggest at least one

Table 1. ANTHRANILIC ACID SYNTHESIS BY AN ENZYME PREPARATION FROM *Aerobacter aerogenes T*17*

Basal reaction mixture† plus	Anthranilic acid formed (mμmol. after 75 min. at 37°)			
	1	2	3	4
Phosphoenolpyruvic acid (2 μmol.)	+	+	−	+
Reduced diphosphopyridine nucleotide (1 μmol.)	+	+	+	−
Diphosphopyridine nucleotide (1 μmol.)	+	−	−	−
	475	430	133	5

* Strain used by Doy and Pittard (ref. 8).

† *Tris* buffer *p*H 8·3 (50 μmol.), shikimic acid-5-phosphate (1 μmol.), magnesium chloride (5 μmol.), ribose-5-phosphate (2 5 μmol.), L-glutamine (5 μmol.), ammonium sulphate precipitated enzyme preparation (1 mgm. protein). Final vol. 1 ml.

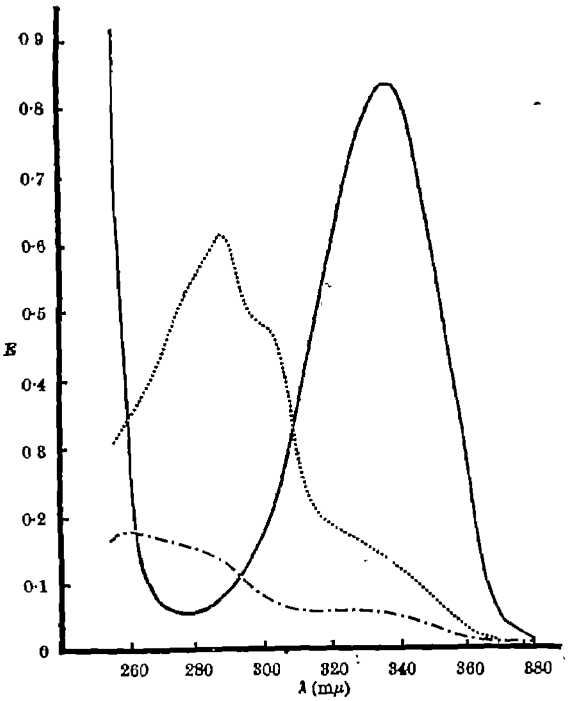

Fig. 2. Formation of phenylpyruvic acids as the result of tryptophan inhibition of anthranilic acid synthesis. *a*, Reaction mixture as for Fig. 1*a* except 1·7 mgm. protein used and the test incubated 45 min. Spectra measured immediately in ethyl acetate and after overnight storage at 30°. ——— Immediate spectrum. Overnight spectrum as in Fig. 1*a*. *b*, Reaction mixture as above with addition of L-tryptophan (0·5 μmol.). ·–·–·– Immediate spectrum. . . . Spectrum after storage overnight in ethyl acetate

common intermediate containing the pyruvate side-chain as proposed by Davis[3] rather than a pathway branching at 5-phosphoshikimic acid[4]. However, the possibility remains that the effect of phosphoenolpyruvic acid is an indirect one, and more information is needed about the metabolism of compounds such as 3-enolpyruvylshikimate-5-phosphate[5].

Several amino-acids have been shown to inhibit the first reaction specific to their own formation. Moyed[6] has shown that tryptophan inhibits the conversion of shikimic acid to anthranilic acid by cell-free extracts. Our experiments show that this inhibition is accompanied by the formation of the phenylpyruvic acids (Fig. 2), indicating that intermediates which would be converted to anthranilic acid are diverted by tryptophan along the phenylalanine–tyrosine pathways. This provides evidence that end-product inhibition by tryptophan, like that of other amino-acids, concerns the first specific reaction.

The observation of the formation of phenylpyruvic acid, 4-hydroxyphenylpyruvic acid and 4-hydroxybenzoic acid in the absence of the nitrogen source, glutamine, for anthranilic acid synthesis agrees with similar results using suspensions of whole cells[7]. This supports the suggestion of the above authors that the nitrogen for anthranilic acid formation may be introduced in the first reaction specific to the tryptophan pathway.

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ELECTRIC BIREFRINGENCE INVESTIGATIONS OF DEOXYRIBONUCLEOPROTEIN FROM RAT THYMUS

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NUMEROUS physico-chemical studies have been made on deoxyribonucleoprotein (DNP), but only rarely have reproducible results been obtained and there have been few estimates of the dimensions of the molecule. In the present work DNP has been investigated chiefly by the method of electric birefringence, which was used by Benoit for DNA[1] and by O'Konski *et al.* for proteins and viruses[2–4]. Satisfactory reproducibility has been obtained on material which, judging by its very large molecular size, may be undegraded. The birefringence Δn and relaxation time T of decay of birefringence on removal of the electric field give information about the electrical properties and the length ($2a$) of the molecule respectively, the latter by the following relationships:

$$T = \frac{1}{6D} \qquad (1)$$

$$D = \frac{k_1}{a^3}\left(\ln \frac{2a}{b} - k_2\right) \qquad (2)$$

where D is the rotary diffusion coefficient, $2b$ is the molecular diameter and k_1 and k_2 are constants.

Preparations of DNP were made from rat thymus by homogenization in 0·9 per cent sodium chloride solution followed by dispersion in water to give, finally, a rigid gel. Electric birefringence and centrifugation studies were made on dilutions of the gel in 10^{-3} *M* disodium hydrogen phosphate solution, *p*H 7. DNA concentrations were measured by the Dische diphenylamine method[5], and protein concentrations by a modification[6] of Zamenhof and Chargaff's method[7] involving measurement of the ultra-violet absorption of the copper-biuret complex. The curves of decay of birefringence were investigated initially by drawing graphs of log Δn against time. In later experiments T was measured by a method involving comparison of these decay curves with those produced by two condenser resistor circuits of known time constants[6].

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The results showed that two main molecular components were present in the DNP preparation; their decay times will be referred to as $T_{\max}$ and $T_{\min}$. The relaxation times were found to be independent of concentration of DNP over a sevenfold range and the mean values of $T_{\max.}$ in a series of ten experiments was 165 msec. with standard deviation of ± 33 per cent of the mean. The error in measuring $T_{\min.}$, however, was as much as 100 per cent[6], the values usually being about 1/50th of $T_{\max}$.

The Kerr law states that:

$$\Delta n = B\lambda E^2 \qquad (3)$$

where B is the Kerr constant, λ is the wave-length of the light used and E is the electric field strength. This law was found not to be obeyed by DNP, Δn depending on a power r of the field of less than two. The reproducibility in measurement of Δn at a given voltage was good, the agreement being within 5 per cent for two samples diluted to the same concentration from one gel and within about ± 15 per cent for different preparations measured at the same concentration.

A progressive decrease in Δn but not in T was observed on keeping the DNP dilutions at 4° C. This was enhanced if the dilutions were incubated at 37° (Table 1). The approximate constancy of T indicates that 'ageing' is not a process of degradation along the length of every molecule. It could be due to a decrease in the number of molecules of the long component; however, the ratio of initial birefringence of the short component to that of the long component at the instant of removal of the electric field (which depends on both the number and the length of the molecules) remains approximately constant. In fact, decay curves of the same initial height from DNP samples of different ages could be superimposed exactly, indicating that the distribution of molecules remained constant. In contrast to this effect of ageing, both irradiation and re-precipitation of the DNP[6] with 1·4 M sodium chloride solution caused a decrease in Δn and no change in T but an increase in ratio of the initial birefringence of the short to the long component, indicating that the number of molecules of the latter had decreased.

A possible explanation of the ageing effect is that the DNP consists of laterally bonded three-dimensional aggregates or particles of unit DNP molecules, the change of Δn with time being due to lateral disaggregation, that is, partial or complete breakage and removal of some of the sub-units by enzymatic action. Such changes would not affect the overall length since this would depend on the unbroken sub-units, and the change in axial ratio would not affect T significantly since it occurs only as a logarithmic term in equation (2).

The finding that Δn depends on a power of E of less than 2 indicates that saturation of birefringence occurs, presumably because the alignment of the molecules in the electric field is not countered sufficiently by Brownian motion. Experiments on the effect of proteolytic enzymes (trypsin and subtilisin) and of urea added to the DNP, and on DNA extracted from the DNP[6], all show that the power r increases on destruction or removal of the protein component, indicating that the DNA moiety is considerably smaller in volume than the DNP.

Centrifugation of the DNP at 12,000g for 30 min. yielded a gel residue. The DNP concentration of the clear supernatant liquid was usually about 30–50 per cent of the total value. Similar centrifugation of DNA prepared from the DNP caused, as would be expected, no phase separation. However, the relaxation times of decay of birefringence of DNA were found to be only slightly less, $T_{\max.}$ being about 100 msec. The very large volume of a DNP aggregate or particle could explain this very high sedimentation-rate compared with that of DNA although the lengths of the two molecules could be very similar.

If axial ratios of about 5 : 1 and 50 : 1 are assumed for DNP and DNA respectively, the very large values of 15,000 Å. and 17,000 Å. are obtained for the lengths of the major axis, $2a$, using equations (1) and (2). The latter value for DNA is considerably greater than that usually found from measurements of rotary diffusion coefficient (even allowing for denaturation caused by use of a medium of low ionic strength) although it is within the range found for various DNA preparations by Reichmann *et al.*[8]. It is possible that the DNA here is cross-linked by residual protein present, but, after addition of proteolytic enzymes to DNP, similar values of $T_{\max}$ are obtained and it seems fairly unlikely that either the protein fragments, or the enzymes themselves, would cross-link the constituent DNA (which, presumably, is released by the enzyme action) to give values of $T_{\max}$ similar to those of DNA extracted from the DNP.

In summary, although constancy of T is clearly not a good proof of constancy of molecular volume, when considered with the fair reproducibility of Δn, it indicates that these DNP preparations do not consist of mere randomly sized particles of gel but that they may be aggregates or particles of more or less constant size formed by lateral association of unit DNP molecules. A detailed account of other investigations on the properties of DNP, including their concentration dependence, will be given elsewhere.

I thank Prof. J. S. Mitchell for advice and the Nuffield Foundation for a grant with which I began this work.

Table 1

	DNP conc. (per cent)	Time after dilution of gel (hr.)	$T_{\max.}$ (msec.)	$T_{\min.}$ (msec.)	$\Delta n/\lambda$* (cm.$^{-1}$ $\times 10^{-8}$)
Exp. 1.	0 036	0	168	2	4 08
		2¼ at 37° C.	131	8	3 23
		24 at 4° C.	157	7	3 73
Exp. 2.	0·044	0	182	2	7 80
		4 at 4° C.			6 55
		24 at 4° C.	118	2	5 60
Exp. 3.	0·042	0	138	3	8 52
		4 at 4° C.	138	5	7 35
		24 at 4° C.	126	4	6 22

* All values measured at the same field strength.

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ACUTE RADIATION INJURY: PATHOPHYSIOLOGICAL ASPECTS OF THE MASSIVE LEAKAGE OF ALBUMIN INTO THE GASTROINTESTINAL TRACT

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PHYSIOLOGICAL accumulation of albumin in, and leakage through, the gastrointestinal wall is documented[1–4,12]. Quantitative studies of this leakage have shown that the bulk of the 'catabolism' of albumin occurs via the gastrointestinal tract, both in man[4] and in animals[5,6].

It is thus reasonable to assume albumin leakage from the gastrointestinal tract when this tract is in a pathological state. This was first demonstrated in ulcerative colitis and terminal ileitis by Steinfeld *et al.*[7] and in 'exudative gastroenteropathia' by Citrin *et al.*[8], Gordon[9] and Schwartz and Jarnum[10]. These findings have since been confirmed[11–13].

Cancer of the stomach, too, has proved to be associated with a pathological leakage of albumin[2,3,12] as well as a pathological distribution of albumin[13].

It is therefore reasonable to suspect that damage to the gastrointestinal capillary system as well as the known injury of the epithelium in the acute radiation syndrome would give rise to excessive loss of protein. Barandun *et al.*[14] have found, moreover, an increased percentage of PVP-^{131}I in the cœcal contents of mice exposed to 1,000 r. In order to investigate the clinical significance of these injuries the following experiments were conducted.

Following irradiation of rabbits by a medical cobalt-60 γ-irradiation source (group I, 250–400 r.; group II, 650–850 r.; and group III, 1,200–1,600 r.) the degradation and distribution of albumin were investigated by means of 70–100 μc. intravenously administered ^{131}I-labelled rabbit albumin. Plasma activity, total body activity and urinary and fæcal excretion of radioactivity were determined daily. In the first two groups the ^{131}I-albumin was injected 42 hr. after irradiation, and in the last group after 18 hr.

The degradation and distribution of albumin, determined by the methods of Matthews[15] and Campbell *et al.*[16], in controls and irradiated animals are tabulated in Table 1, as are the half-life values. Initial plasma volumes and amounts of intravascular albumin are also given. A clearly elevated degradation can be observed in the irradiated rabbits except in rabbit No. 4 which showed a nearly normal value. The half-life is inversely proportional to the radiation dosage. Figs. 1, 2 and 3 also show the cumulative urinary excretion of radioactivity, which normally amounted to 30–40 per cent of the administered dose in 11 days, but was 60–70 per cent in irradiated groups I and II. Fæcal excretion of radioactivity in controls was < 0·07 per cent during the period of investigation (11–13 days). Groups I and II showed an increased excretion (1·03–1·76 per cent and 0·17–0·45 per cent respectively), as did group III during the 4–7 days these animals lived (0·17–0·71 per cent). The ratio of intravascular to extravascular albumin was 1 : 1·5 in controls and in group I. A lower extravascular albumin pool was recorded in group II, possibly indicating a non-steady state. In the last group (Fig. 4), in which the animals died, extensive extravascular retention of ^{131}I-albumin was found.

On analysis of the plasma activity curve in its exponential parts, the half-life of the initial distribution in controls was found to be equal to that in group I (half-life = 0·7–0·8 day), whereas in group II the half-life value was ~ 0·2 day. In group III, on the other hand, a half-life of 0·4–0·5 day was found, probably because of the earlier start of the ^{131}I-albumin study in those animals (18 hr. after irradiation).

In group III both animals died. The rabbit which had received 1,200 r. showed, on the sixth day of the experiment, a high urinary excretion of radioactivity (Fig. 4), and simultaneously a steeper slope of the plasma activity curve was observed. This is interpreted as a massive re-absorption of degradation products of ^{131}I-albumin which has leaked into the gastrointestinal tract, denoting at least a partial restitution of the re-absorption capacity of the intestine. No corresponding signs of re-absorption were observed in the animal which had received 1,600 r. Autopsy revealed general œdema of the gastrointestinal tract, most pronounced in the ileum and colon, which were partially dilated. The changes were most accentuated in rabbit No. 8 (1,600 r.), in which 47 per cent of the retained dose was found in

Table 1. DATA ON RABBITS INVESTIGATED ACCORDING TO IRRADIATION DOSES

Animal		Initial plasma vol. (ml.)	Initial intravascular albumin pool		*T*/2 *IV** day	*T*/2 *RT*† days	Ratio *IV*/*EV* albumin‡	Degrad. per cent of intravascular albumin	Degrad. (gm./24 hr.)	Degrad. (gm./kgm./24 hr.)
			gm.	gm./kgm.						
Controls	1	104	4·9	1 9	8 5	8·6	1:1·5	19·5	1 0	0·36
	2	150	7 0	1·6	12·6	11·1	1:1·5	14·7	1·0	0·23
Group I	3	123	5·8	1·3	6·0	6·6	1:2	28·2	1·6	0·37
	4	128	6·0	1·3	8·3	7·7	1:1·5	20 6	1·2	0·25
Group II	5	104	4·9	2·1	5·1	5·0	1:1·5	25·2	1·2	0·5
	6	122	5·7	2·2	5·5	5·1	1 1	23·0	1·3	0·5
Group III	7	127	6·0	2·3	3·3–5·3	—	1:2 5	—	—	—
	8	107	5·0	1 9	3·7	—	1:2 5	—	—	—

* *T*/2 *IV* = half-life of plasma activity.
† *T*/2 *RT* = half-life of retained dose (total body activity).
‡ *IV* = intravascular, *EV* = extravascular albumin.

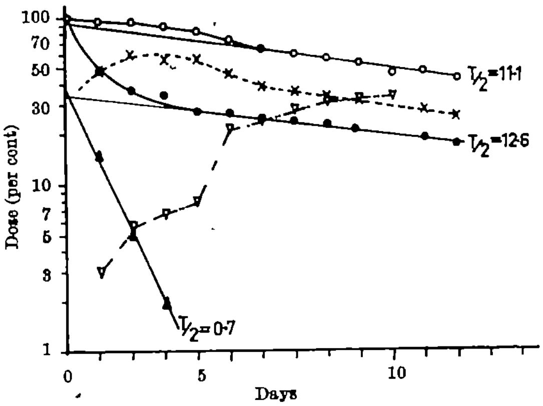

Fig. 1. Control rabbit. Total activity in plasma compartment (●) and total body activity (○); derived extravascular activity (×); cumulative urinary excretion of radioactivity (▽); ▲, denotes the slope of the initial distribution

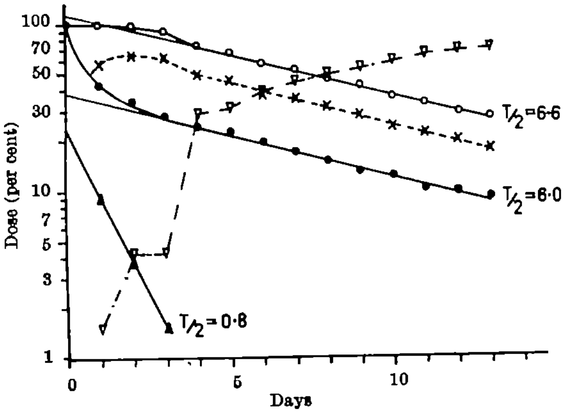

Fig. 2. Rabbit irradiated with 275 r. Symbols as in Fig. 1

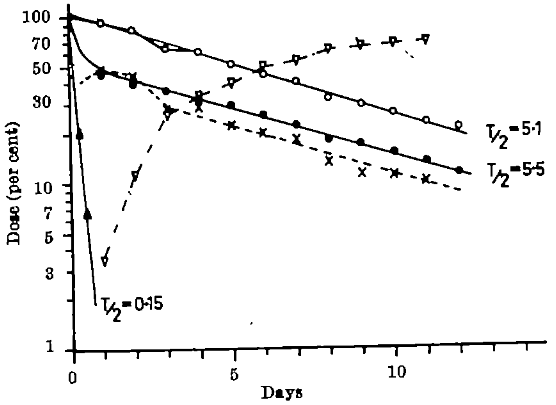

Fig. 3. Rabbit irradiated with 865 r. Symbols as in Fig 1

the gastrointestinal tract, its walls and contents, particularly in the ileum (no blood). In rabbit No. 7 (1,200 r.) 20 per cent of the retained dose was located in the gastrointestinal tract, but the highest values were referable to the upper portion. Other organs showed in both animals the same relative values for retained dose.

Values for degradation and distribution of ^{131}I-rabbit albumin in the controls are consistent with those found in earlier investigations[15]. A shorter half-life was found in all irradiated animals: the longer the exposure to irradiation, the shorter was the half-life. The injection of ^{131}I-albumin was given after irradiation to avoid denaturation of the labelled protein. Corresponding values for the half-life of retained dose (total body activity) and half-life of plasma activity were obtained in the controls and in groups I and II, demonstrating the validity of the methods of measurements. Common to all irradiated groups was an increased catabolism of albumin, strongly indicative of an increased leakage of albumin through the gastrointestinal wall. This was also evident from the increased fæcal excretion of radioactivity. With lower radiation doses the gastrointestinal epithelium and the capillary bed appear to be the initial sites of damage. When LD_{50} is reached, a more general, or more pronounced, disturbance of gastrointestinal permeability seems to occur, yet no extravascular retention of albumin is apparent. For doses exceeding LD_{50}, on the other hand, the retention points to definite disturbances of permeability, at least in the gastrointestinal tract.

The extension and significance of the capillary damage in other organs are now being investigated. Although there is considerable accumulation of albumin in the intestinal wall, the bulk escapes into the intestinal lumen and is thereby lost to the circulation.

In groups I and II the determinations of plasma activity and total body activity were suggestive of a decreased leakage around the twelfth to fourteenth day after irradiation. The excessive excretion of radioactivity in rabbit No. 7 (1,200 r.) indicates at least partial restitution of the re-absorption capacity of the intestine, since radioactive degradation products are excreted through the kidneys. The continuous

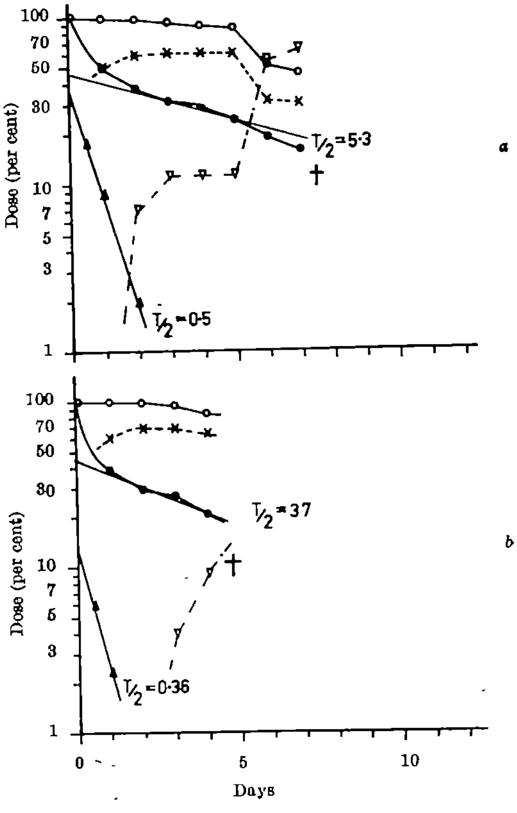

Fig. 4. Rabbits irradiated with 1,200 (*a*) and 1,600 r. (*b*). Death is denoted by +. In rabbit No. 7 (*a*) the *T*/2 for the last three days is not indicated in the diagram, but corresponds to 3·3 days

leakage of serum albumin results, however, in hypovolæmia, shock and death, as was conspicuously evidenced by the 1,600 r. animal. This rabbit died earlier than the other and about half the total albumin pool (retained dose) was located in the gastrointestinal tract—either in the wall or as degradation products in the lumen.

This preliminary work, which is part of a more extensive investigation now in progress, demonstrates excessive losses of albumin into the gastrointestinal tract after total-body irradiation.

These substantial losses of albumin are due to the fact that the epithelium, and evidently the capillaries, of the gastrointestinal tract constitute the most sensitive system during this phase of radiation injury.

The implication is that this important mechanism, hitherto not definitely recognized, at least partly underlies the shock which characterizes the acute radiation complex. Injuries to other capillary systems in the organism are at present being investigated. This work raises the question of therapeutic aspects concerning adequate substitution with colloids such as plasma, albumin and blood.

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CHEMICAL PROTECTION AGAINST ALPHA-PARTICLE IRRADIATION

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AND

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CELLS are in general considerably more resistant to ionizing radiation when they are anoxic, and many agents which modify radiation damage do not act *per se*, but rather by bringing about a change in oxygen concentrations in the vicinity of the cells under test[1,2]. The ratio of radiosensitivities of well-oxygenated to anoxic cells depends on the linear energy transfer of the radiation used, being in general smaller with radiations of high than of low linear energy transfer[3,4]. It follows that a 'chemical protector' which acts by inducing anoxia[5,6] will likewise be less effective when the linear energy transfer of the radiation is high. Reports on reduced effectiveness of chemical protection against radiations of high linear energy transfer have been based on observations with organized systems[1,7] in which oxygen tension was not under control. The evidence from such experiments cannot therefore be regarded as demonstrating a general feature of 'true' chemical protection, as distinct from the induction of anoxia.

Preparations of free-living cells, and particularly of bacteria, can be irradiated under well-controlled gas conditions, and therefore afford a suitable system for testing the dependence on linear energy transfer of true chemical protection. Both glycerol[8,9] and cysteine[10] have been shown to afford protection to bacteria irradiated in anoxic as well as in fully oxygenated conditions. We have therefore tested the ability of these compounds to protect cells of *E. coli B/r* against 27-MeV. and 5·2-MeV. α-particles (linear energy transfer 25 and 86 keV./μ respectively). Preliminary experiments with 8-MeV. electrons showed that glycerol (used at 1 *M*) and cysteine (0·15 *M*) were efficient protective agents when they were incorporated in agar gel, on which were placed 'Cellophane' carriers with the bacteria spread on these in a monolayer, and in a concentration such that an easily countable number of colonies would grow out when the 'Cellophane' carriers were transferred after irradiation to the surface of nutrient agar. This method of preparing samples was well suited for use in α-particle irradiations.

The beam of α-particles, from the Medical Research Council Cyclotron at Hammersmith Hospital, was defined by a horizontal slit 6 cm. × 1 mm. It was convenient to use 'Cellophane' carriers of about 2 cm. diameter, and it was necessary therefore to arrange that they were scanned by the 1 mm. high beam. The irradiation vessels were placed tangentially on the circumference of a wheel, 30 cm. in diameter, which was rotated once per second in a vertical plane, so that each carrier, moving downward through the beam, was exposed for approximately 1 m.sec. in each revolution. The beam current was so adjusted that each dose was delivered during at least ten revolutions of the wheel, to average the effect of variations in beam current during the exposure. The wheel carried sixteen irradiation vessels, eight of which, usually, contained agar gel incorporating the protective compound, while the other eight contained agar gel only. One sample in each group of eight was protected from α-particle bombardment by a disk of copper 0·25 mm. thick, and these samples, together with others not taken into the cyclotron chamber, served as controls.

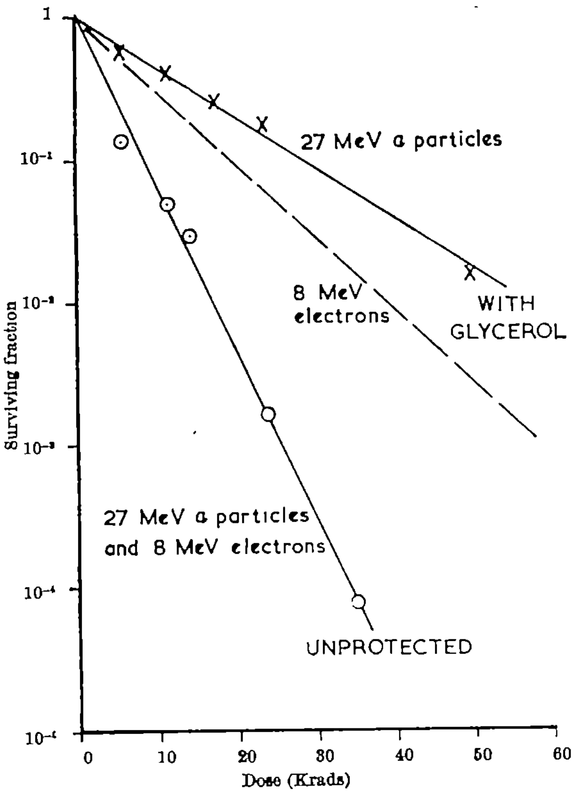

Fig. 1. *E. coli B/r* exposed to 27-MeV. α-particles or 8-MeV. electrons

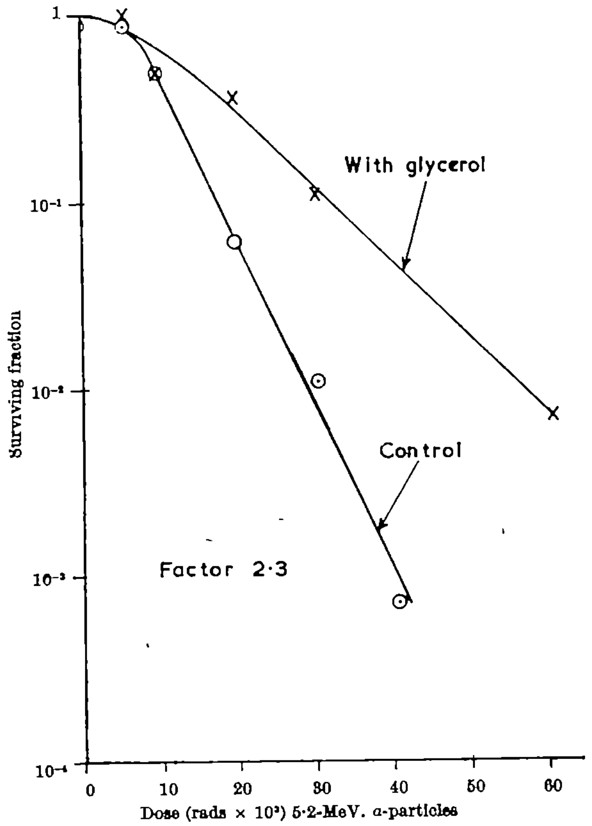

Fig. 2 *E coli B/r* exposed to 5·2-MeV. α-particles

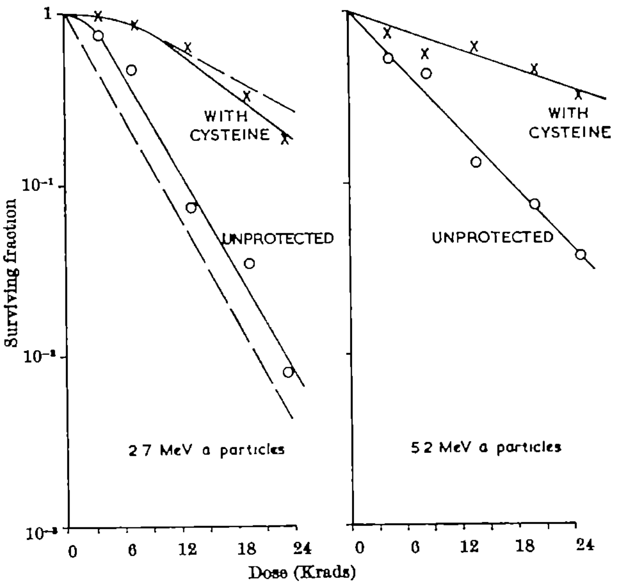

Fig. 3 *E. coli B/r* exposed to 27-MeV. and 5·2-MeV. α-particles. Broken lines in left-hand diagram show survival curves for 8 MeV. electrons in similar conditions

The α-particle beam (current 1 μamp., reduced to 0·04 μamp. by the slit) passed between the parallel plates of an ionization chamber used as monitor. Absolute doses were determined from the defined geometry, and were confirmed by using a Faraday cup.

Figs. 1 and 2 show the extent to which glycerol protected against 8-MeV. electrons and 27-MeV. α-particles, and against 5·2-MeV. α-particles; 'protection factors' against these radiations were respectively about 2·3, 3·2 and 2·0. Data for protection by cysteine are shown in Fig. 3. Survival in the presence of cysteine was not reduced to a sufficiently low level to enable us to estimate protection factors; it can be seen that cysteine protected the cells to roughly the same extent against all three qualities of radiation, at least up to doses of 24 kilorads.

Extension of these preliminary data awaits the completion of modification of the cyclotron, now under way. We are of the opinion that the results warrant an interim report because work in the field of chemical protection is being actively pursued in many places, and our data have a bearing on some suggested mechanisms of 'true' chemical protection:

(1) *Reduction in yield or effectiveness of radiolysis products of water, which are agents of radiation damage.* This mechanism has been supported by some authors, particularly in respect of protection by glycerol[9,11]. Protective action by scavenging of OH or H radicals, or by dehydration, could be expected to decrease with increasing linear energy transfer, since short-lived radiolysis products of water are produced in lower yield as linear energy transfer increases; the yields of OH and H radicals in irradiated water are less by factors 4–5 with polonium α-particles than with cobalt-60 γ-rays[12]. Our results show that glycerol protects bacteria to roughly the same degree against 8-MeV. electrons and 5·2-MeV. α-particles.

(2) *Production of anoxia within the cell.* It is sometimes suggested that chemical protective agents may act by depleting the cell of oxygen, even though there is sufficient oxygen in its immediate vicinity to confer full radiosensitivity in the unprotected state. The operation of such a mechanism would

also lead to reduced protection factors at linear energy transfer values where the overall oxygen enhancement ratio is low.

(3) '*Mixed disulphide' formation*. This mechanism has been postulated by Eldjarn and Pihl[13] to account for protection by sulphur compounds when 'direct action' on biological target molecules is involved. Our data on cysteine protection are not inconsistent with this mechanism of action, but it could not account for protection by glycerol. It may be that two (or more) different mechanisms of action must be invoked to account for true chemical protection. There are, however, striking similarities in the radiobiological action of these two dissimilar compounds: both protect bacteria much more effectively against radiations delivered in aerobic than anaerobic conditions; and, when *E. coli B/r* is irradiated in suspension, both compounds tend to change the shapes of survival curves, causing these to become sigmoid[14]. It seems likely that true chemical protection of bacteria by these compounds has a common mechanism, different from any so far proposed.

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EFFECT OF PRETREATMENT WITH NITRIC OXIDE AND N-ETHYLMALEIMIDE ON THE LEVEL OF SULPHYDRYL COMPOUNDS IN BACTERIA AND ON THEIR SENSITIVITY TO X-IRRADIATION UNDER ANOXIA

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NITRIC oxide increases the sensitivity of anoxic bacteria, insect, plant and mammalian cells to X-irradiation[1–4]. At concentrations less than 100 μM, this gas is as effective as oxygen in raising the radiosensitivity of *Shigella sonnei* in aqueous suspension[5]. The radiosensitivity is increased almost to the maximum level by 15 μM, but pretreatment or post-treatment with nitric oxide at this concentration is without effect on the sensitivity to irradiation under anoxia[5]. It has been found, however, that the pretreatment of *Shigella flexneri* with 1 mM nitric oxide increases the sensitivity to a subsequent X-irradiation under anoxic[6,7] but not aerobic conditions[8]. A similar increase in the radiosensitivity occurs when *E. coli B/r* is pretreated with the sulphydryl reagent *N*-ethylmaleimide (NEM) (refs. 9 and 10). Again the bacteria are rendered more sensitive to X-irradiation under anoxia, but the aerobic sensitivity is not increased to the same extent.

It has long been known that sulphydryl compounds are among the most effective agents for protecting against the effects of X-rays, but more recently it has become evident that sulphydryls play an essential part in the protection afforded cells by anoxia, as has been shown in experiments on bacteriophage and transforming principle. When protected from indirect effect by the addition of nutrient broth or other organic substances, neither bacteriophage nor transforming principle shows any appreciable oxygen effect[11–13]. When inside the cell, however, bacteriophage[14,15] and transforming principle[16] are in contact with sulphydryls such as glutathione and are protected against X-irradiation by anoxia. Anoxic protection is observed also if sulphydryl compounds are added to a suspension of bacteriophage or transforming principle[17–19] while it is reduced when phage-infected bacteria are treated with NEM (ref. 20). In all these experiments, anoxia is protective when, and only when, sulphydryl compounds are present.

It seemed of interest, therefore, to test whether the reduction in the protection afforded by anoxia to *Shigella* of either strain following treatment with nitric oxide[7] was due to the depletion of the intracellular sulphydryl compounds.

Experiments were carried out on *Shigella sonnei* grown overnight on nutrient agar, collected, washed and resuspended at about 10^{10} cells per ml. in 0·15 M sodium chloride, 0·1 per cent serum albumin, 10^{-4} M Ca^{++}, 10^{-4} M Mg^{++} and 0·02 M phosphate buffer at pH 6·8. The cells were exposed to nitric oxide in four circular flasks fitted with stopcocks and a magnetic stirring bar[7]. The flasks were evacuated and flushed with nitrogen three times to remove oxygen and were filled with a 50 per cent mixture of nitric oxide and nitrogen. This gas mixture was obtained from the Matheson Co. and was passed through a solution of sodium hydroxide to remove nitrogen dioxide. Cells gathered from nutrient agar plates would tolerate exposure to 1 mM nitric oxide for periods up to 90 min. with a 40–50 per cent loss of colony-forming ability. Cells grown in Difco nutrient broth were killed more rapidly by this procedure and were not used in these experiments. After each flask was exposed to nitric oxide for the required period, it was evacuated and flushed with nitrogen three times. A small sample was taken for use in the X-ray experiments, while the bulk of the cells were centrifuged preparatory to measuring the sulphydryl content of the bacteria. The cells from each flask were spun and the pellets were extracted with 0·3 M trichloroacetic acid. The extract was spun at 20,000g for 15 min. and the clear supernatant was then estimated for sulphydryl content by the nitro-prusside method[11].

The irradiations were carried out with X-rays generated at 250 kV. and 15 m.amp. with about 1 mm. aluminium and 2 mm. 'Pyrex' glass added filtration. The dose-rate in the irradiation vessel was determined by measuring the rate of oxidation of acid ferrous sulphate solution and was close to 3,000 rads per min. Nitrogen anoxia was obtained by passing oxygen-free nitrogen through the irradiation vessel while stirring magnetically for at least 10 min. before starting the irradiation.

The effects of exposure to nitric oxide or NEM on the sulphydryl content of the bacteria are shown in Fig. 1. Cells from experiment II exposed to nitric oxide for 60 and for 70 min. were used in X-ray experiment. The numbers of normal and nitric oxide-treated bacteria that survive X-irradiation under oxygen or under anoxia are shown in Fig. 2. Results with NEM-treated cells are shown in Fig. 3. It is seen in these figures that cells are less well protected by anoxia after pretreatment with either agent. The addition of cysteine to the pretreated cells just before irradiation increases the protection afforded by anoxia to a level slightly beyond that obtained with untreated cells.

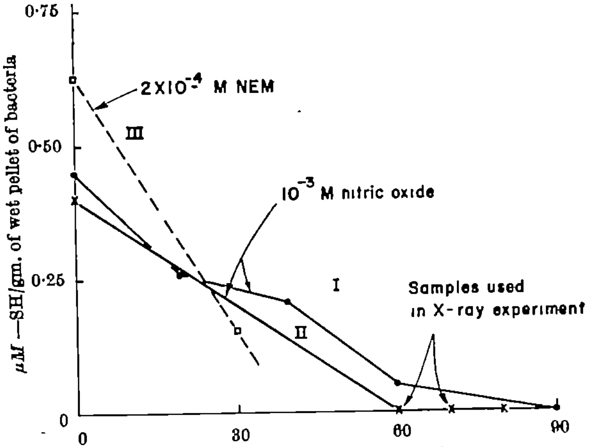

Fig. 1. The sulphydryl content of *Shigella sonnei* after exposure to 1 m*M* nitric oxide or 0·2 m*M* *N*-ethylmaleimide at 22° C.

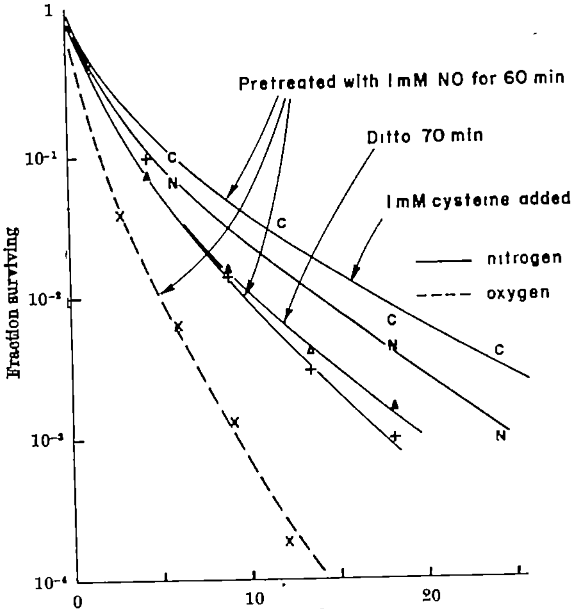

Fig. 2. Survival of colony-forming capacity as a function of the dose of X-rays delivered with or without oxygen. Survival curves are given for *Shigella sonnei* both untreated and after pretreatment with 1 m*M* nitric oxide for 60 or for 70 min. The cysteine was added to the pretreated cells just before irradiation

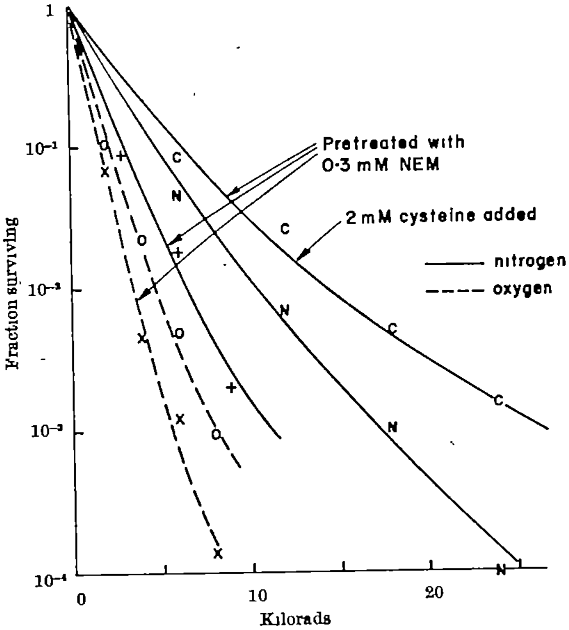

Fig. 3. Survival of colony-forming capacity as a function of the dose of X-rays delivered with or without oxygen. Survival curves are given for *Shigella sonnei* both untreated and after pretreatment with 0·2 *M* *N*-ethylmaleimide for 30 min. The cysteine was added to the pretreated cells just before irradiation

These results are in accord with those of Dale, Davies, and Russel[7] and Bridges[9,10] in showing that bacteria can be rendered more sensitive to anoxic X-irradiation by pretreatment with either nitric oxide or with *N*-ethylmaleimide. They also show that the amount of sulphydryl in the bacteria is drastically reduced by these treatments. There are several reasons for thinking that the increase in the anoxic radiosensitivity is due to the reduction in the level of the intracellular sulphydryl compounds in the cells used in this experiment. (1) The full level of protection under anoxia is readily restored to the pretreated cells by the addition of cysteine just prior to irradiation. (2) Similar effects on radiosensitivity are obtained following a reduction in the level of endogenous sulphydryl compounds by either substance. (3) With a suspension of free bacteriophage, sulphydryls have been found necessary for anoxic protection, while the anoxic protection of phage-infected cells was reduced by pretreatment with NEM (ref. 20).

The main features of these results are in accord with the reaction mechanism proposed[16,19,20] to explain the results obtained with bacteriophage:

$$RH \xrightarrow{\text{radiation}} R^{\cdot} \tag{1}$$

$$R^{\cdot} + \text{—SH} \longrightarrow RH + \text{—S}^{\cdot} \tag{2}$$

where *R*H is biologically active DNA and *R*· is a DNA radical formed by the loss of a hydrogen atom. Evidence that DNA is the sensitive site in bacteria is seen in the work on radiosensitization of bacteria and transforming DNA as the result of the incorporation of unnatural pyrimidines[22]. Protection by anoxia is the result of reaction (2) and is blocked if oxygen or nitric oxide reacts with the DNA radical $R^{\cdot}$, or if the amount of —SH is depleted by pretreatment with NEM or NO. It is to be expected that sensitization to anoxic irradiations would require the reduction of sulphydryls in the cell to some critical level at which the cells remain viable, but which is inadequate for efficient anoxic protection. The factors which determine the level to which sulphydryl compounds may be reduced without killing the cell have not been fully explored as yet, but might be of importance for the avoidance of anoxic protection as an adverse factor in the treatment of partially anoxic malignant tumours by X-rays.

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CONTAMINATION OF COMMERCIAL PREPARATIONS OF GLUCOSE OXIDASE WITH BETA-FRUCTOFURANOSIDASE

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THE glucose–glucose oxidase system is frequently used for the generation of hydrogen peroxide in various oxidative reactions. Using this system in experiments with thyroid particles prepared in 0·25 *M* sucrose we found that the enhancement of organic iodine formation usually resulting from addition of glucose and crude glucose oxidase also occurred in the absence of glucose. It has been known for some time that sucrose is not a substrate for purified glucose oxidase[1]. As this effect of glucose oxidase alone did not occur with saline-prepared particles (Table 1), it seemed possible that the oxidase preparation was contaminated with β-fructofuranosidase. Subsequent work confirmed this supposition. Apparently sufficient sucrose remained in the packed particle pellet to form enough glucose as substrate for the oxidase.

Sheep thyroid tissue was homogenized in Krebs–Ringer phosphate solution *p*H 7·4 (1 gm./3 ml.), with an all-glass homogenizer. Nuclei and debris were removed by centrifugation for 10 min. at 600*g*. The supernatant solution was then sedimented at 39,000 r.p.m. (Spinco model *L* centrifuge, No. 40 rotor) for 45 min. and the resulting pellet was washed and centrifuged twice with 12 ml. of the Ringer medium. The washed pellet was re-suspended in Ringer-phosphate solution and an amount equivalent to 100 mgm. of fresh thyroid tissue was added to Ringer-phosphate medium containing 10^{-6} *M* potassium iodide and 2 μc. iodine-131 in a total volume of 2·5 ml. (refs. 2 and 3). Incubations were at 37° and reactions were stopped after 1 hr. by freezing. Chromatography was in ethanol : *n*-butanol : 0·1 *M* ammonium carbonate (*p*H 7·4) (2 : 1 : 1) on Whatman No. 1 paper and sections thereof were counted in a well-type scintillation counter for origin and remaining radioactivity (mainly or entirely iodide).

Table 1. COMPARISON OF IODINATION BY SALINE-PREPARED AND SUCROSE-PREPARED SHEEP THYROID PARTICLES

Particles	Glucose oxidase alone	Glucose oxidase and glucose (10^{-3} *M*)
	mμmoles organic iodine per hr.	mμmoles organic iodine per hr.
Saline-prepared	47	197
Sucrose-prepared	230	200

Each flask contained particles from 0 1 gm. of sheep thyroid, 10^{-3} *M* glucose, 10^{-6} *M* I^-, Ringer-phosphate medium *p*H 7·4 in a total volume of 2 5 ml. Flasks with saline particles had 0·5 mgm. and sucrose particles had 0·25 mgm. of glucose oxidase (Prep. *B*). Temp. 37° C.; duration 1 hr.

To inhibit the glucose oxidase, reactions were performed anaerobically in Thunberg tubes which had been evacuated and re-filled with nitrogen three times. In some cases comparable aerobic studies were carried out. Reactions were carried out at room temperature (see Table 3 for conditions) for 1 hr. and terminated by placing the tubes in a boiling-water bath for 5 min. Sucrose, raffinose, trehalose, maltose, lactose were commercial preparations of high purity. α-Fructofuranoside was kindly supplied by Dr. H. G. Fletcher. Glucose oxidase preparations were obtained from Squibb and Co., through the kindness of Dr. D. Perlman (Prep. *A*), Nutritional Biochemical Corp. (Prep. *B*), Takamine Chemical Co. (Prep. *C*), Pfanstiehl Co. (Prep. *D*) and Worthington Corp. (Prep. *E*). Reducing sugar was determined by the Somogyi–Nelson method.

Glucose oxidase preparations from Squibb and Co. and the Nutritional Biochemical Corp. were compared for their effect on the formation of protein-bound iodine (origin radioactivity) in systems containing the oxidase, glucose, sucrose or no added substrate. The results are listed in Table 2. It can

Table 2

	No glucose oxidase mμmoles organic iodine per hr.	Prep. *A* glucose oxidase mμmoles organic iodine per hr.	Prep. *B* glucose oxidase mμmoles organic iodine per hr.
Particles	68	76	80
Particles + sucrose	86	47	202
Particles + glucose	59	256	197

Each flask contained particles from 0·1 gm. sheep thyroid, 2·5 ml. Krebs–Ringer phosphate medium *p*H 7·4, 10^{-4} *M* I^{-}. Concentrations were 1×10^{-3} glucose, 8×10^{-3} *M* sucrose and 0·5 mgm./flask glucose oxidase. Temp. 37° C.; duration 1 hr.

be seen that organic iodine formation by thyroid particulate preparations was enhanced by glucose oxidase (Prep. *B*) in the presence of both sugars but not without substrate. Preparation *A* stimulated synthesis only in the presence of glucose.

As glucose oxidase is inactive anaerobically the presence of β-fructofuranosidase was ascertained in the absence of oxygen. Glucose and fructose were allowed to stand anaerobically with glucose oxidase (Prep. *B*) and were found not to lose reducing power. Thus, the anaerobic technique used here appeared to be satisfactory for our purposes. A comparison of five glucose oxidase preparations was made, under aerobic and anaerobic conditions. Significant quantities of β-fructofuranosidase activity was shown in two of the preparations. The results are listed in Table 3. It is somewhat surprising that there was not a greater difference between anaerobic and aerobic experiments. This may be partly due to the relatively low activity of glucose oxidase at *p*H 7·3 and partly due to the fact that a portion of the reducing sugars is present as fructose.

Table 3. PRODUCTION OF REDUCING SUGAR BY CRUDE GLUCOSE OXIDASE

	Glucose oxidase preparation				
	A mμmoles*	*B* mμmoles*	*C* mμmoles*	*D* mμmoles*	*E* mμmoles*
Aerobic	0	612	484	52	26
Anaerobic	0	848	525	87	0

* Reducing sugar formed in 1 hr. at 24° C.

0·5 mgm. glucose oxidase, 3 mgm. sucrose in 1 ml. of 0·1 *M* sodium phosphate buffer *p*H 7·3.

Trehalose was found to be oxidized at 0·00028 of the rate of glucose in the glucose–oxidase preparation of Keilin and Hartree[1]. As some of the cruder preparations can form considerable quantities of reducing sugar anaerobically (Table 4), it is possible that such hydrolysis also occurred in the earlier work. Raffinose was also hydrolysed at a significant rate (Table 4). Bealing has reported formation of reducing sugar from trehalose and raffinose (as well as several other oligosaccharides) in crushed mycelium preparations of *Penicillium spinolosum* and certain *Aspergillus* species[4]. On the other hand, we found practically no hydrolysis of maltose, lactose or α-fructofuranoside under the same conditions. Maltase activity has been reported in samples obtained from several of these suppliers[6] but was found absent in yet another preparation[7].

Table 4. EFFECT OF CRUDE GLUCOSE OXIDASE ON THE HYDROLYSIS OF NON-REDUCING SUGARS

Sugar	mμmoles per hr. of reducing sugar produced
Trehalose	315
Sucrose	795
Raffinose	87

Trehalose (3 mgm.), sucrose (3 mgm.) and raffinose (4·5 mgm.) in 1 ml. of 0·1 *M* sodium phosphate buffer *p*H 7·3 were incubated anaerobically at 24° C. with 0·5 mgm. (Prep. *B*) glucose oxidase for 1 hr.

It has been known for some time that glucose oxidase preparations may be contaminated with catalase and mutarotase[5]. From the work of Bealing[4] on crushed mycelial preparations of *P. spinulosum* one might expect to find certain oligosaccharases in partially purified glucose oxidase preparations. This proved to be the case. Several commercial preparations of glucose oxidase are contaminated with β-fructofuranosidase and probably with other oligosaccharases. Such contamination must be kept in mind when glucose oxidase is used as a peroxide-generating system with cell fractions prepared with sucrose solutions. An example is given in which enough glucose is formed from sucrose to make the latter sugar equally as good a peroxide source as glucose for the formation of protein-bound iodine by sheep thyroid particles.

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PERIPHERALLY EVOKED CORTICAL REFLEX IN THE CUNEATE NUCLEUS

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THE anterior cerebral hemispheres of the cat can modify the excitability of subcortical neurones at various sites, including such sensory nuclei as those of the dorsal columns[1–4]. The excitatory influence of the cortex on neurones of the dorsal column nuclei is mediated largely, if not exclusively, by the pyramidal system[4]. Since cutaneous stimulation excites pyramidal tract neurones[5,6], it was suggested[4] that peripheral stimulation would produce a double discharge in the dorsal column nuclei, the first being due to the ascending afferent volley and the second to a descending, reflexly produced, pyramidal volley. The cortical outflow should be direct, for pyramidal tract fibres enter the hilus and ventral aspect of the cuneate and gracile nuclei and end on neurones within[7,8]. A gross potential change, recordable at the surface of these nuclei, could be expected, for Magni *et al.*[9] produced a gross potential in the cuneate nucleus by directly stimulating the pes pedunculi after destroying the remaining mesencephalic tissue.

A second, or late, surface negative wave has been observed in recordings from the cuneate nucleus[10]

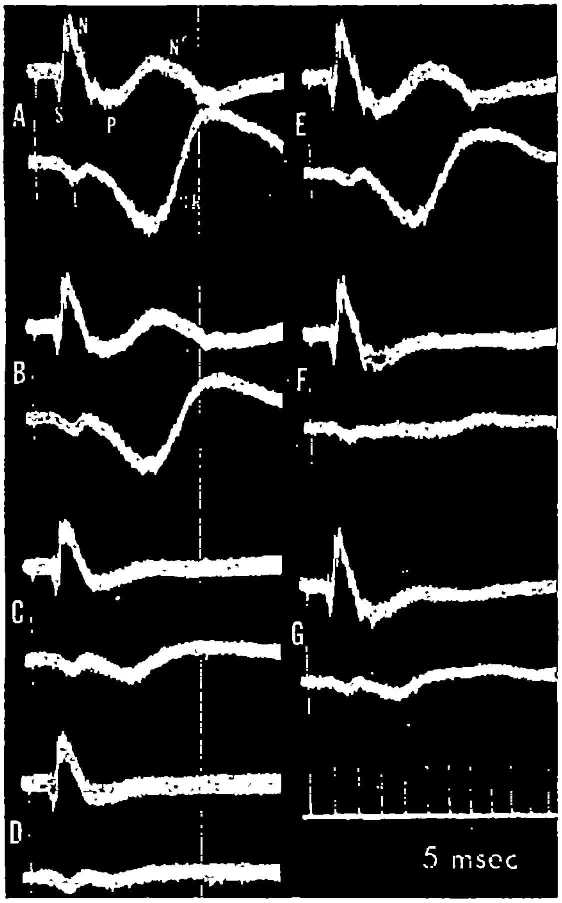

Fig 1. Upper trace of each pair, recorded with silver-ball electrode from surface of cuneate nucleus, shows 'normal' cuneate response, with second negative wave (*N*°) superimposed on a low amplitude positive wave (*P*). Lower trace of each pair, recorded with penetrating electrode about 0 5 mm. deep in medullary pyramid contralateral to cuneate electrode, shows killed-end recording of adjacent lemniscal response (*L*) and corticofugal reflex discharge in pyramid (*CR*). Time-scale applies to both traces of each pair. Responses produced by 0·1 msec. shock to forepaw ipsilateral to cuneate electrode. Superimposed traces throughout. *A*, 1 stimulus/sec., *B*, 2 stimuli/sec.; *C*, 4 stimuli/sec.; *D*, 6·25 stimuli/sec.; *E*, control response just before ice applied to anterior cerebral cortex; *F*, response 15 sec. after ice removed from both cortices (30 sec. application); *G*, response 10 min. after removal of ice, showing slight recovery

and is shown in the upper traces of Fig. 1. The more usual recording from the cuneate surface shown in the literature consists of a brief initial spike signalling the afferent volley, a longer negative wave and a very prolonged positive wave. The negative wave, 4–5 msec. in duration, presumably signals activity in cuneate neurones; concomitant activity occurs in the medial lemniscus (lower traces of Fig. 1). As suggested by Therman[11], the later positive wave has been regarded as a surface sign of activity in the underlying reticular neurones. However, this wave appears maximally over the dorsal column nuclei and reverses in sign within the cuneate nucleus; Anderson, Eccles and Schmidt[12] ascribed it to presynaptic inhibitory action of peripheral origin.

This prolonged surface positivity may be small in many preparations. Another phenomenon—the second surface negative response in the cuneate—is then clear. As shown in Fig. 1, this wave appears about 12–14 msec. after forepaw stimulation and lasts 18–20 msec. Like the late positive wave, this potential appears maximally over the dorsal column nuclei and reverses sign deep within the cuneate nucleus. It lags behind the underlying corticofugal reflex discharge[6] by about 3 msec. (5 msec. in Fig. 1, since the corticofugal reflex was recorded rostral to the cuneate nucleus). When the intensity or frequency of forepaw stimulation was altered the second negative wave and the corticofugal reflex varied together. Fig. 1 *A–D* show that the second wave disappeared just a little before the pyramidal discharge failed (rate of. 6·25/sec.). Similarly, when the stimulus intensity was decreased to near zero, the second cuneate wave disappeared just before the pyramidal reflex discharge; the latter failed when the first cuneate wave had diminished to about 7 per cent of its maximum amplitude.

The second negative wave could be eliminated by any method which stopped the corticofugal reflex discharge. Very deep anæsthesia or administration of small amounts of barbiturate to the chloralose-anæsthetized cat removed both the pyramidal reflex and the late cuneate response. The late wave is precluded by decerebration, and is also eliminated when both anterior cerebral hemispheres, from the ansate and coronal fissures rostromedially over the cruciate fissure to the anterior pole, are removed by suction. This is the tissue from which pyramidal tract fibres arise[13]. Transection of the pyramidal tracts alone at the olivo-trapezoidal level was sufficient to remove all trace of the response, even though activity in the adjacent medial lemniscus remained unaltered above the transection. The second negative wave of the cuneate thus appears to be a reflexly produced discharge descending from the cerebral cortex exclusively via the pyramidal tracts. The effect is bilateral. Fig. 1 *E–G* show elimination and partial recovery of the late wave following transient icing of both anterior cerebral hemispheres. When ice was applied directly to the exposed cortex, the second response disappeared in 4–10 sec.

Direct stimulation of a strand of dorsal column fibres isolated from the adjacent spinal cord except at its rostral connexion with the dorsal column nuclei (similar to the method used previously with pyramidal tract fibres[14]) produced a normal cuneate response and the second negative wave. The system appears to be a closed loop: cuneate nucleus–thalamus–cerebral cortex–cuneate nucleus. This positive feedback affects less than half the cuneate neurones[4]; the remaining neurones undergo a prolonged inhibitory effect[3] which is probably presynaptic in nature[12].

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INFLUENCE OF *p*H ON INTERFERON PRODUCTION AND ACTIVITY

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THE decreased growth of attenuated polioviruses in acid medium[1] led to the interesting suggestion by Lwoff that local acid production in inflamed areas could function as a homœostatic mechanism, limiting virus growth[2]. An alternative explanation for the different behaviour of attenuated and virulent viruses could be the increased interferon production of attenuated viruses, as recently described[3]. In order to investigate a possible correlation between these two phenomena, we examined the influence of bicarbonate concentration on the production and action of interferon.

The interferon used in this work was produced in a continuous line of rat tumour cells (*DE7R* cells), cultured in a medium consisting of Hanks's saline solution, enriched with 20 per cent inactivated calf serum and 0·5 per cent lactalbumin hydrolysate. The serum content of the medium was decreased to 3 per cent at the time of virus inoculation.

Interferon titrations were carried out in tube cultures of secondary rat embryo cells. Only embryos aged 18–20 days were used for preparing these cultures, all animals belonging to the highly inbred *R*-strain (originally obtained from Dr. Mühlbock, Amsterdam). Cultures at least 5 days old were used for the assay of interferon, since we found that younger cultures were less susceptible. The culture medium at the time of the titration of interferon consisted of Hanks's saline solution with 3 per cent inactivated calf serum and 0·5 per cent lactalbumin hydrolysate, enriched with choline, folic acid, nicotinamide, calcium pantothenate, pyridoxin hydrochloride, thiamine and riboflavin at a concentration of 1 γ/ml. Two different varieties of this medium were used; a low bicarbonate medium containing 0·1 per cent sodium bicarbonate and a high bicarbonate medium containing 0·3 per cent sodium bicarbonate. The *p*H's of the two media at the time of preparation were respectively 6·8 and 7·2.

The influence of the acidity of the medium on the yield of interferon was determined in cultures of *DE7R* cells inoculated with Sindbis virus. Three different multiplicities of infection were used, at both *p*H 6·8 and 7·2. In order to keep the *p*H as close as possible to its original value, bicarbonate was added several times. Culture fluids were collected when the cytopathological effect of the virus was between 75 and 100 per cent. The infectious virus was destroyed by overnight dialysis at *p*H 2. The interferon content of the dialysed fluids was then determined as follows: two-fold dilutions of the fluids to be tested, starting at 1/5, were carried out, using culture medium as a diluent. The interferon dilutions were added to tube cultures of rat embryo cells, inoculating 1 ml. into each tube and using 2 tubes per dilution. These cultures were then incubated overnight at 37° C. 500 $TCID_{50}$ of Sindbis virus were added the next morning as challenge virus. The interferon titre was read after 72 hr. as the highest dilution giving in both tubes at least 50 per cent protection against the cytopathological effect of this challenge virus. The results of this experiment show that, at each of the three multiplicities used, two- to four-fold higher yields of interferon were obtained in acid medium (Table 1).

Table 1. Interferon Titre obtained in *DE7R* Cells at Different *p*H and with Varying Multiplicity

Medium	Multiplicity of infection	Interferon titre
Low bicarbonate (*p*H 6·8)	10	1/5
	0·1	1/160
	0·001	1/80
High bicarbonate (*p*H 7·2)	10	None
	0·1	1/40
	0·001	1/20

The influence of the bicarbonate content on the action of interferon was determined, using Sindbis, vaccinia and herpes virus as challenging agents. Two titrations were carried out each time, one in low bicarbonate and one in high bicarbonate medium. The results of these experiments are summarized in Table 2.

Table 2. Titration of Three Different Interferon Batches in High and Low Bicarbonate Medium

Interferon batch	Challenge virus	Interferon titre: Low bicarbonate medium (0·1 per cent)	Interferon titre: High bicarbonate medium (0·3 per cent)
1	Sindbis	1/320	1/40
2	Sindbis	1/40	1/5
3	Sindbis	1/160	1/20
	Vaccinia	1/320	1/80
	Herpes	1/80	1/20

It can be seen that consistently higher titres were obtained when the interferon was assayed in low bicarbonate medium. Control experiments without interferon showed that the results obtained in the interferon titrations were not due to an inhibition of the challenge virus in the low bicarbonate medium.

The different titres obtained in high and low bicarbonate media were not due to a difference in interferon uptake by the cells under these two conditions, as shown in the following experiment. Two series of tubes were incubated overnight with two-fold interferon dilutions, one series in low bicarbonate medium, the other in high bicarbonate medium. The next morning the interferon was removed and every series was divided into two ; to one-half of the tubes, low bicarbonate medium was added, to the other half, high bicarbonate medium. The challenge virus was added afterwards. The interferon titre obtained in cultures incubated in high bicarbonate medium, but at the time of challenge changed to low bicarbonate medium, was approximately the same as the titre obtained in cultures where the bicarbonate content had always been low. On the other hand, cultures incubated in low bicarbonate medium, but at the time of challenge changed to high bicarbonate medium, gave the same low titre as was obtained in the series where the bicarbonate concentration was consistently high.

The possibility of using bicarbonate for reversing the cell-virus equilibrium obtained under the influence of interferon was studied in rat embryo cells infected with vaccinia virus. Rat embryo cultures were incubated with interferon in low bicarbonate medium and the next day 500 $TCID_{50}$ of vaccinia virus were added. After four days no sign of cytopathological effect was noticed in these cultures, whereas the control cultures were completely destroyed. On the fifth day the interferon was removed from the protected cultures and the cell sheets were washed twice with phosphate buffered saline. To half of these cultures fresh low bicarbonate medium was added; to the other half, fresh high bicarbonate medium. In four days the cultures with high bicarbonate medium were destroyed by the virus, contrary to the cultures with low bicarbonate medium, which showed very little sign of cytopathological effect. This clearly demonstrated that the protective effect of interferon obtained in acid conditions could be reversed by increasing the bicarbonate content of the medium.

These experiments show that the slightly acid conditions obtained in a low bicarbonate medium are favourable for the production, as well as for the activity, of interferon. This observation could explain the low plating efficiency of attenuated polio strains in low bicarbonate medium, since these viruses are characterized by an increased capacity to induce interferon production. Such interpretation is in agreement with the observations of Vogt, who found that this low efficiency of plating is caused by a reduced growth-rate of the virus, due to a decreased cell susceptibility which is only established after a preparatory period of several hours[1]. This preparatory period would correspond to the time necessary for the production of interferon and for the establishment of its protective action.

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ANTIGENS OF *BRUCEI* TRYPANOSOMES

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THE antigenic variation which occurs in relapsing chronic infections with African trypanosomes was first recognized more than fifty years ago by Franke (1905) and by Rodet and Vallet (1906). This work, and much subsequent investigation by Ehrlich and others, has been reviewed by Taliaferro[1], and little has been added in more recent years. Agar-gel diffusion and immunoelectrophoretic techniques are now being applied to problems of trypanosomal immunity[2-4] and have been used to show a striking difference in precipitins between variants[5]; however, the precise nature of the antigens of African trypanosomes has remained indeterminate[4].

Some new observations are summarized here and will be described in detail in full elsewhere; a few preliminary results have already been briefly reported[4-6].

A syringe-passaged laboratory strain of *Trypanosoma rhodesiense*, originally isolated from man in 1923[7], was used for most experiments, including antigen fractionation. A recently isolated strain of *T. brucei*-group trypanosomes (*ANT* 32), kindly provided by Mr. M. P. Cunningham, East African Trypanosomiasis Research Organization, Uganda, was used for comparison. Relapse variants of each strain were isolated either after sub-curative arsenical drug treatment[8] or from chronically infected rabbits[9]. Trypanosomes were separated from heavily infected rat blood by centrifugation, washed three times in normal saline, lysed in water and broken ultrasonically or with ballotini in the Mickle disintegrator; this crude antigen source was usually freeze-dried. Rabbit anti-trypanosome sera were prepared by injection of crude antigen combined with Freund's adjuvant, followed by alum-precipitated antigen[10].

Nature of the antigens. At 37° C. the crude antigen source was stable in dilute mineral acid for at least 3 hr. and in dilute alkali (pH 9) for at least 18 hr., but at 100° C. reactivity with antiserum in agar gel was lost after 15 min. in acid or alkali; in neutral solution the major portion of the precipitin pattern was selectively lost after 30 min. at 100° C. Selective loss of the same major antigenic group was also produced by pepsin, 0·05 M sodium metaperiodate or by an acetylating mixture[11] (acetic anhydride in pyridine). The minor antigen group which resisted these procedures also differed from the larger group by its ability to pass through a dialysis sac and by its incomplete precipitability with cold 0·5 N trichloroacetic acid.

The separateness of the two groups was confirmed by an estimation of their molecular size[12] which correlated closely with that of macromolecular components separable in the analytical ultracentrifuge[4]. The major and minor groups (previously[4] called '*A*' and '*B*') which were present in both trypanosome strains, and in their variants, will therefore be referred to as the '4 S' and '1 S' antigens respectively. Capacity to react with antibody appeared to be insensitive to chymotrypsin, papain or carboxypeptidase treatment; but separation of the hydrolytic products by paper electrophoresis, partition chromatography or dialysis, showed that no antigenic peptides were present and that retention of antigenicity in the incubates was due to an unhydrolysed protein residuum. Inability to react with antibody after incubation of the antigen in 4 M urea may indicate the involvement of protein ternary structure in the precipitin reaction. Ribonuclease, deoxyribonuclease, ptyalin, β-amylase, lysozyme and acid and alkaline phosphatase pre-treatment had no effect on antigenicity.

Although this evidence indicated that the antigens were predominantly protein, the possible presence of a polysaccharide component was inferred from the sensitivity to metaperiodate and to acetylation, and from the persistence of antigenicity in the aqueous phase after trichloro-trifluoroethane (Imperial Chemical Industries, Ltd., 'Arcton 113') extraction[13]. The crude antigen preparation was also Molisch-positive[14], although the constituent responsible was afterwards found to be ribonucleic acid. Earlier analyses[14] had shown that the only detectable carbohydrate present in whole cell hydrolysates of *T. rhodesiense*, apart from nucleotide pentose, was a trace of glucosamine (0·2–0·3 µgm./mgm. protein), but a larger-scale fractionation was devised to investigate this point.

Analysis for possible polysaccharide and lipid antigens. Fractionation, based on a number of standard procedures for extraction of polysaccharide antigens from micro-organisms[15], consisted essentially of successive treatment of crude antigen with cold 0·5 *N* trichloroacetic acid[16] (TCA), extraction of the TCA-insoluble residue with 45 per cent aqueous phenol[17], separation, purification and further extraction with acid ether-ethanol[18] of the aqueous, phenolic and solid interfacial phases, and of the dialysed original TCA-soluble extract. Precipitin tests at all stages showed the absence of polysaccharide, lipopolysaccharide or phospholipid antigens, and the predominant association of antigenicity with a protein component.

Although lipid staining[14] (oil red *O*, fettrot 7*B*), and pre-staining (sudan black *B*), of electrophoretic separations on paper and on starch gel indicated that lipid or lipoprotein antigens were absent, crude antigen was fractionated with lipid solvents to check this aspect further. Serial extraction with chloroform-methanol at 0° C.[19], ether and ether–ethanol at $-70°$ C.[20], acid ether–ethanol at $-10°$ C.[18], and a final extraction of the residue with chloroform–methanol under reflux for 30 min., showed that antigen activity was not associated with unbound lipid, lipoprotein or phospholipid.

Separation of protein antigens. Zone electrophoresis at *p*H 8·6 of the crude antigen on Whatman 3 *MM* paper[14] showed at least three mobile proteins and an immobile residue, and at least four proteins were separated on starch gel, using a discontinuous buffer[21]. Analyses of eluates of electrophoretic fractions, chemically and by ultra-violet absorbance at 260 mµ and 280 mµ, determination of the sedimentation coefficient of crude antigen components (by courtesy of Dr. P. A. Charlwood), and electrophoresis of ultracentrifugal fractions showed the presence of a group of three nucleoproteins, mainly ribonucleoprotein, with S_{20w} values of 43, 54 and 72, and a group of at least two smaller proteins with S_{20w} values of 1 and 4. The latter group migrated anodically on electrophoresis at *p*H 8·6, whereas the nucleoprotein group was largely immobile.

Antigenicity was associated with 1 *S* and 4 *S* proteins, but predominantly with the latter. The nucleoprotein group appeared to be very feebly antigenic, as only one of four antisera tested gave a detectable reaction indicative of an antigen of corresponding molecular weight when determined by the gel-diffusion method[12]. The approximate molecular weight of the 1 *S* and 4 *S* proteins (1–2 × 10^4 and 4–8 × 10^4 respectively) from ultracentrifugal observations corresponds to estimates of about 10^4 and 5 × 10^4–10^5 determined by the gel-diffusion method[12] for the 1 *S* and 4 *S* antigen groups distinguished earlier.

Treatment of the crude antigen with non-denaturant ents used to indicate specific reactive groups in nes[22] showed that antigenicity was not affected rmalin-acetamide, formalin-alanine, *p*-chloro- ibenzoate or methanol-hydrochloric acid pretreatment, but was abolished by propylene oxide or diazobenzenesulphonic acid treatment.

The evidence thus far would therefore suggest that the antigenicity of *T. rhodesiense* is associated with at least two groups of soluble unconjugated proteins of molecular weight 10^4 and 5 × 10^4–10^5 approximately, principally with the latter, and that the haptens are probably ternary hydrogen-bonded structures involving, in the case of the larger 4 *S* proteins, phenolic or other hydroxyl-containing substituents such as the free side chains of tyrosine, serine and threonine, all which are present in trypanosomal protein[14].

Antigen location. The antigenic proteins are apparently mainly cytoplasmic, as the highest level of antigenicity per unit weight of trypanosomal protein occurs in the 'cell sap' fraction of centrifuged homogenates prepared in aqueous sucrose solutions. The centrifugal procedures conventionally used for the separation of nuclei, mitochondria, microsomes and cell sap in mammalian cell homogenates[23] were applied to trypanosome homogenates prepared by shaking with ballotini in a Mickle disintegrator. Three fractions which sedimented after 1·4 × 10^4*g*-min., 4·2 × 10^4*g*-min., and 6·3 × 10^6*g*-min. respectively, together with a final water-clear supernatant, were examined microscopically and chemically.

Separation of nuclear, microsomal and cell sap fractions corresponded to that observed in mammalian cells, but in the second fraction, which normally contains the mitochondria of mammalian cell preparations, some concentration of kinetoplast-containing fragments was observed. This is of interest in view of the unique mitochondrial-nuclear type of structure found in the kinetoplast of Trypanosomidae[24,25], and of some Bodonidae[26].

Aqueous 0·25 *M* sucrose–5 m*M* magnesium sulphate solutions gave better fractionation of cellular components than 0·44 *M* or 0·88 *M* sucrose solutions, as judged by microscopic appearance and distribution of protein and nucleic acids. Cell fractions were tested for antigenicity by serial dilution (times 5) in Ouchterlony gels; irrespective of the homogenization medium, antigenic activity in the cell sap was 10–50 times greater per unit weight of protein than in any other fraction (Table 1). After repeated washing, antigenicity was retained in the nuclear and microsomal, but not in the 'mitochondrial', fraction; incubation of the unwashed fractions in 0·4 *M* deoxycholate[30] did not release more antigen. Fractional precipitation of a 1 ml. sample of cell sap with ammonium sulphate in steps of 10 per cent saturation gave three protein peaks at 30, 50 and 80 per cent saturation respectively, with corresponding peak antigen titres, of which the last was the highest.

Table 1. ANTIGENICITY OF SUB-CELLULAR FRACTIONS OF *Trypanosoma rhodesiense*

Fraction	Protein[27] (µgm./ml.)	DNA[28] (µgm./ml.)	RNA* (µgm./ml.)	Antigenic titre/mgm. protein
Nuclear	1,570	420	0	3–15
(Mitochondrial)	780	0	70	6
Microsomal	610	0	290	8
Cell sap	1,310	0	35	100–500

Washing and suspension medium: aqueous 0·25 *M* sucrose–5 m*M* magnesium sulphate. Trypanosomes homogenized 40 min. with No. 12 ballotini in Mickle disintegrator and centrifuged 1·4 × 10^4*g*-min., 4·2 × 10^4*g*-min. and 6·3 × 10^6*g*-min. to separate nuclear, 'mitochondrial' and microsomal deposits respectively; the cell sap fraction is the clear supernatant which remained after 6·3 × 10^6*g*-min. The 'mitochondrial' fraction consisted mainly of kinetoplast-containing cell fragments. Antigenic titre is the reciprocal of the smallest dilution giving a visible precipitin reaction on an Ouchterlony gel.

* Determined by difference from total nucleic acid content[29] and DNA content.

Attempted detection of variant antigen synthesis. The almost complete restriction of trypanosomal antigens to soluble cytoplasmic proteins, and their highly specific nature, suggested that detection by gel-diffusion of selective antigen synthesis in a cell-free system might be possible, especially as the lowest limit of detection of precipitinogenic activity in our trypanosome preparations was of the order of 0·7 μgm. protein. Recent experiments[31] on the ribosomal control of specific hæmoglobin synthesis in cell-free reticulocyte preparations suggested a similar cross-over experiment. Microsomal and cell sap fractions of parent and relapse variant strains of *T. rhodesiense* were mixed and tested with corresponding homologous antisera after 1 hr. incubation at 37° C. in phosphate-buffered Krebs saline *p*H 7·4 containing 5 mM Mg^{++}, 17·4 μM 3-phosphoglycerate and 4·4 μM adenosine triphosphate. No formation of heterologous antigen was detectable in mixtures of heterologous microsomal and homologous cell sap fractions, but this may have been due to lack of sensitivity of the test method.

Antigen specificity. The 4 S group contains at least four proteins with very similar antigenic determinants which are, however, distinguishable by immunoelectrophoresis. These are present in varying proportions relative to each other in washed nuclear and microsomal fractions and in cell sap.

The specificity of reaction of rabbits immunized with different variant trypanosome homogenates was tested with mixed antisera[32] against crude antigen. Some produced antibodies which were clearly variant-specific, but antibodies common to, or cross-reacting with, a number of variants, including some of another strain, were present in all animals.

Further analysis of this apparently non-specific response showed that a quantitative difference also existed. When cell sap fractions from two antigenic variants were tested against antiserum to one or other of them, the concentration of 'common' antigen was found to be about 10 times greater in the homologous than in the heterologous fraction. The trypanolytic power of immune serum was completely variant-specific. It was abolished by absorption with cell sap but not by absorption with a 1 S antigen preparation; this suggests that lysis may be associated with the 4 S antigen.

A comparison of sera from rabbits, either infected, or immunized with crude antigen, showed that in both cases the 4 S antigens were the main precipitinogens; the washing of trypanosomes during the preparation of crude antigen did not appear qualitatively to alter their antigenic character[4]. Variants isolated from the infected rabbits were in some cases identical with those obtained from rats, suggesting that antigenic variation may be independent of the host species. A variant can also appear more than once in the same infection, and this may reflect the short interval (1–2 weeks) during which specific precipitins to a variant could be detected.

Discussion

Our investigation has shown that at least three groups of precipitinogens in *Brucei*-group trypanosomes can be separated and characterized; their probable distribution within the cell has also been established. This information could now provide a consistent terminology for trypanosomal antigens and a coherent basis for further work on the immunology of African trypanosomiasis. For example, the variant changes in trypanosomal antigens established as cytoplasmic proteins should be susceptible of analysis in terms of present knowledge of the intracellular control of protein biosynthesis. In many ways, this antigenic variation resembles the changes in phase in the free-living ciliates *Paramecium* and *Tetrahymena*[33]; a similar mechanism may contribute to the chronic relapsing nature of other parasitic protozoal infections where parasites can survive in the presence of protective antibody[34,35].

The association of antigenicity with the bulk of the proteins of the trypanosome cytoplasm also suggests that antibody interaction with glycolytic, respiratory or other soluble enzymes could occur; respiratory inhibition has in fact already been used as an index of antibody action in trypanosomiasis[36]. In this context, a relation of antigenicity with drug-sensitive enzyme loci may be reflected in the reported association of suramin- and antrycide-resistance, and 'antibody-fastness' in trypanosomes[37]. Some confirmation of this report is provided by our *T. rhodesiense* strain which, on maintenance in a series of chronically infected rabbits, became significantly less sensitive to suramin, but not to mapharside, pentamidine, antrycide or ethidium. Preliminary experiments with trypanosomes taken from rats several hours after drug treatment suggest that suramin, unlike ethidium, can affect the 4 S antigens.

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EFFECTS OF VI ANTIGEN ON BACTERIAL HÆMAGGLUTINATION AND HÆMOLYSIS

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It has been known for many years that Vi antigen interferes with agglutination by O antibodies of typhoid bacilli containing both antigens. Other bacterial antigens, too, have similar effects, such as certain K antigens of *Escherichia coli*. Spaun[1] reported that this inhibitory effect of Vi antigen can be demonstrated also on red blood cells which had been artificially coated with both O and Vi antigens—an observation that was confirmed by Ceppellini and Landy[2,3]. Recently, it was shown that Vi antigen interferes with O agglutination of latex (polystyrene) particles as well[4]. At the present time, the mechanism whereby Vi antigen inhibits these and other agglutination reactions has not yet been fully elucidated. Several possibilities present themselves. (1) Because of spatial arrangement of the two antigens, Vi antigen interferes with the reaction of O antigen and homologous antibodies. (2) Treatment with two antigens of passive carriers, such as latex particles or erythrocytes, may result in competition between the antigens for surface areas or in the replacement of one by the other. This explanation, however, does not apply to bacteria naturally containing both antigens. (3) The Vi antigen may inhibit agglutination *per se* rather than interfere with the combination of O antigen and antibody. The present investigation was undertaken in an attempt to elucidate the mode of action of Vi antigen as inhibitor of agglutination.

Bacterial hæmagglutination and hæmolysis tests were carried out as described previously[5,6]. Briefly, sheep red blood cells (2·5 per cent) were washed three times. Bacterial antigens, alone and in combination, were added to the sediment in amounts sufficient to make a 2·5 per cent erythrocyte suspension. The cells were then kept in a water-bath at 37° C. and washed three times. For the hæmagglutination tests, these modified erythrocytes (0·2 ml.) were added to bacterial antisera in serial dilutions (0·2 ml.); the mixtures were incubated at 37° C. for 30 min. and then centrifuged at 1,300*g* for 3 min. The resulting hæmagglutination was read grossly. For the hæmolysis tests, guinea pig serum (Carworth Laboratories, New City, New York, in a dilution of 1 : 10; 0·1 ml.) was added to the above-mentioned mixtures of antisera and modified red blood cells. The suspensions were incubated at 37° C. and the resulting hæmolysis read grossly. The following antigens and antisera were used. Crude antigens of *Escherichia coli* 0111, *Salmonella enteritidis*, *Shigella sonnei*, and *Staphylococcus aureus* were prepared as described previously[5,7]. In addition, highly purified lipopolysaccharides from *Escherichia coli* 0111, *Salmonella enteritidis*, and *Shigella sonnei* were kindly made available by Prof. O. Westphal, Freiburg, Germany. Vi antigen was prepared according to Schubert, Edwards and Ramsey[8] and kindly supplied by Dr. A. H. Harris, Division of Laboratories and Research, New York State Department of Health, Albany, New York. All antisera were obtained from rabbits. Vi antiserum was kindly supplied by Dr. P. R. Edwards, Communicable Disease Center, Atlanta, Georgia; *Shigella sonnei* antiserum from Division of Laboratories and Research, New York State Department of Health, Albany, New York; *Salmonella enteritidis*, *Escherichia coli*, and staphylococcal antisera were produced in this laboratory. Phosphate hæmagglutination buffer of *p*H 7·3 (Difco) was used as diluent in all experiments.

It was reasoned that, if Vi antigen inhibits agglutination *per se* rather than interfering with the attachment of O antibody to O antigen on the surface of the erythrocyte, then immune hæmolysis by O antibodies and complement should not be similarly inhibited, for hæmolysis does not depend on aggregation of the red blood cells. Accordingly, repeated experiments were carried out on the effect of Vi antigen on agglutination and hæmolysis of sheep erythrocytes which had been modified with enterobacterial O and Vi antigens. Using standard amounts of both antigens, it was found that Vi antigen inhibits hæmagglutination but not hæmolysis. In view of the fact that fewer O antibodies per red blood cell are needed for hæmolysis than for agglutination, the effects of Vi antigen (1 : 100) on these two immunological reactions were determined with sheep erythrocytes which had been modified with varying amounts of O antigen. The results of a typical experiment are recorded in Table 1. It can be seen that Vi antigen reduces the agglutinating titre of the O antiserum more than four-fold and up to sixteen-fold. In contrast, the hæmolytic titre of the identical O antiserum, even with minimal amounts of O antigen on erythrocytes, is reduced only two-fold, and this reduction is irregular both with regard to amounts of O antigen on the erythrocyte surface and time allowed for hæmolysis. Similar results were obtained with other enterobacterial O antigens, both crude and highly purified, and with heterogenetic staphylococcal antigen as well. In all these experiments the presence of O

Table 1. EFFECT OF VI ANTIGEN *E. coli* 0111 SUPERNATE HÆMAGGLUTINATION AND HÆMOLYSIS

	E. coli 0111 supernate									
	1 : 20		1 : 40		1 : 80		1 : 160		1 : 320	
Tests	B	Vi	B	Vi	B	Vi	B	Vi	B	Vi
	Antibody titres (reciprocal)									
Hæmagglutination	6,400	400	3,200	200	800	< 200	800	< 200	< 200	< 200
Hæmolysis										
15 min.	3,200	1,600	1,600	800	200	< 200	< 200	< 200	< 200	< 200
30 min.	3,200	3,200	1,600	1,600	400	400	< 200	< 200	< 200	< 200
60 min.	3,200	3,200	1,600	1,600	800	400	200	< 200	< 200	< 200
120 min.	3,200	6,400	1,600	1,600	800	800	200	200	< 200	< 200

B, buffer; Vi, Vi antigen.

antigen on the surface of the erythrocytes did not affect the agglutinating or lytic titre of the Vi antiserum.

A second series of experiments was carried out to determine whether Vi antigen inhibits O hæmagglutination after O antigen-modified red blood cells had interacted with the corresponding O antibody. Accordingly, erythrocytes which had been treated with O antigen were added to O antiserum in serial dilutions in duplicate. After incubation at 37° C. for 30 min., Vi antigen (1 : 100; 0·1 ml.) was added to one series and buffer to another. The mixtures were again incubated for 15 min. at 37° C. and centrifuged. It was found that a definite reduction in the agglutinating titre of the O antiserum was effected by Vi antigen. Centrifugation of antigen-modified red blood cells after incubation with the corresponding antiserum strikingly enhances agglutination. It is unlikely that the attachment of O antibody to the antigen-modified erythrocytes is enhanced by centrifugation; rather, the aggregation of antibody-sensitized erythrocytes is facilitated. Therefore, the results of the foregoing experiment suggest that Vi antigen inhibits aggregation of red blood cells rather than the attachment of O antibodies to the erythrocytes.

Finally, experiments were undertaken to determine whether Vi antigen reverses agglutination effected by O antibodies. To this end, erythrocytes which had been modified with O antigen were added to O antiserum in serial dilutions in duplicate; the mixtures were incubated for 30 min. at 37° C., and the resulting agglutination was read after centrifugation. Vi antigen was added to one series, and buffer to the other. The mixtures were again incubated for 30 min. at 37° C. and centrifuged. It was found that Vi antigen reverses moderate agglutination effected by the higher dilutions of the O antiserum.

The results of these experiments, taken together, suggest that the major effect of Vi antigen as inhibitor of agglutination consists of interference with aggregation by O antibody of O antigen-modified erythrocytes—and possibly of bacterial cells as well—rather than with attachment of O antibody to the corresponding antigen on the cell surface. This inhibitory effect on aggregation may be due to interference, by steric hindrance, with the reaction of O antibody fixed to one cell with O antigen attached to another cell and/or with the non-immunological factors responsible for agglutination. A degree of specificity appears to be associated with the inhibitory activity of Vi antigen, for this antigen seemingly does not interfere with agglutination by its own homologous antibody.

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EFFECTS OF PHYTOHÆMAGGLUTININ ON THE MORPHOLOGY OF CULTURED LEUCOCYTES

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RECENTLY interest has been taken in the mitosis-stimulating phytohæmagglutinin. This substance is a hæmagglutinin extracted from beans of the genus *Phaseolus*, and it was first used in the production of anti-serum for hog cholera[1], and later for the separation of viable leucocytes by Li and Osgood[2], who also described the preparation of a crude extract. A more refined product is now obtainable, prepared by the more elaborate method of Rigas and Osgood[3]. It is non-toxic *in vivo*[4] and has been used to separate leucocytes from marrow[5] and blood[6] for cultures, in which neither mitoses nor motility of the cells were affected. Its potentialities as a mitogenic agent were first realized by Hungerford *et al.*[7], who used a modified version of the Li and Osgood method to culture cells for examination of their chromosomes; this method of chromosome examination is now widely used. As a result of our studies of the cells during culture in order to determine the effect of phytohæmagglutinin on the cells we are able to record the following observations.

Leucocytes from peripheral blood were separated by the use of dextran, and cultured by a method slightly modified from that of Hungerford *et al.*[7]. Eight cultures were set up in which no phytohæmagglutinin was used, and ten set up with the addition of phytohæmagglutinin. Samples were withdrawn at intervals by means of a sterile needle and centrifuged in a Wintrobe tube sufficiently to sediment the cells but not to pack them. The bulk of the supernatant medium was removed, and the cells, resuspended in a small drop of medium, were spread on slides. Films were stained with: (1) Jenner–Giemsa, (2) the Feulgen reaction to check nuclear detail and (3) Washburn's per-oxidase stain.

A differential count of 200 cells was made on the Jenner–Giemsa films.

In these experiments phytohæmagglutinin-*M* (Difco) was used. The batch used (No. 450506) gave reasonably good yields of mitoses, but a previous batch (No. 448242) had no mitogenic properties. Slides made from cultures showed only two types of cell: polymorphonuclears and lymphocytes. A few monocytes were present initially in both cultures with and without phytohæmagglutinin, but were not present after 20 hr.

Changes in cell types during culture (Fig. 1). The rates of increase in the percentages of lymphocytes are shown in Fig. 1. It will be seen that in the presence of phytohæmagglutinin the rate of increase

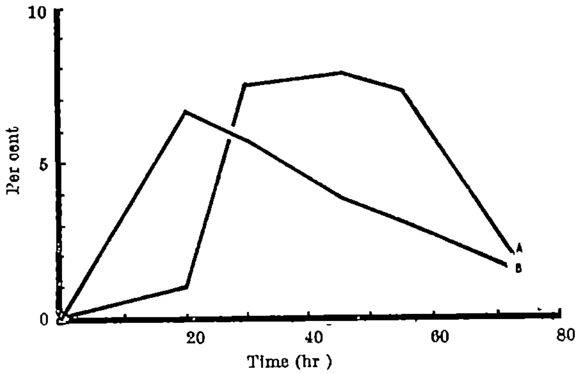

Fig. 1. Mean rate of increase in percentage of lymphocytes. *A*, culture without phytohæmagglutinin; *B*, culture with phytohæmagglutinin

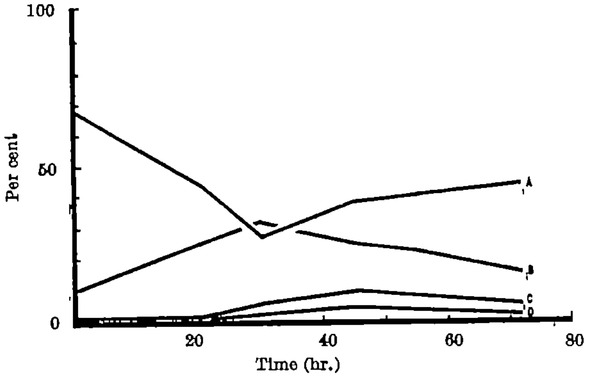

Fig. 2. Mean variation in the percentage of the lymphocyte types present in cultures with phytohæmagglutinin. *A*, normal lymphocytes; *B*, nucleoli possessing lymphocytes; *C*, 'blast' cells; *D*, mitotic cells

in the percentage of lymphocytes was rapid over the first 20 hr., and then gradually diminished during the remainder of the period of the culture.

In the case of cultures incubated without phytohæmagglutinin the initial rate of increase of lymphocytes was slow during the first 20 hr., rising rapidly in the next 10 hr., thereafter falling quickly.

These trends may be partially accounted for by the degenerative changes observed in the polymorphonuclear cells, which show two methods of degeneration; by vacuolation of the cytoplasm, and by nuclear pyknosis. The predominant degeneration in cells cultured without phytohæmagglutinin was by the pyknotic method, which appears to be the slower of the two methods, and so cells remain intact for a longer period of time, with a consequent slower initial rate of disappearance. In cultures with phytohæmagglutinin the predominant type of degeneration is by vacuolation, which, being faster, may cause the rapid initial rate of increase of lymphocytes already observed.

Change in morphology of cell types. The polymorphonuclear cells did not show changes other than the degenerative ones mentioned here. The lymphocytes, however, did show some changes in cultures with or without phytohæmagglutinin. Initially the lymphocyte population could be divided into three groups of large, intermediate and small lymphocytes respectively, the majority comprising the intermediate type. In non-phytohæmagglutinin cultures the population remained as such except for some degeneration by cytolysis until near the end of the period of culture when degenerative changes appeared in the nuclei. Although a few cells in these cultures did seem to develop nucleoli at various times no mitoses were seen. In the phytohæmagglutinin cultures a number of other types of lymphocytes were observed in addition to the normal lymphocytes. The percentages of these types present at the times of sampling are shown in Fig. 2. It will be seen that the first type to appear was the nucleoli-possessing cells. These were at first about the same size as the normal cells, but grew in size as the period of culture went on, showing increasingly basophilic cytoplasm, and the nuclei became larger and lighter in colour. From the graph it will be seen that these cells are most probably derived from the initial normal lymphocyte population.

After 20–30 hr. a new type of lymphocyte appears and these cells are very much larger than the initial cells, resembling primitive 'blast' cells, with deep blue cytoplasm, large nuclei and a number of nucleoli. Coincident with the appearance of this type is the appearance of mitotic cells, which reach a peak at about the same time, or slightly after the blast cell peak. Some mitotic figures do appear before the blasts, but these are smaller and are probably derived from the larger nucleoli-possessing cells, which are therefore probably capable of division. As the numbers of these more primitive cells decrease, the numbers of normal lymphocytes increase, presumably due to the new cells formed by the dividing cells. Some lymphocytes degenerate by cytolysis at all stages, the peak of degeneration being between 20 and 30 hr.. and the main cell types involved are the intermediate lymphocytes, the nucleoli-possessing cells and the blast forms. There is now some evidence that this type of degeneration may be a response to decreasing amounts of metabolites present in the medium due to increased metabolism of the more primitive cells. Larger lymphocytes which were present initially degenerated rapidly by vacuolation while the small lymphocytes become pyknotic.

The results presented here show that some of the lymphocytes in phytohæmagglutinin cultures undergo regressive changes, almost the reverse of the normal maturation sequence (Fig. 3). It seems probable that

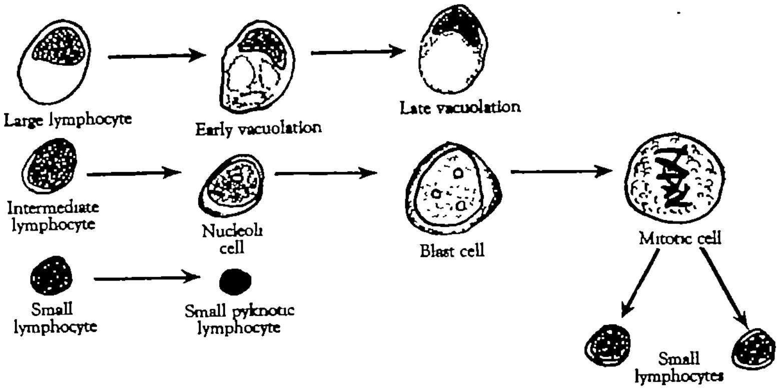

Fig. 3. Diagrammatic representation of morphological changes occurring in lymphocytes during culture with phytohæmagglutinin

concentrations of 0·2 mgm. per cent gave the best results in terms of sharply defined lines (Fig. 1). It was later found that incorporation of the antigen in agar layer over gelified antibody-charged agar gave still better results (Fig. 2). This change was adopted in our routine.

Horse immune sera and their γ-globulins against antigens from human tumours[4] were assayed by this method. Titres obtained by the described method were consistently the same as those obtained by the Ouchterlony method. Pre-immune and other non-immune control sera or γ-globulins did not form lines of precipitation.

Overnight standing of charged tubes results in the same pattern found after 1 hr. centrifuging, indicating that the effect of the gravity field is to accelerate diffusion.

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CHEMISTRY

Chemiluminescence and Oxidation

Most chemiluminescent reactions are known to involve molecular oxygen or other compounds (peroxides, ozonides) liberating oxygen[1]. This striking correlation between chemiluminescence and oxidation was first established three centuries ago by Robert Boyle, who performed experiments with shining wood, fish and flesh, and found that their light disappeared on removing the air[2]. However, no satisfactory theory of the phenomenon has been offered so far.

We suggest that this action of oxygen on chemiluminescence may be explained on the basis of the following considerations.

(1) Many oxidation processes (oxidation of fats, lubricants, fuels, oxidative destruction of polymers, etc.) are chain reactions. The active radicals are the peroxy-radical RO_2 and radical R containing no oxygen, the former being a product of oxidation of the latter:

$$R + O_2 \xrightarrow{k_2} RO_2 \qquad (1)$$

(2) A weak luminescence has been detected recently in various chemical reactions at moderate temperatures[3]. It was shown that in liquid-phase radical reactions excitation occurs by the elementary processes of radical combination (or disproportionation). Immediately after recombination the energy of this exothermal process is concentrated on the newly formed molecules of a product, and a small portion of them emits energy in the form of light while the remaining molecules lose it by collision. The quantum yield (the ratio of photons emitted to the number of recombinations) is extremely low, namely, 10^{-8}–10^{-15}.

(3) The products of recombination of RO_2 and R radicals are compounds of different spectroscopic properties. The former are oxygen-containing molecules (for example, ketones, alcohols, aldehydes, etc.) which are luminescent in the visible and in the near ultra-violet. The latter show no luminescence in these spectral regions. Hence the former should transform the energy of the reaction into radiation more efficiently than the latter do.

(4) In the absence of oxygen only R radicals exist and recombine:

$$R + R \xrightarrow{k_4} \text{products } (+\text{ light}) \qquad (2)$$

Admission of oxygen results in the appearance of RO_2 and in new modes of recombination:

$$R + RO_2 \xrightarrow{k_5} \text{products } (+\text{ light}) \qquad (3)$$

$$R + RO_2 \xrightarrow{k_6} \text{products } (+\text{ light}) \qquad (4)$$

The higher $[O_2]$ the greater $[RO_2]$ and the contribution of reaction (4) to the total light intensity which results in brighter chemiluminescence. At a certain oxygen concentration all R-radicals are replaced by RO_2 and a further increase in $[O_2]$ does not lead to increasing of $[RO_2]$ and of the luminescence intensity.

Our hypothesis has been verified in experiments on oxidation of hydrocarbons initiated by thermally decomposing substances[4]. At a gradual consumption of dissolved oxygen and after $[RO_2]$ and intensity

Table 1. Quantitative Data on Hydrocarbon Oxidation at 60° C.

Parameters measured		Numerical values measured: Using the chemiluminescence method	Numerical values measured: Using other methods	Unit
$k_3/\sqrt{k_3}$ * for	ethylbenzene	$(5\cdot8 \text{ to } 6\cdot5)\times10^{-4}$	$(5\cdot4 \text{ to } 6\cdot0)\times10^{-4}$	$l.^{1/2}$ mol.$^{-1/2}$sec.$^{-1/2}$
	cumene	$2\cdot8\times10^{-3}$	$3\cdot1\times10^{-3}$	
Concentration of dissolved oxygen $[O_2]$†	in benzene	$(0\cdot91 \text{ to } 0\cdot96)\times10^{-3}$	$0\cdot95\times10^{-3}$	mol. l.$^{-1}$
	in chlorobenzene	$1\cdot40\times10^{-3}$	$1\cdot40\times10^{-3}$	
	in ethylbenzene	$1\cdot72\times10^{-3}$	$1\cdot75\times10^{-3}$	
Concentration of O_2 in saturating gas		20·4	20·9‡	per cent
		4·4	4·6§	
		0·24	0·26§	
Decomposition constant of the initiator (*a,a*₂-azobisisobutyronitrile)		$(1\cdot10–1\cdot17)\times10^{-5}$	$1\cdot15\times10^{-5}$	sec.$^{-1}$
Efficiency of initiation (cage effect)		55–60	~ 60	per cent
$k_2/\sqrt{k_4}$ for	methyloleate	3×10^2	5×10^2	$l.^{1/2}$ mol.$^{-1/2}$sec.$^{-1/2}$
	ethylbenzene	$2\cdot7\times10^4$	no data	
	tetraline	4×10^4	$2\cdot2 \times 10^4$¶	

* k_3—constant of the elementary reaction $RO_2 + RH \xrightarrow{k_3} RO_2H + R$
† Saturation with air
‡ Oxygen concentration in air
§ Measured mass-spectrometrically
¶ At 45° (ref. 5).

Diffusion of Antigen into Antibody-charged Agar in Wintrobe Hæmatocrit Tubes accelerated by Centrifugation

THE methods of immunodiffusion and precipitation in gel media offer, over precipitation in fluid media or in interfaces, the advantage of creating automatically self-diluting gradients of concentrations of one or the two reactants, and thus, in a single tube or plate, the optimal conditions for reaction and aggregation (zone of equivalence). The chances of detecting a reaction in a single tube by the ring precipitation method are dependent on the pre- or post-zonal conditions since excess of one reactant dissolves the precipitate of antigen–antibody complex. Hence the need for serial dilutions even for qualitative evaluation.

Abandoned to the spontaneous forces that govern diffusion of the reactants in gel media, both single[1] and double[2], considerable time is required before the final reading of each test. With agar-diffusion Ouchterlony plates, lines of precipitation may appear as late as 14 days. This required recharging the reservoirs with reactants three times or more with consequent need for larger amounts of the reactants.

Given the dependence of the diffusion process on temperature (2°–3° C.) it becomes difficult to control this factor over long periods of time.

The factors that affect immunodiffusion are well known[3]. Some are of general nature such as temperature, pH, ionic strength of the solvents, trace elements and viscosity of the gel. These affect both the primary stage, that is, the antigen–antibody reaction, and the secondary stage, that is, the formation of the visible, light-scattering aggregates or precipitates. These can be controlled. Other factors are particular to each system, such as the nature, molecular weight and charge of the reactants, their relative concentrations and the presence of non-reacting proteins. These can only be controlled to an extent and they affect primarily the second stage.

For the simple detection and quantitation of an antibody, when identification of different antibodies is not required, such as in routine evaluation of immunization procedures, single diffusion in tubes was adequate. To reduce the time element it was decided to use Wintrobe hæmatocrit tubes providing constant dimensions and applying an increased gravity field by centrifuging.

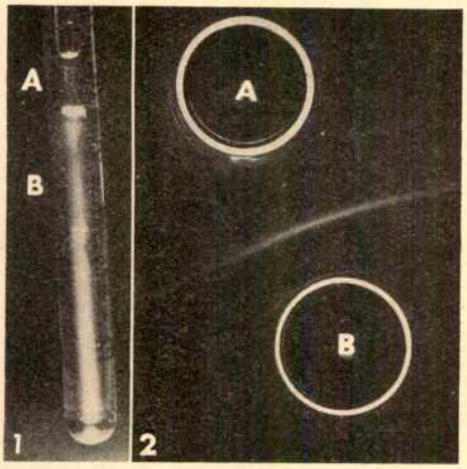

Fig. 1. 1, Modified Oudin method. Wintrobe tube after centrifugation for 1 hr. at 1,500g. 2, Ouchterlony agar plate. *A*, Antigen: mixture of purified antigens from human tumours (ref. 4); *B*, horse immune γ-globulin prepared against *A*. In 1 the γ-globulin solution is incorporated in the agar. In 2 it is deposited in the reservoir

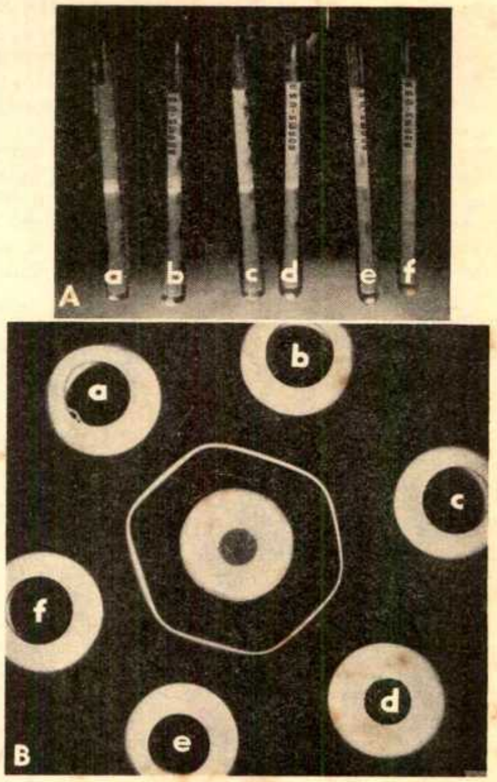

Fig. 2. *A*, Modified Oudin technique. The γ-globulin (Fig. 1) and its dilutions (10^0, *a* to 10^{-5}, *f*) are incorporated in agar in the lower half of the Wintrobe tube. After centrifuging for 1 hr. at 1,500g the lines of precipitation appear at, or near, the interface. In *B* the same system in Ouchterlony double diffusion show the correspondence of the dilutions (10^0, *a* to 10^{-5}, *f*). The titre was $10^{-5\cdot5}$, as determined in extrapolating tubes

The application of a gravity field to expedite diffusion has been suggested by Crowle[3]. It finds application only when a single antigen–antibody system is being tested, but not when separation of antigens in a mixture due to their differential diffusion-rate is expected. In this case the centrifugation would force the precipitate to move down and upset the resolution provided by the different diffusion-rates of each antigen.

In a single antigen–antibody system centrifugation can accelerate the collision of antigen–antibody molecules thus contracting the time otherwise required by simple diffusion forces.

'Ionagar' (Colab) was dissolved at 1·5 per cent in the following mixture of pH 7·6: 0·8 per cent sodium chloride, 9 parts, 0·15 M phosphate buffer, 1 part. Cadmium sulphate was added to make a 0·0005 per cent solution. Merthiolate was used as preservative at 0·01 per cent. Melted, sterile agar is cooled at 45° C. All equipment is kept at 37° C.; reagents at 25° C. Antibody (γ-globulin solutions and sera) dilutions are added to an equal volume of melted agar, mixed well without bubbling and added to Wintrobe hæmatocrit tubes up to mark 5. Agar is allowed to gel at 25° C. Agar surface must be smooth and free of air bubbles. Antigen and its dilutions overlayed on to the agar up to the 5·5 mark. Tubes are capped and centrifuged at 25° C. for 1 hr. at 1,500g. Antigen dilutions corresponding to protein

I thank, Dr. E. L. Fireman for providing the five-year-old samples used for this work.

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BIOPHYSICS

Proof that the Replication of DNA involves Separation of the Strands

IN their description of the specific pairing of the nucleotides of deoxyribonucleic acid (DNA), Watson and Crick[1] proposed that this pairing was the basis for a mechanism of duplication: if unwinding occurred at the time of replication, each strand of the double helix could act as a template for the creation of a new molecule so that each daughter molecule would be a 'hybrid' of a parental strand and a new strand. Since then there have been several demonstrations that DNA replication does indeed result in an equal division of the parent molecule between the two daughters[2,3]. This, however, has not put the hypothesis beyond the realm of cavil. The DNA which divides between the two daughters might, the argument goes[4,5], consist of a pair of double helices : if so, no unwinding of the strands would be necessary to produce the observed result, and the mechanism of replication could be radically different from that proposed by Watson and Crick.

Decision could be reached if some DNA were found which (*a*) becomes hybrid on replication and (*b*) can be isolated intact in its entirety and, in this state, shown to be two-stranded. Bacterial DNA is unsuitable; it becomes hybrid[2], but it has not yet been isolated intact and so there is no unambiguous estimate of its native length-to-mass ratio (that is, strandedness). Conversely, *T*2 bacteriophage DNA is known to be largely or totally two-stranded[6], but the transfer of parental *T*2 DNA to the progeny is accompanied by so much fragmentation that only small sections of the recipient molecules are known to be hybrid[7]; these small hybrid sections could conceivably be four-stranded without markedly disturbing the ratio of total length to total mass. However, the case of λ-bacteriophage DNA appears to be straightforward. Parental λ-DNA is transferred to progeny particles which, though typical in other respects[8], contain DNA which is half parental and half new (that is, is manifestly hybrid)[9]. Further, the entire DNA of λ resides in a single molecule which can be extracted intact[10,11]. If this molecule can be shown to have the ratio of total length to total mass of two-stranded DNA, then strand separation must occur.

To prepare labelled λ DNA for autoradiography, lysogenic *E. coli CR*34 (λ) thy$^-$ was induced by irradiation with ultra-violet light and then incubated in *A* medium[9] supplemented with 2 μgm./ml. tritiated-thymine (11·2 c./m*M*). After 100 min., the bacteria were lysed with chloroform. The resulting phage was sedimented with 10,000-fold excess of unlabelled λ. resuspended and extracted with phenol[12]. This preparation of λ-DNA was diluted to a total concentration of 5 μgm./ml. in *M*/100 phosphate buffer *p*H 7, heated at 75° C. for 20 min.[11], and collected on glass microscope slides for autoradiography. Though most labelled molecules were seen to be folded or tangled, some could be measured. These ranged up to 23μ in length (Fig. 1). This estimate of length, when combined with the reported value of 46×10^6 for the molecular weight[11], gives a ratio of about 2×10^6 mol. wt./μ. This is the expected ratio for a DNA double helix in the *B* configuration[13]. Further support for the conclusion that λ-DNA is two-stranded came from the finding that the density of grains per unit length along these molecules was approximately the same as that along *T*2 DNA, labelled with the same tritiated-thymine and exposed under film for the same time; if *T*2 DNA is largely two-stranded so must be λ-DNA.

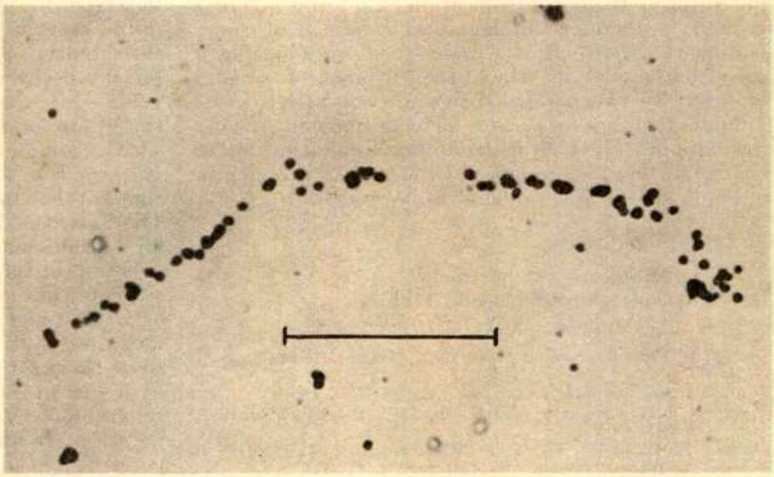

Fig. 1. Autoradiograph showing two molecules of λ-bacteriophage DNA, labelled with tritiated-thymine and exposed under Kodak '*AR* 10' stripping film for 56 days. The scale represents 10μ

In short, λ-DNA is known to form hybrids on replication. It is shown here to have the ratio of total length to total mass of two-stranded DNA. Therefore the replication of DNA must involve separation of the polynucleotide strands and the Watson–Crick model for DNA replication seems to be correct.

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evidence for formation of dislocation loop after irradiation. Both copper and gold have stacking fault energies[4-6] which are considerably lower than for the other four metals[6,7]. These results would therefore seem to be consistent with the conclusion that stacking fault energy is an important factor in dislocation loop formation.

A second important factor in the formation of dislocation loops during irradiation is the rate of production of point defects since it is this which determines the dynamic equilibrium concentration of point defects in the lattice during irradiation. The rate of production of point defects would depend on the flux density and the nature of the bombarding particle. Recently, for example, Beevers and Nelson[8] have observed dislocation loops in molybdenum after bombardment with hydrogen and helium ions at rates of production which were estimated to be about 10^6 higher than for the neutron irradiations.

There are many factors, including dislocation density and distribution, specimen shape and size, and grain structure, as well as irradiation conditions such as flux density and irradiation temperature, which influence the size of dislocation loop in a neutron-irradiated metal. The development of a quantitative theory of dislocation loop formation and growth is therefore difficult. The effect of these factors can, however, be understood by adopting a simplified model in which the surfaces of a solid bounded by two parallel planes are considered to be unfillable sinks for point defects created during irradiation. The sink distribution is assumed not to change during irradiation and the rate of production of point defects is considered to be constant.

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A Room-Temperature Diffusion Constant for Hydrogen in Proton-irradiated Steel

Recent measurements of hydrogen diffusion-rates in α-iron by Johnson and Hill[1] gave approximately 2×10^{-7} cm.^{2}/sec. for the value of D at room temperature; and in steel by Frank, Lee, and Williams[2] gave approximately 1×10^{-7} cm.^{2}/sec. Johnson and Hill measured re-emission of hydrogen from cylinders previously soaked in hydrogen at high temperatures and pressures, while Frank *et al.* used a mass spectrometer to measure the emission of hydrogen and deuterium from thin sheets. Frank, Swets and Fry[3], observing the build-up of hydrogen permeating through thin sheets of steel, obtained for D at room temperature values about an order of magnitude higher.

Because a knowledge of hydrogen diffusion-rates and mechanisms is important both for scientific and for technical problems, it is desirable to obtain measurements by as many independent methods as possible. I have made a determination of hydrogen diffusion at room temperature by studying the retention of tritium in proton-irradiated steel, which gives a value for the diffusion coefficient of about 10^{-10} cm.^{2}/sec. This number is believed reliable within a factor of 2.

Samples of mild steel were obtained which had originally been used for measurements of tritium and argon-37 production cross-sections by high-energy protons[4] and that had since been stored at room temperature for five and a quarter years. Tritium was originally produced throughout the bulk of the samples by irradiation with a beam of 1 BeV. protons, having a total flux of about 4×10^{12} protons/cm.^{2} The original tritium concentration, as determined approximately one month after the irradiation, varied with depth in the 10-in. long target from about 3×10^8 to about $2{\cdot}5 \times 10^9$ atoms/gm. The mild steel had been subjected to no special preparation, and presumably contained hydrogen, carbon, and other impurities in the amounts typical of non-vacuum-cast commercial steels.

From a slice selected from a known depth in the original target, the tritium was extracted and measured by techniques similar to those used for the initial determination of the tritium concentration in the samples[4]. The slice was 0·84 cm. thick. Correcting for the decay of tritium (12·4 year half-life) in the years since the original measurement, I determined the approximate fraction of tritium retained in the slice. Assuming an infinite slab, I then calculated a value of D of about 10^{-10} cm.^{2}/sec. Using the actual shape of the samples would lower this value somewhat. The difference between the diffusion-rate determined for tritium and the rate for hydrogen in the same sites is probably less than a factor of 2.

There thus exists a difference of about three orders of magnitude between D at room temperature as measured by tritium retention and what are believed to be the most reliable recent measurements of D for hydrogen in iron and steel. At present, the tritium-retention method has been used to determine only the values of D for room temperature. Until the temperature dependence of D is measured by this method, it is premature to attempt to explain the reasons for the smallness of our result. There are at least three important considerations to be borne in mind in comparing our determination with those made by more classical methods:

(1) The tritium the retention of which I measured is unequivocally introduced interstitially in the bulk of the iron or steel as the result of high-energy nuclear reactions of protons with the atoms of the metal. This is one great advantage of this technique compared with other methods of charging samples with hydrogen, which may preferentially introduce it into the structure of the metal at those locations from which it is most easily re-emitted.

(2) The same irradiation which produces the tritium produces simultaneously other radiation-damage to the metal.

(3) The concentration of tritium measurable in this way is extremely low—in this experiment, atom concentrations of about 10^{-13}. The over-all hydrogen concentration, however, is probably typical of ordinary commercial steels, and hence in a region of maximum metallurgical interest. This concentration may be low compared with that introduced by soaking samples in a hydrogen atmosphere at high temperature or pressure and then quenching them.

Neutron Capture in Tin Isotopes at Stellar Temperatures

THEORIES of stellar nucleosynthesis have emphasized the importance of neutron capture (s- and r-processes occurring in giant stars and supernovæ respectively) in producing the heavy elements[1,2]. For the s-process, cosmic isotopic abundances are predicted to show an inverse proportionality to the neutron capture cross-sections. While some nuclei can be produced by only one process or the other, the abundances can be most accurately measured for isotopes of a single element. Thus the many isotopes of tin provide the best opportunity to test the theories and to determine such parameters as the relative contribution of supernovæ to the material of the solar system. Tin-116 cannot be formed by the r-process, whereas tin-122 and tin-124 can only be so formed. In addition, the r-process is expected to be only a minor contributor[2] to the production of tin-117, -118, -119, and -120.

Stellar temperatures corresponding to neutron energies of 10–60 keV. have been suggested as typical for nucleogenesis. We have measured radiative capture cross-sections for seven of the tin isotopes by use of neutrons having an energy distribution of 18–46 keV. (mean energy, 30·4 keV.; full width at half maximum, 16 keV.), using the Oak Ridge National Laboratory 3-MV. pulsed Van de Graaff accelerator and the ^{7}Li(p,n) reaction near threshold[3]. We plan to extend the measurements to several energies from 10 to 60 keV. with better resolution, but as the neutron energy distribution in a star already covers many resonances in each isotope no significant change in the conclusions derived from the present results is anticipated.

The capture cross-sections were measured with a Moxon–Rae detector[4], using two *RCA*-7046 fourteen-stage photomultipliers and graphite converters 1 in. thick. The overall time resolution was 3–4 nanosec., permitting excellent neutron discrimination by time-of-flight, using an 8-cm. flight path to the isotope samples. Each sample weighed 30–35 gm. Corrections of a few per cent were made for resonance self-protection, average neutron path length in the sample, and isotopic impurities. The results are shown in Table 1.

The abundance attributed to giant-star nucleosynthesis, N_s, is found by subtracting an estimate of the supernovæ contribution, N_r (Table 1, col. 4), from the observed abundances N (Table 1, col. 3). The predicted inverse proportionality is shown by the approximate constancy of the product $N_s\sigma_c$ (Table 1, col. 5) for the s-process nuclei tin-116, -117, -118, -119, and -120. The increased relative importance of the r-process corresponding to these data is seen in comparing the ratio N_r/N_s (Table 1, col. 7) with the latest published estimates[2], which are shown in col. 8.

Table 1. 30-KEV. NEUTRON CAPTURE CROSS-SECTIONS

(1) Nucleus	(2) σ_c (mb.)	(3) Isotopic abundance N*	(4) N_r†	(5) $N_s\sigma_c = (N - N_r)\sigma_c$	(6) $N_r\sigma_c$	(7) N_r/N_s†	(8) N_r/N_s‡
Sn 116	92 ± 19§	0·1424	0	13·1	—	0	0
117	390 ± 82§	0·0757	0·040	13·9	—	1·12	0·08
118	59 ± 12§	0·2401	0·045	11·5	—	0·23	0·02
119	243 ± 51§	0·0858	0·040	11·1	—	0·87	0·07
120	35 ± 7§	0·3297	0·045	10·0	—	0·16	0·03
122	23 ± 5	0·0471	0·0471	—	1·1	—	—
124	23 ± 4	0·0598	0·0598	—	0·8	—	—

Note: σ_c computed for natural tin from these results is 93 ± 20 mbarns (mb.).

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‡ See ref. 2.

§ This is the r.m.s. absolute error, the relative errors are estimated at ± 10 per cent.

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METALLURGY

Some Factors influencing Dislocation Loop Size in Neutron-irradiated Metals

THE nature of radiation damage in metals has mainly been inferred from changes in physical properties resulting from irradiation. This evidence cannot generally be interpreted unambiguously. A more direct experimental approach has recently been made possible with the development of the technique of transmission electron microscopy which makes possible the direct observation of dislocations in metals. The damage resulting from neutron irradiation has been examined in a number of metals using this technique. In some metals dislocation loops were observed while in others no structural changes were found subsequent to irradiation. The reasons for these differences in behaviour are not yet understood.

Dislocation loops in metals result from the aggregation of point defects into disks on close packed planes. These disks afterwards collapse to form a circular area of stacking fault bounded by a dislocation loop. The radius at which collapse occurs will depend on the energy of the disk relative to that of the collapsed configuration. Since the stacking fault energies of metals may differ by more than an order of magnitude, it seems reasonable to expect that the stacking fault energy would be an important factor in determining whether or not dislocation loops are observed in a particular metal.

Transmission electron microscopy investigations have been carried out on six metals, including copper[1], gold[1], aluminium[1], nickel[1], iron[2] and molybdenum[3]. Irradiation conditions were similar in all cases and total integrated fluxes were in the same range. Dislocation loops were observed only in copper and gold, while the other metals investigated showed no between solid and liquid and dependence on distance along the tube suggest that the effects will not be marked when particle collisions become frequent. The behaviour of suspensions of spherical particles in tubes has been satisfactorily explained in the past[9,10] by assuming, after Vand[11], that "the suspension behaves as though there were a layer of pure liquid at the tube wall". The thickness of this layer appeared to be independent of Reynolds number, length and diameter of tube, etc., and depended only on the particle diameter. Whitmore's "reduced concentration" theory[12] could also be considered in terms of the fluid layer hypothesis. The possible presence of particle migration complicates the issue in that such factors as tube-length, particle Reynolds number and particle concentration appear to be relevant. Until such time as particle migration effects at higher concentration are better understood, however, the behaviour of suspensions will need to be explained in terms of the simple models at present in use.

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Packing of Equal 0-Spheres

A SPHERE can be defined as the locus of a point at a constant distance from a fixed point. A sphere in n dimensions is bounded by an $(n-1)$ dimensional envelope. This value $(n-1)$ can be used to define the sphere; thus an 0-sphere is a sphere in one dimension, that is a pair of points on a line[1].

Recent experiments[2,3] have confirmed that loose random packings of 2-spheres have densities of the order of 0·60. This value has been established by empirical methods only; no satisfactory theoretical treatment for a random pack has yet been found. The only alternative to direct measurement appears to be to produce a model packing with the aid of a computer and measure the density of that. Simulated random packings of 2-spheres have been produced by Monte Carlo methods[4]. Two methods have been used; the first involves placing spheres at random in a unit cube box, the second starts with a close packed array and gives each sphere a random move. This second method is useful when studying variation of distribution function in a sphere pack but not in estimating the density of a normal random arrangement. The first technique can be applied to the simple case of the packing of 0-spheres. In this case a computer is not required to generate the necessary random co-ordinates; random number tables serve equally well. A reference line is chosen and random numbers form co-ordinates on this line. These co-ordinates represent the centres of 0-spheres and the chosen diameter can be marked off. Spheres are placed until an overlap occurs, this one is rejected, more are placed, all overlaps being rejected until all the spaces left on the line are less than the chosen diameter. This represents the random packing, and the ratio of line covered to total length is the density. If part of a sphere overlaps either end of the reference line it is added at the other end.

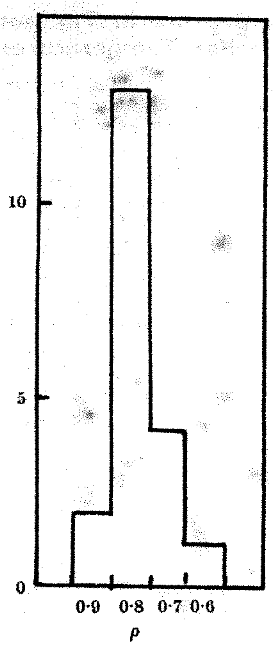

Fig. 1. Histogram for spheres of diameter 10 on 100 unit line

Since two digit random numbers were available a 100 unit line was chosen as the reference line. This means that diameters and positions can only be specified to an accuracy of 1 in 100. The edge effect which has to be considered when packings of 2-spheres are being investigated does not appear since there is no container to introduce it.

Table 1. RESULTS OF TRIALS WITH SPHERES OF DIAMETERS 10 AND 2

Diameter	Definition	No. of trials	No. of spheres on 100 unit line	Frequency N	Average density
10	1·0	20	9	2	0·78
			8	13	
			7	4	
			6	1	
2	0·01	5	40	1	0·75
			38	2	
			37	1	
			35	1	

The results of twenty trials with spheres of diameter 10 are shown in Table 1 and Fig. 1 shows the histogram of frequency N against density ρ. The mean value obtained for the density of the random pack is 0·78. To increase the definition on the reference line the tabled random numbers were taken in pairs giving random four digit numbers. With a 100 unit line this defines the position of the sphere to 0·01. Five trials with diameter 2 gave a value of 0·75.

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1·0–13·0. Despite the fact that the Reynolds numbers are some hundred times greater than those of Segré and Silberberg[1], the final positions adopted by rolling particles are in excellent agreement with the positions reported by these workers.

The apparatus consisted essentially of a vertical glass tube of length 42·0 cm. and internal diameter 0·94 cm., the upper end of which passed through the base of a glass cup and formed, in effect, a re-entrant mouthpiece. A rectangular water-filled 'Perspex' box surrounded most of the length of the tube to reduce optical distortion. The lower end of the tube was connected to a reversible variable-speed gear pump, the efflux from which was returned to the glass cup.

The methyl methacrylate spheres, of diameter 0·23–0·34 cm., were suspended in an aqueous solution of lead nitrate. The rotation of certain particles was prevented by drilling a fine hole in one side of the sphere, the offset centre of gravity counteracting rotation except when the largest hydrodynamic couples were present. A small quantity of ferric chloride sealed in the hole allowed the spheres to be moved in the tube with the aid of a magnet; a thin surface layer on the symmetrical particles served a similar purpose. Each run was started with a sphere about 8 cm. below the upper end of the tube, either close to the tube centre or in contact with the wall. After the sphere had been carried about 30 cm., a photograph was taken and compared with an equally enlarged photograph of a 'Perspex' graticule within the liquid-filled tube, this method giving the radial position of the sphere to within 0·01 cm. The sphere never moved radially during the brief period when the liquid was accelerating and, by the time the photograph was taken, radial movement had again effectively ceased.

Some typical results are given in Table 1.

It is apparent that the rolling spheres reach equilibrium when r/R is in the range 0·50–0·65, as previously reported[1], while all the non-rolling spheres move nearer to the tube axis. The initial position of the sphere has little influence on the final position, though it should be mentioned that if a sphere was located exactly on the tube axis, there was little tendency towards radial movement. For a symmetrical sphere, therefore, the axis of the tube corresponds to a position of unstable equilibrium; once the sphere is slightly displaced rolling starts and there is an increasing tendency for the sphere to 'run away' from the tube axis. In the case of some slightly asymmetrical spheres, a form of uneven rolling developed, and it is significant that as the roll-rate increased the particles moved away from the tube axis; when rolling was arrested they moved towards the tube axis.

In previous attempts to explain the behaviour of spherical particles in tubes in terms of a Magnus-type effect[1,4], it was necessary to assume that the spheres travelled with a velocity different from that of the liquid. A calculation of Simha[7] was used in order to show that the spheres lagged the liquid; the rotation of the spheres then carries them towards the tube axis if the Magnus force acts in the usual direction. In order to find the direction of the force for the current range of Reynolds numbers, the liquid density was changed a little so that symmetrical spheres had a slight tendency to rise (or fall) relative to the undisturbed fluid. A downward-settling sphere in downward-flowing liquid was found to behave much as before, though reaching equilibrium somewhat closer to the wall, but an upward-settling sphere in a downward-flowing liquid tended to drift gradually away from the wall and the rotation to die away. Thus the radial force resulting from an imposed axial translation is in the direction of the orthodox Magnus force, and if the results mentioned in this communication are to be explained in terms of the sphere's axial translation through the liquid, the sphere must lead and not lag the fluid.

Table 1. FINAL POSITIONS OF ROLLING AND NON-ROLLING SPHERES IN TUBE

Run no.	Sphere diam. (cm.)	Starting position	Av. vel. of liquid (cm./sec.)	d (cm.)	$\frac{r}{R}$
		Rolling spheres			
1	0·23	*W*	1·32	0·09	0·56
2		*W*	2·46	0·06	0·63
3		*W*	4·66	0·11	0·52
4		*C*	1·32	0·09	0·56
5		*C*	2·46	0·06	0·63
6		*C*	4·66	0·08	0·58
7	0·30	*W*	1·32	0·08	0·51
8		*W*	2·46	0·07	0·53
9		*W*	4·66	0·10	0·47
10		*C*	1·32	0·08	0·51
11		*C*	2·46	0·06	0·55
12		*C*	4·66	0·08	0·51
		Non-rolling spheres			
13	0·23	*W*	1·32	0·29	0·14
14		*W*	2·46	0·26	0·20
15		*W*	4·66	0·25	0·22
16		*C*	1·32	0·22	0·29
17		*C*	2·46	0·32	0·07
18		*C*	4·66	0·23	0·27
19	0·34	*W*	1·32	0·13	0·36
20		*W*	2·46	0·16	0·30
21		*W*	4·66	0·19	0·23
22		*C*	1·32	0·10	0·42
23		*C*	2·46	0·14	0·34

R = tube radius = 0·47 cm.
d = final distance between tube wall and sphere surface.
r = final distance between sphere centre and tube axis.
W = indicates that sphere started on tube wall and C close to tube centre.

All spheres, irrespective of density or asymmetry, tended to move inwards if initially placed close to the tube wall; this also applied to a slowly settling sphere in a stagnant liquid. It is therefore clear that a short-range hydrodynamic force of repulsion is present between the particle and wall, even in the presence of particle rotation. The interplay between this inward-directed 'wall effect' and the outward-directed 'Magnus effect' produces the phenomenon observed by Segré and Silberberg, though both effects are little understood at the present time. Thus, Goldsmith and Mason[8] have recently quoted a theory, backed by experiment, which shows that spherical particles should migrate radially in Poiseuille flow only if the particles are deformable; no movement should occur with rigid spheres. It is probable, however, that these workers observed no migration with rigid spheres because of the very low particle Reynolds numbers used ($< 10^{-6}$), much lower than those used by Segré and Silberberg (10^{-3}–6×10^{-2}) or in the present work (1·0–13·0). Conversely, Rubinow and Keller[4] have accepted the findings of Segré and Silberberg regarding the annular concentration of particles and attempt to make their theory fit the facts by the introduction of an arbitrary function having the property of reversing the sign of the radial force at a fixed value of the radius. The mathematical complexity of the system seems to be largely responsible for the current slow rate of theoretical progress.

The influence of particle migration on the behaviour of concentrated suspensions is a matter of conjecture. However, the weak nature of the radial forces involved, their susceptibility to small density differences

GEOLOGY

Carbonatitic Lavas

RECENTLY, von Knorring and du Bois[1] described a vesicular lava of high carbonate content from western Uganda which shows close geochemical affinities with intrusive carbonatites. They stated: "It must be emphasized that the lavas examined are fresh and unaltered and that the carbonated nature cannot be attributed to alteration after solidification". Descriptions of extrusive equivalents of carbonatites are rare, but two other examples have been reported recently from Africa. Bailey[2] concluded that certain carbonate-rich tuffs in Northern Rhodesia were primary, and not alteration products of more normal rocks. On p. 58 of his report, he wrote: "Indigestible though the fact may be to some philosophical systems —the pyroclastics around the Rufunsa vents are effusive carbonatite". Reference is made, in both papers, to the 1954 eruption of Oldonyo Lengai in Tanganyika, during which showers of dust and ashes, largely composed of calcium and sodium carbonates, were observed to fall on the flanks of the volcano[3].

We have presented experimental evidence for the magmatic origin of carbonatites elsewhere[4,5]. Evidence from the system $CaO—CO_2—H_2O$ indicates that simplified carbonatite magmas can exist through a wide range of pressure at moderate temperatures. The lowest pressure reached in these experiments was 27 bars. Since then, Wyllie and Raynor[6], at the University of Leeds, have confirmed the existence of these melts down to pressures of 10 bars, and the minimum pressure could be considerably less than this. Addition of other components would lower the pressure even further; alkali carbonates which melt at low temperatures without dissociation at 1 bar can be especially effective in this way. On the basis of the experimental evidence, therefore, it can be concluded that carbonatite magmas could reach levels very near to the surface (10-bars pressure is developed by a rock overburden of about 140 ft.), and such magmas could be erupted as tuffs. If the alkali-bearing carbonatitic magmas persist down to pressures approaching 1 bar, then the eruption of vesiculating carbonatitic flows appears to be a reasonable possibility.

Experimental studies of multicomponent systems related to carbonatites are now well advanced. Preliminary data for the system $CaO—MgO—CO_2—H_2O$ have already been reported[5]. Fluorine is known to be an important constituent in many carbonatites, and phase relations in the system $CaO—CaF_2—CO_2—H_2O$ have therefore been studied[7]. Apatite is ubiquitous in carbonatites and investigation of the systems $CaO—CO_2—H_2O—P_2O_5$ and $CaO—CaF_2—H_2O—P_2O_5$ has confirmed that calcite and apatite can be precipitated together from a melt through a wide pressure and temperature interval, extending to below 650° C.[8] In the system $CaO—CO_2—H_2O—SiO_2$, calcite is precipitated along with a silicate mineral (calcio-chondrodite) from a low-temperature melt[9]. More detail on the crystallization of alkali carbonate melts has been obtained in the systems $CaO—Na_2O—CO_2—H_2O$ and $CaO—K_2O—CO_2—H_2O$[10]. The significance of these systems is evident from the eruption of alkali carbonates at Oldonyo Lengai and from the widespread fenitization associated with carbonatite complexes.

Von Knorring and du Bois concluded that the existence of carbonatitic lava flows was evidence for the primary origin of carbonatite magmas. The problem of whether carbonatite magmas are primary or derivative is also being studied experimentally. Systems such as $CaO—CO_2—H_2O—NaAlSiO_4$, $CaO—CO_2—H_2O—NaAlSi_3O_8$, $CaO—CO_2—H_2O—KaAlSi_3O_8$, and $CaO—CO_2—H_2O—NaFeSi_2O_6$ are being investigated, and it is hoped that the data obtained will indicate which of these suggested origins is more likely. However, whatever the ultimate origin of carbonatites may be, the nature of the reactions involving alkalis and basic oxides, with either solid-vapour or solid-liquid-vapour equilibria, is of fundamental importance for understanding the processes involved in the development, emplacement, or eruption of carbonatites and carbonatitic rocks.

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PHYSICS

Influence of Particle Rotation on Radial Migration in the Poiseuille Flow of Suspensions

Segré and Silberberg[1] recently observed that when an initially uniform dilute suspension of rigid spheres passes through a circular tube in laminar flow, the spheres tend to concentrate into an annular region about half-way between the tube centre and tube wall. It was suggested that the inward motion of the spheres resulted from a force, akin to the Magnus effect, which was present because the spheres rotated as they passed through the tube. Work by Tollert[2], Saffmann[3] and Rubinow and Keller[4] has also indicated the presence of an inward force of the above type, while the 'minimum energy dissipation' theory of Jeffery[5] shows that the spheres should move inwards and eventually travel along the tube axis. Unfortunately no satisfactory explanation has been given of the fact that, in the above experiment[1], the inner spheres moved outwards while the outer spheres ceased to move inwards when they reached the region of the annulus.

I have recently made observations with single particles which clearly indicate that the rotation of the spheres results in an outward movement and not inward as previously suggested[1-4]. In the absence of rotation, the spheres normally drift towards the tube centre. This behaviour is obtained when the densities of the sphere and liquid are equal, the tube Reynolds number is in the range 100–500 and the relative particle Reynolds number[6] is in the range high resolution the induced dipole spectrum shows some interesting features.

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GEOPHYSICS

Origin of Sudden Commencements

Geomagnetic storms fall into two distinct categories: recurrent and sporadic. Recurrent storms are produced by continuous streams of particles issuing from the solar *M*-regions. Swept round by the rotating Sun, the corpuscular streams overtake the Earth in its orbit at monthly intervals. By contrast, a sporadic magnetic storm marks the arrival of a single cloud of plasma, ejected at the time of a solar flare.

Sudden commencements are more frequent among storms of the sporadic type. This has been known for some time[1], but the observation has not received a satisfactory explanation. Recent theories on the nature of sudden commencements seem to provide a key to the answer. According to these theories[2], a sudden commencement results when the velocity of the solar plasma exceeds the ambient Alfvén velocity, thereby creating a hydromagnetic shock wave. A shock wave of this kind could form at the stream boundary, either in interplanetary space or in the region of impact at the Earth[2]. On the latter hypothesis it follows that the solar plasma must advance into the Earth's field with a velocity exceeding the local Alfvén velocity:

$$V_A = \frac{B}{\sqrt{\mu_0 \rho}} \quad (\text{M.K.S.})$$

Fig. 1 gives the values of V_A calculated for the Earth's exosphere in the plane of the magnetic equator ($B = B_0\,(R_E/r)^3$) out to a distance of 10 Earth-radii. Beyond that point the calculations become uncertain because of field irregularities. The ion-density was taken to be:

$$\rho = 2{\cdot}58 \times 10^9\, m_H \left(\frac{R_E}{r}\right)^3 e^{3{\cdot}03\, R_E/r}\ \text{kgm./m.}^3$$

in accordance with recent results obtained by the study of nose whistlers[3].

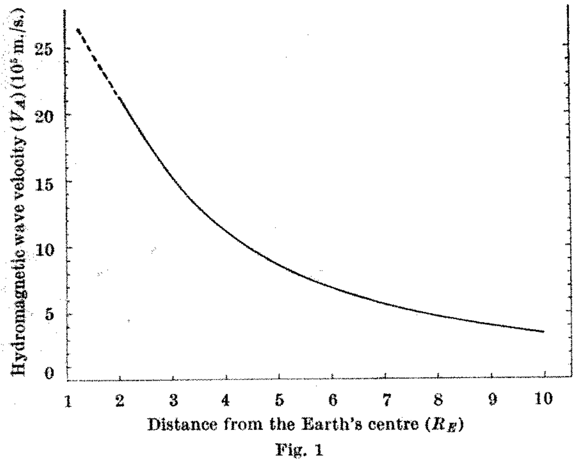

Fig. 1

A plasma cloud ejected at the time of a solar flare is propagated towards the Earth with a mean velocity of ~ 6 × 10^5 m./sec. (ref. 4). The cloud front probably reaches the Earth with a speed of 7 × 10^5 m./sec. In extreme cases (great magnetic storms) the velocity of approach may exceed 2 × 10^6 m./sec. Because of the low value of the Alfvén velocity near the boundary of the geomagnetic field (Fig. 1) a shock wave is likely to develop in that region (~ 10 R_E), particularly when the velocity of the incident plasma is higher than usual.

As regards the corpuscular streams which produce recurrent magnetic storms, the situation is quite different. In this case the velocity of approach of the stream surface (v_1) is no longer equal to the velocity of individual stream particles (v). Instead the relation:

$$v_1 = \left(\frac{1}{\omega^2 a^2} + \frac{1}{v^2}\right)^{-\frac{1}{2}}$$

holds, where ω is the solar (synodic) angular velocity of rotation and a the astronomical unit. The maximum possible value of v_1 is 4 × 10^5 m./sec. (as $v \rightarrow \infty$). Adopting the most probable value of v (6 × 10^5 m./sec.) (ref. 4) we obtain $v_1 = 3{\cdot}3 \times 10^5$ msec. Comparison with Fig. 1 shows that the velocity of the advancing stream surface could easily be less than the critical value necessary for the creation of a shock wave. This would explain the absence of sudden commencements among recurrent storms. The margin is narrow, however. Variations in field strength and density in the exosphere must inevitably lead to fluctuations in the hydromagnetic wave velocity. As a consequence, we should expect to find some recurrent storms with sudden commencement-characteristics, as well as some sporadic storms with gradual onset, a prediction which is amply confirmed by observation.

In principle the hydromagnetic shock could develop ahead of the solar plasma long before it reaches the Earth. The comparative rarity of sudden commencements among recurrent storms indicates that, as a rule, $V_A > 3{\cdot}3 \times 10^5$ m./sec. in the interplanetary medium. Since $V_A = 2{\cdot}2 \times 10^{16} \frac{B}{\sqrt{n}}$ m./sec., where n is the number of protons/m.^{3}, we deduce that $n < 4{\cdot}4 \times 10^{21} B^2$. This relation gives an upper limit to the ion-density when the field strength is known. Measurements obtained from satellite *Explorer X* (1961 κ) showed B = 1–2 × 10^{-8} w/m.^{2} at a distance beyond 30 R_E (ref. 5). Assuming this to be a representative figure, we conclude that the density of interplanetary gas near the Earth's orbit does not exceed 10^6 particles/m.^{3} under normal conditions.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Optical Echoes from the Moon

Experiments have been conducted to focus pulsed optical radiation on to the surface of the Moon and to detect the echoes.

A ruby optical maser radiating pulses of approximately 50 joules energy, 0·5-msec. duration, at 6934 Å. was used as the source. The transmitting optical system included a Cassegrainian telescope of 12-in. diameter. The echoes were received on a Cassegrainian telescope of 48-in. diameter, passed through an interference filter of 7 Å. band-width and were detected with a photomultiplier tube of spectral response type S-20, cooled to liquid nitrogen temperature. The field of view of the receiving telescope was 0·2 milliradians.

The photoelectron count obtained in a 0·5-msec. interval at the expected time-delay was compared with the counts obtained in 0·5-msec. intervals where no echoes would be expected and where the only relevant contributions to the count were those due to noise, that is, to Earthlight and scattered light (photoelectric dark current was negligible).

Table 1

	1 Eastern standard time	2 Region of the Moon	3 No. of flashes	4 No. of intervals	5 Average noise	6 *S.D.*	7 Average count in expected interval
May 9, 1962	21.56–22.07 hr.	Albategnius 15° S., 8° E.	11	15	1·11	0·28	1·91
May 10, 1962	21.52–22.34 hr.	Copernicus 10° N., 20° W.	23	16	1·39	0·22	1·74
May 11, 1962	21.57–22.50 hr.	Tycho 43° S., 10° W.	30	22	1·42	0·16	1·83
	22.54–23.15 hr.	Longomontanus 50° S., 20° W.	16	17	1·52	0·19	2·32

The experiments, conducted at Lexington, Massachusetts, on May 9, 10 and 11, gave positive results as indicated in Table 1. Column 3 indicates the number of consecutive flashes utilized; column 5 is the average number of photoelectrons due to noise in a 0·5-msec. interval column; 6 is the standard deviation of the noise count; column 7 is the average count in the 0·5-msec. interval where an echo was expected. The expected time delay of the signal was computed from ephemeris data and the position of the illuminated area on the Moon. Column 4 gives the number of range-intervals per trace used to obtain the data in columns 5 and 6.

I thank J. Daley, jun., of Lincoln Laboratory, for help in setting up and operating the telescopes, and G. Hardway and S. Kass, of the Raytheon Co., for help in the use of the maser. The work was supported in part by the U.S. Army Signal Corps, the Air Force Office of Scientific Research, and the Office of Naval Research.

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Fundamental Band of the Quadrupole Spectrum of the Hydrogen Molecule

The application of interferometric techniques to astrophysical problems in the near infra-red shows considerable promise. The abundance of hydrogen in planetary atmospheres is probably of major importance.

Herzberg[1] observed the 2–0 and 3–0 bands of the quadrupole spectrum of hydrogen using pressures of 10 atmospheres and optical paths of 10–55 km. atmospheres. In addition, an induced dipole spectrum of hydrogen has been found by Chisholm, MacDonald, Crawford and Welsh[2] by making measurements in the pressure-range of hundreds to thousands of atmospheres.

We have observed the 1–0 band of the quadrupole spectrum of hydrogen. The spectrograph used was of the echelle type described previously[3]. An 8-metre 'White' multiple reflexion absorption tube allowed the optical path to be varied from 32 to several hundred meters. The pressures of hydrogen gas used varied from 1 to 12 atmospheres. The lines observed were $Q(1)$, $S(0)$, $S(1)$ and $S(2)$. The wave numbers of these lines have been measured to a precision of a few thousandths of a wave number. The results are $Q(1) = 4{,}155{\cdot}243$, $S(0) = 4{,}497{\cdot}830$, $S(1) = 4{,}712{\cdot}895$ and $S(2) = 4{,}916{\cdot}990$ all expressed in wave numbers *in vacuo*.

The intensities of the quadrupole lines have been observed to depend only on the mass of gas in the absorbing path (pressure independent). We have been unable to see any pressure shift or appreciable pressure broadening in the pressure-range of 1–12 atmospheres. The quadrupole lines are exquisitely sharp. The breadth of the lines closely approaches the breadth expected from the Doppler effect.

Probably the most surprising feature of the 1–0 quadrupole spectrum is its relatively high intensity. The intensities of the lines is 10–100 times as great as might be expected from the observations of Herzberg[4] on the overtones and the calculations of James and Coolidge[5]. We have been able easily to observe $S(1)$ with atmospheric pressure and an optical path of less than 100 metres.

In addition to the quadrupole spectrum we have also observed the induced dipole spectrum, which consists of extremely broad features underlying the quadrupole spectrum. The intensity of the dipole spectrum has been observed by Hare and Welsh[6] to depend on the square of the pressure. We have been able to verify this last observation. Furthermore, at as equivalent to 10–20 per cent of the major component, of a substance having an R_F value similar to that of DL-inositol. The R_F value of the minor component in three different solvent systems[3] and its electrophoretic mobility on paper in borate buffer[4] were found to be identical with that of an authentic specimen of DL-inositol. A positive reaction with the Scherer test for inositols, as adapted by Nagai and Kimura[5] for use on paper chromatograms, was also obtained.

By hydrolysis of a 'ferric phytate' preparation from several kilograms of soil, sufficient for the mixture of the two inositols was obtained for preparative chromatography on a cellulose powder column[6]. The minor component was isolated and found to be optically inactive; its hexabenzoate had a melting-point of 213° C., not depressed by admixture with authentic DL-inositol hexabenzoate.

A sample of component VI, inositol hexaphosphate, was isolated as the barium salt, converted to the free phosphate by treatment with a cation exchange resin ('Zeo-Karb 225'), the solution adjusted to *p*H 4·0 and heated for 4 hr. at 110° C. The resulting mixture of unchanged hexaphosphate and lower esters was fractionated on 'Dowex-1(*C*1)' as before and the various components hydrolysed. The unchanged hexaphosphate and each of the lower esters produced by the hydrolysis at *p*H 4·0 contained DL-inositol.

It is known that boiling 95 per cent acetic acid saturated with hydrogen chloride brings about the reversion of *myo*-inositol to DL-inositol[7,8]; but no such change has been reported when aqueous acid is used. It was found that hydrolysis of inositol hexaphosphate (Ciba, Ltd.) under the conditions used for hydrolysis of the soil preparations gave rise to *myo*-inositol only. Addition of inositol hexaphosphate to soil phytate preparations before hydrolysis did not increase the amount of DL-inositol present in the hydrolysate, as judged by visual examination of the paper chromatograms.

Reversion of *myo*-inositol hexaphosphate to DL-inositol hexaphosphate during extraction from soil was considered to be very unlikely. However, a sample of *myo*-inositol hexaphosphate (Ciba, Ltd.) was put through the extraction procedure and hydrolysed. Only *myo*-inositol was detected in the hydrolysate.

Although *myo*-inositol occurs to a considerable extent in Nature in the form of phosphate esters, none of the other inositols has previously been found in combination with phosphate. The fact that DL-inositol hexaphosphate has now been shown to occur in soil organic matter but is apparently absent from plant and animal tissues[9] suggests that it is of microbiological origin.

Scyllo-Inositol Hexaphosphate

Hydrolysis of component VII with 6 *N* hydrochloric acid at 110° C. for 40 hr. liberated material which gave a positive Scherer reaction for inositols. Paper chromatography in acetone–water (4 : 1) and *n*-butanol–acetic acid–water (4 : 1 : 1) of the hydrolysis product showed that its R_F value in these systems was identical with that of *myo*-inositol (preceding communication). A mixture of the inositol derived from component VII and *myo*-inositol failed to separate in these solvent systems.

When sufficient of the inositol from component VII became available its hexa-acetate was prepared. This had a melting-point of 301° C. which was not depressed by admixture with authentic *scyllo*-inositol hexa-acetate. The identity of the inositol with *scyllo*-inositol and the absence of other inositols were confirmed by: (1) Paper chromatography in phenol–water (4 : 1)[3]. This system distinguished between *myo*-inositol and *scyllo*-inositol provided that chromatograms were irrigated for at least 24 hr. This is contrary to the observations of Hawthorne[10] but in agreement with those of Angyal, McHugh and Gilham[3]. (2) Comparison of the infra-red spectra. The absorption band at 873 ± 11 cm.$^{-1}$ attributed to deformation of equatorial C—H bonds is shown by all the inositols except *scyllo*-inositol[11].

Scyllo-inositol has long been known to occur free in certain plant and animal tissues, but there is no record of its occurrence in combination with phosphate[9]. Recently, Hawthorne[10] examined lipids extracted from the liver of *Raia clavata* (the common ray) and also from the seeds (acorns) of *Quercus robur* (the common oak), both of which contain free *scyllo*-inositol, but found only *myo*-inositol in the hydrolysates of the phospho-inositide fraction.

Caldwell and Black[2] found that organic phosphates accumulated in soil parent materials (for example, clay–sand mixtures) if organic and inorganic nutrients were added, the whole inoculated with a water extract of surface soil, and incubated for several months at 30° C. The presence of *myo*-inositol hexaphosphate, and material apparently identical with what has here been shown to be *scyllo*-inositol hexaphosphate, was demonstrated in these materials after incubation. This investigation gives no indication which of the many types of micro-organism present in soil are responsible for the build-up of inositol phosphates.

Examination of organic phosphates extracted from yeasts[12–15] and *Penicillium chrysogenum*[16] has shown that although in one case[12] a material referred to as 'phytin' was isolated, later workers[13,14] either failed to repeat the preparation or considered that the 'phytin' was not inositol hexaphosphate. It is obviously necessary to carry out further investigations of groups of micro-organisms likely to be present in soil, with the view of determining which of them possess the ability to synthesize inositol hexaphosphate.

I thank Prof. S. J. Angyal, University of New South Wales, Sydney, for his advice, and for the preparation of the hexabenzoate derivative and its comparison with an authentic sample of DL-inositol hexabenzoate, and also for samples of *scyllo*-inositol and *scyllo*-inositol hexa-acetate.

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are shown as percentages of the applied nitrogen. They may be converted to parts of inorganic nitrogen per million parts of dry soil by multiplying by 5·6.

In the first week after urea application, the average *p*H value and contents of ammonium, and hence of free ammonia, in the urea-treated soil were high and the dry-matter yield was only 57 per cent of that of the nil-nitrogen control. The urea-treated soil smelt of ammonia during this first week, and the results of daily analyses showed a rapid accumulation of ammonium ion, with a maximum on the fourth day after urea application. In the second week, both the *p*H value and the ammonium content fell and the yield rose to 92 per cent of the control. In the following three weeks the *p*H value and the ammonium content decreased further, but little or no nitrate was formed. The nitrite-ion content, however, increased and reached a maximum of 24 per cent of the applied nitrogen in the fourth week. In this and the subsequent week the dry-matter yields fell to 32 per cent of those of the nil-nitrogen controls. In the fifth and sixth weeks after urea application, the nitrite-level fell and nitrate contents increased sharply. The dry-matter yield also increased in the sixth week to reach a value of 82 per cent of that of the nil-nitrogen control. The total inorganic nitrogen accounted for only half the applied nitrogen; the other half was lost probably as gaseous products.

Throughout the experiment, visual symptoms of damage to the plants growing in the urea-treated soil were recorded. During the first three weeks of bioassay, visible damage was confined to necrosis at the leaf tip accompanied by some loss of turgidity. In the fourth and fifth weeks, the leaves rapidly lost their bright sheen and became flaccid, and at harvest many of the plants appeared to be dead. During the sixth week of bioassay the plants in contact with the urea-treated soil appeared to be as healthy as the controls.

These results confirm that phytotoxicity to seedlings can occur soon after the application of urea to soil, due, at least in part, to the accumulation of free ammonia produced by the hydrolysis of urea. They also show that nitrite can accumulate in urea-treated soil under conditions similar to those of this experiment. This nitrite accumulation is accompanied by marked phytotoxicity and reduction in plant yield. The later phase of urea-induced plant damage is thus probably due to toxic levels of nitrite.

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FORMS OF INOSITOL HEXAPHOSPHATE IN SOILS

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DL-Inositol Hexaphosphate

An investigation of the 'phytate' fraction of soil organic matter by Smith and Clark[1], using a step-wise elution with increasing concentration of hydrochloric acid from an anion-exchange resin column, showed that in addition to small amounts of lower inositol phosphates there was present an organic phosphate which was eluted from the column after the main fraction, inositol hexaphosphate, had been removed. This material was referred to as an "isomer of inositol hexaphosphate". A further examination of this by Caldwell and Black[2] showed that on hydrolysis a substance was released which gave positive Scherer and Salkowski reactions but did not support the growth of *Saccharomyces carlsbergensis* or of a *myo*-inositol-requiring mutant of *Neurospora crassa*. The 'isomer' was therefore considered to be the hexaphosphate of an inositol other than *myo*-inositol.

In an investigation of the 'phytate' fraction of some Australian soils the following techniques have been used. The soil is first washed with 0·2 *N* hydrochloric acid, then extracted for 4 hr. at 60° C. with *N* sodium hydroxide. The extract is made strongly acid (*p*H 0·5) and the precipitated humic acid removed by centrifuging. Sufficient solid sodium hydroxide is added to neutralize the resulting fulvic acid solution, followed by excess sodium hypobromite. Oxidation is continued at 5° C. overnight and completed next day at 60° C. for 1 hr. The solution is acidified, extracted with ether to remove bromine, adjusted to $pH \simeq 2{\cdot}0$ and concentrated ferric chloride solution added until no further precipitation takes place. The precipitate is suspended in water and converted to the sodium salt by addition of sodium hydroxide solution.

Examination of material prepared in this way from four soils of widely differing characteristics was made by gradient elution, with 0–1·5 *N* hydrochloric acid, from a column of anion-exchange resin, 'Dowex-1(*Cl*)'. Aliquots of the fractions collected were analysed for total phosphorus by the method used by Caldwell and Black[2]. The results indicated the presence of seven components. Comparison with results obtained in an identical separation carried out on a mixture of inorganic phosphate and commercial phytin (Ciba, Ltd.) showed that component I was inorganic phosphate, IV and V were apparently identical with material present in commercial phytin, that is, presumably lower phosphates of inositol, VI was inositol hexaphosphate and VII the material described by Smith and Clark[1].

Hydrolysis of components II–VII was carried out in 6 *N* hydrochloric acid at 110° C. for 40 hr., the hydrolysates evaporated, de-ionized and examined by paper chromatography[3]. Components II and III contained no inositol; VII, *myo*-inositol only; but IV, V and VI contained in addition to the major component, *myo*-inositol, a smaller amount, estimated

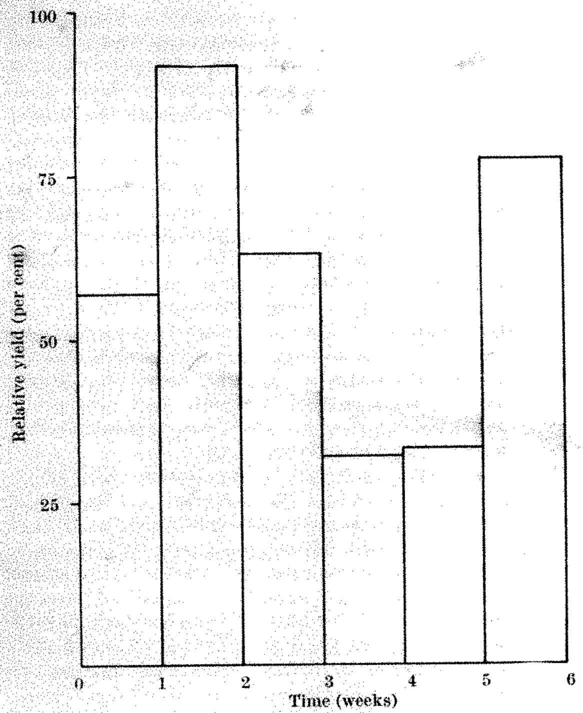

Fig. 1. Dry-matter yields from urea-treated soil at each weekly bioassay as percentages of yields from nil-nitrogen control

mixed throughout the soil there was little or no loss of seedlings at the germination stage. Two or three weeks later, however, in the urea-treated soil the plants lost their healthy appearance and many died. There was no adverse effect on the plants in the same soil treated with equivalent ammonium nitrate. The following is an account of an investigation into the possible cause of this later phase of urea-induced phytotoxicity, which cannot be ascribed to the presence of biuret.

The effects of the successive transformation products arising from addition of urea to soil were bioassayed at weekly intervals over a six-week period. The technique used was a modification of that of Stanford and DeMent[2] which has proved valuable for making determinations of short-term crop responses to nutrients or toxic materials applied to soil. The test soil, a sandy loam (*p*H 7·4), was sieved to pass ¼-in. mesh. Half the sieved soil was mixed mechanically with crystalline urea containing 1 per cent biuret at a rate equivalent to 0·6 gm. nitrogen per pot. The other half of the soil was also mixed mechanically, though no nitrogen was added. The treated and untreated soils were placed in 5-in. diameter plastic pots with two replicates of each treatment at each time of bioassay. The pots, placed on saucers in a heated glasshouse, were given a uniform overhead watering and were then covered with saucers to reduce loss of moisture. Further watering was given when necessary. At weekly intervals, 12 maize seeds, var. John Innes Hybrid, were sown in a 50:50 (w/w) mixture of silver sand and sandy loam in bottomless 3½-in. plastic pots standing on Petri dish bases. One week after sowing, by which time the seedlings were 2–3 in. high and had developed a root system, the 3½-in. pots were removed from the Petri dishes and placed on the urea-treated and untreated soils in the 5-in. pots. Water was applied to the saucers under the 5-in. pots to encourage rapid downward growth of the roots into the soil. A week after the transfer to the urea-treated and untreated soils, the foliage was cut at the level of the sand–soil mixture and oven-dried at 105° C. The dry-matter yields of the crop from the urea-treated soil are shown in Fig. 1 as percentages of those from the untreated soil for each week of the six-week experiment. Thus, the changing fertility of the treated and untreated soil was assessed over each separate weekly period for a total of six weeks.

At intervals of 2 or 3 days, the soils in the 5-in. pots were sampled for chemical analysis of inorganic nitrogen. The samples were extracted with *N*/calcium chloride and the ammonium, nitrate and nitrite contents of the extracts were determined by colorimetric methods. Frequent *in situ* measurements of the *p*H values of the soils in the 5-in. pots were made. The results, averaged over each week of the bioassay, are presented graphically in Figs. 2 and 3. The results of the analyses for inorganic nitrogen

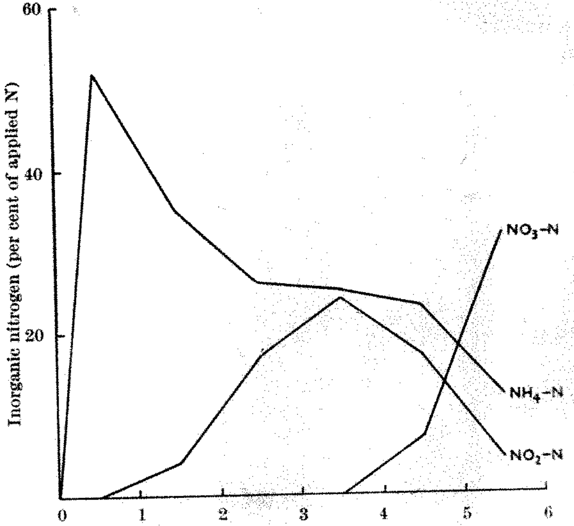

Fig. 2. Inorganic nitrogen contents of urea-treated soil as percentages of the applied nitrogen

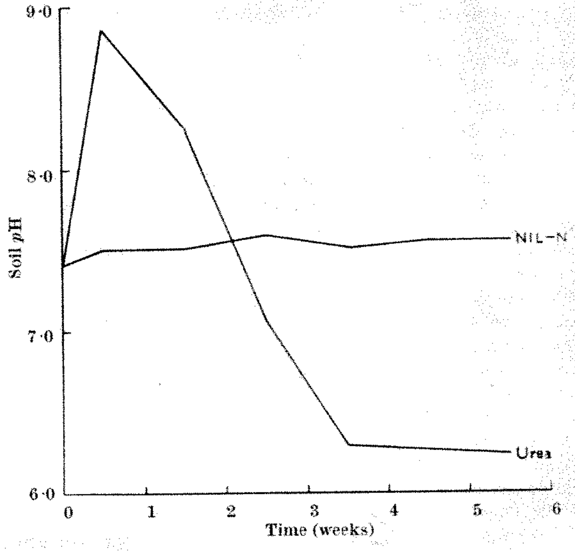

Fig. 3. *p*H values of urea-treated soil and nil-nitrogen control

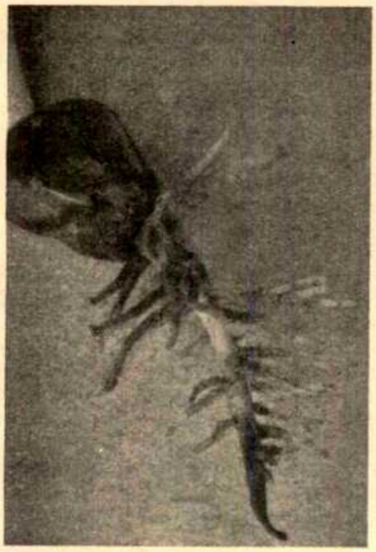

Fig. 1. Maize root damaged by urea

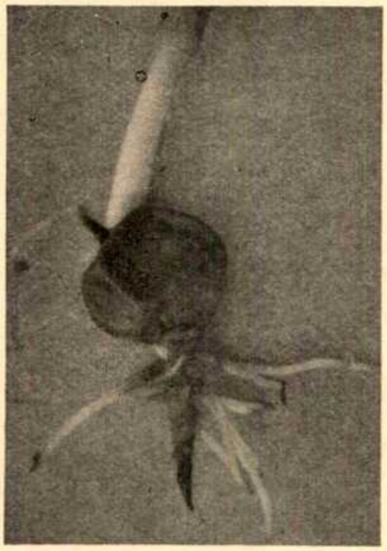

Fig. 2. Maize root damaged by anhydrous ammonia

8-in. diameter pot, equivalent to 70 or 700 lb. nitrogen per acre, were applied as urea or anhydrous ammonia. The former rate was a normal field rate; the latter was considered to give a not unrealistic concentration in the context of combine drilled fertilizer and seed, and had been shown to produce very severe urea damage. The urea was mixed with the soil immediately prior to sowing; anhydrous ammonia gas was injected into the centre of the pot by means of a hand lance either at sowing or two or five days later, spanning the period of ammonia release from the urea. In a ninth treatment no nitrogen source was applied (control). There were six replicates of each treatment. Germination occurred in 5 days, and 7 days after sowing the plants from 4 replicates of each treatment were separated from the soil and the roots washed clean for examination.

At the low nitrogen-rate urea caused no obvious signs of root damage, but about 20 per cent of the seeds showed delayed germination. At the high nitrogen-rate urea produced severe root damage; the roots were very short (mostly less than 1 cm.) and ended in a scorched brown tip (Fig. 1). Damage caused by the anhydrous ammonia was much more severe at the centre of the pot than at the edges, showing that there was little movement of ammonia through the soil. When applied at the high rate at sowing or 2 days later germination was reduced by about 50 per cent; but those plants which did germinate showed damage visually identical with that caused by urea, namely, a shortened root curtailed abruptly with a scorched tip (Fig. 2). At the low rate, only the seedlings near the centre of the pot were damaged. When anhydrous ammonia was applied 5 days after sowing, that is, after the seeds had germinated, damage occurred along the whole length of the roots, which became thin and limp; it was most marked at the high rate of application where some of the plants were killed.

The other two replicates of each treatment were allowed to grow on until the forty-second day after sowing when the roots were washed clean for examination as before. Plants which had not been killed but had had their roots damaged by the anhydrous ammonia treatment had recovered and their growth was similar to that of the control. Of the plants which had received urea, those with the low nitrogen-rate were slightly smaller than the controls but not markedly different, while at the high rate the plants were very small with much-reduced root systems. The lack of recovery from the early damage in the urea-treated plants compared with the recovery of those which received anhydrous ammonia suggests that another factor in addition to ammonia is active in urea toxicity[4].

These observations support the hypothesis that the roots of seedlings can be damaged by the ammonia produced by the hydrolysis of urea in the soil, but they also indicate that at least one other factor may be operative in urea toxicity.

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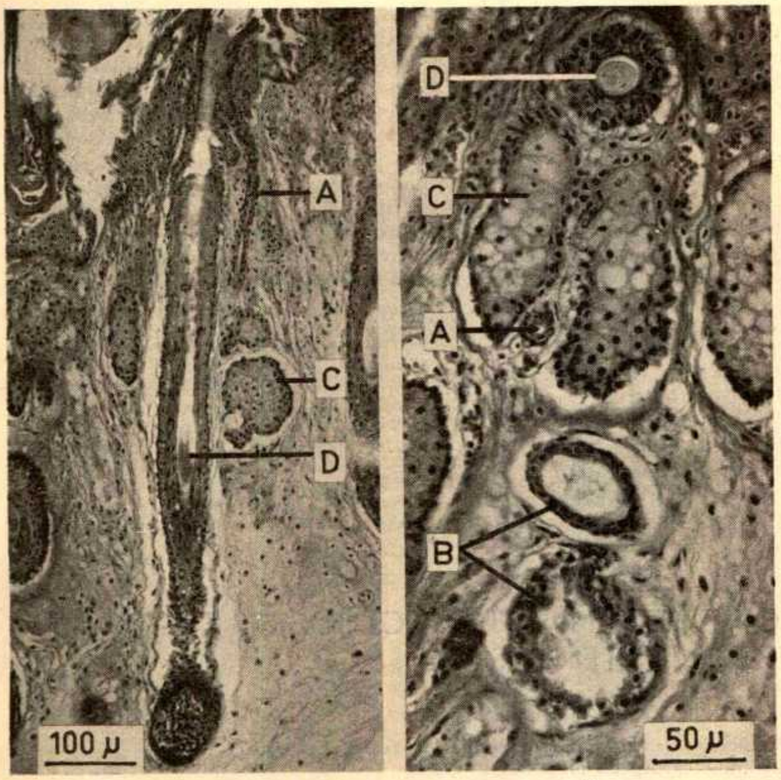
Nitrite Toxicity arising from the Use of Urea as a Fertilizer

In a series of pot experiments with maize as the test crop the fertilizer value of commercial urea containing 1 per cent biuret was compared with that of ammonium nitrate.

The nitrogen sources were either uniformly mixed with the soil at the time of sowing, or placed with the seed ¼ in. below the soil surface. At low rates of applied nitrogen, urea gave a crop response equal to that from ammonium nitrate. At high rates of application, however, the response to urea was much lower than that to ammonium nitrate and the foliage yields were often less than those of the nil-nitrogen controls. The lower yields obtained when urea was placed near the seed were often due to a reduction in the number of germinating seedlings. This damage has been attributed to high concentrations of free ammonia near the seed (ref. 1 and preceding communication). When the nitrogen sources were



a *b*

Fig. 2. *a*, Longitudinal section; *b*, transverse section of follicles from the amnion. *A*, Sweat gland duct; *B*, sweat gland body; *C*, sebaceous gland; *D*, wool fibre

this tissue was formed as a result of plaques having undergone further differentiation and organization. Amniotic plaques have been reported in a wide variety of animals, including ruminants[1-4] and horses[4,5]. Amoroso describes them in the horse as "local heapings-up of amniotic ectoderm", while Hammond, Amoroso and Naaktgeboren and Zwillenberg report that they contain large quantities of glycogen. Hammond observed, significantly, that the plaques "had an appearance very similar indeed to that of the fœtal skin epidermis; distinct layers were present as in the skin; the base adjoining the mesoblast was thrown into papillæ and in the mesoblastic portion of these blood vessels were numerous". In our experience with sheep, amniotic plaques are limited in distribution to the umbilical cord and to a variable area around it. Rarely are plaques found farther than 15 cm. from the point of reflexion of the amnion from the cord. Plaques vary in shape from flat plates, 1–2 cm. in diameter, to small masses of the same area but from which project several club-like projections (up to 2 mm. in length).

Histological sections of fibre-producing tissue (Fig. 2) were similar to those of normal skin. The epidermis and dermis were clearly defined, and follicles were of normal basic structure, although they appeared somewhat shortened. The dermal papillæ were well defined. Sebaceous glands were associated with nearly all follicles, but sudoriferous (sweat) glands were rarely seen. Neither arrector pili muscles nor melanin pigment were observed.

These observations indicate the competence of the amniotic ectoderm to differentiate in a manner similar to that of the normal fœtal epidermis—given adequate inductive stimulus. Further evidence in this direction comes from the recent observations of Payne and Payne[6], who report the occasional production of epidermoid cysts (some of which contained hair follicles and sebaceous glands) from transplants of amnion to the maternal omentum in rats.

In the embryological sense, the primary requirements for the differentiation of the ectoderm with consequent formation of follicle and fibre growth are the presence of an inductive stimulus from the mesoderm and the competence of the ectoderm to react to this stimulus. Both prerequisites must have been met in the present cases, and yet, judging from the apparent rarity of the phenomena, either or both requirements must usually be absent from the amnion, or the time-relationships of their occurrence disturbed. From the point of view of development, the amnion has an origin similar to that of fœtal skin and comprises both ecto- and meso-dermal tissue, a homology which has been emphasized by Naaktgeboren and Zwillenberg. The frequent occurrence of amniotic plaques, their epithelial nature, and the fact that they rarely undergo further differentiation to produce fibres, suggest that the ectodermal component of the amnion is normally competent but that the inductive stimulus of the mesoderm is usually absent during the period of competence.

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TOXIC EFFECT OF UREA ON PLANTS

Damage to Plant Roots caused by Urea and Anhydrous Ammonia

DAMAGE caused to seedlings by urea of low biuret content is often attributed to the ammonia released by the hydrolysis of the urea in the soil[1-3]. If this is so, anhydrous ammonia should cause damage similar to that from urea.

To investigate this hypothesis maize seedlings (J.I. Hybrid, 10 per pot) were grown in a glasshouse in Mitscherlich pots on a sandy loam soil, *p*H 7·4. Nitrogen dressings of 0·25 or 2·5 gm. nitrogen per protein) sometimes seen in the fibroblast cytoplasm. In view of the changed appearance of the surrounding intercellular tissue it can be presumed that the fibroblasts are contributing to the ground substance, producing new (argyrophilic) collagenous fibres or precursor substances, and splitting up the existing bundles of fibres, possibly by secreting an enzyme. Eosinophils have been shown to have considerable chemotactic properties[1] so it is likely that these cells are attracted into the tissues by the fibroblast secretions, to perform a necessary function by acting on or with the secretions so that a pathological condition cannot develop; in extreme cases (for example, œstrous ferret vulval skin) they are attracted right to the source of secretion so that the cells are in contact with each other. In tissues in which the fibroblasts are constantly stimulated to synthesize and secrete, some such regulatory mechanism is essential. The fact that in some of these structures eosinophils are especially numerous during the involutionary process strengthens the idea that they are involved in the removal of substances not required by the organism. Since the eosinophils contain relatively little RNA they are unlikely to be contributing to the extracellular protein themselves.

Although many different suggestions for the function of eosinophils have been put forward from time to time, several authors[1,9,10] have considered that protection against foreign protein is the chief reason for an abnormal accumulation of eosinophils. The ideas suggested by the present findings are thus an extension of this theory to include the regulation of extracellular protein produced under certain normal conditions by the cells of the animal itself.

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COMPETENCE OF THE AMNIOTIC ECTODERM TO DIFFERENTIATE IN SHEEP

By P. G. SCHINCKEL

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DURING a routine examination of placentæ from parturient ewes, Mr. R. Edols of this Laboratory observed one in which he thought that wool fibres were growing on the membranes. Closer examination showed that fibres were growing on the amnion. These were distributed on the distal part of the umbilical cord and the adjacent area of amnion, in a definite but interrupted row, and as isolated islands, along blood vessels passing between the two layers of the amnion. The exact origin of the blood vessels could not be determined because of damage to the cord at parturition. The row was 11 cm. long and rarely more than 3 mm. wide. The fibres were produced from small masses of tissue, in which the general outline of follicles was readily seen (Fig. 1).

Since the discovery of this case, a further 50 placentæ from the same group of experimental ewes have been examined and, in two, fibres (about 1 cm. in length) were found. In these, the number of fibres was much smaller and all were produced from scattered islets of tissue. A fourth case was found in the membranes of one of 40 ewes examined in another group. In this instance only two fibres were found in one isolated amniotic plaque. The phenomenon was not observed in any membranes removed from a third group of 49 ewes, 80–145 days pregnant. All positive cases have been subject to histological examination. The ewes in the first group above were involved in one of Mr. G. Alexander's experiments in which a number of cotyledons were surgically removed from the uterus some months prior to mating. Of the three cases showing fibre growth on the amnion, one ewe had had 12 cotyledons removed, one 36 and one 60. I have been unable to locate any other references in the literature to the presence of fibre-producing follicles on the amnion.

Macroscopically, the fibre-bearing tissue resembled amniotic plaques and there seems little doubt that

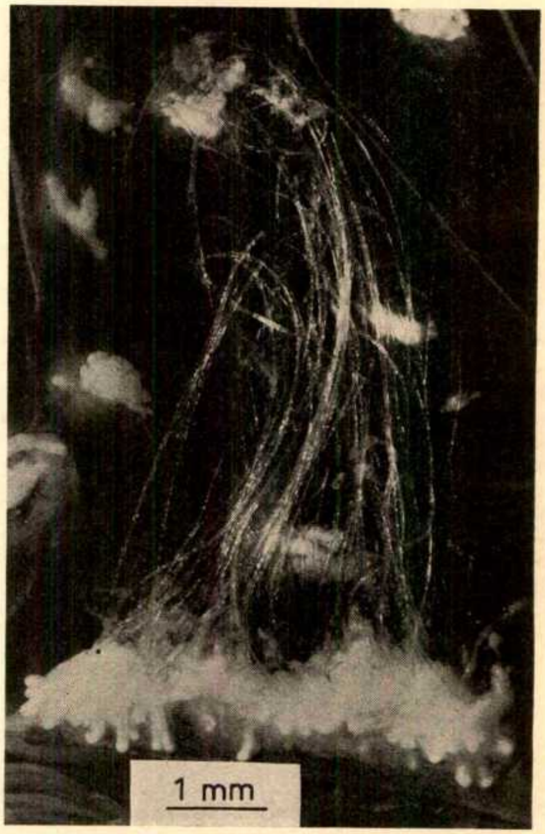
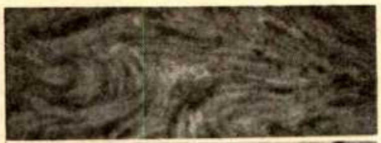


Fig. 1. Photomacrograph of a portion of the fibre-bearing tissue from the amnion showing fibres and outlines of follicles. Blood vessels may be seen beneath the tissue mass



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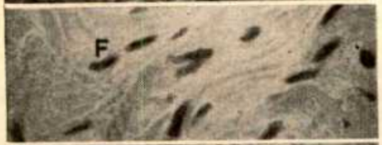

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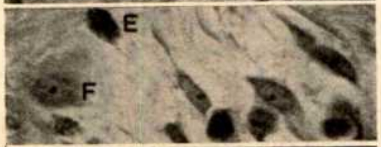

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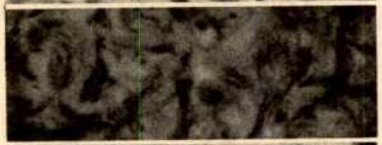

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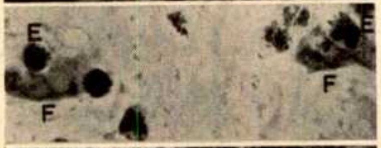

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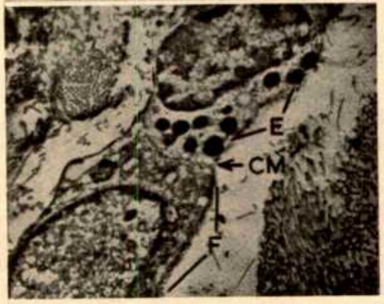

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Figs. 1–5. Photographs with light microscope (all × 600)

Figs. 1 and 2. Vulval skin of spayed ferret. (1) Dense collagenous fibres; little argyrophilia (silver impregnation–methyl blue). (2) Small fibroblast nuclei (*F*) with no visible cytoplasm; no eosinophils (hæmatoxylin–azur II–eosin)

Fig. 3. Vulval skin of pro-œstrous ferret. Enlarged nuclei and nucleoli of fibroblasts; some basophilic fibroblast cytoplasm; many eosinophils (*E*) (hæmatoxylin–azur II–eosin)

Figs. 4 and 5. Vulval skin of œstrous ferret. (4) Loosened and argyrophilic collagenous fibres; increased ground substance (silver impregnation–methyl blue). (5) Enlarged fibroblasts with much basophilic cytoplasm and negative golgi images; numerous eosinophils, some in contact with fibroblasts (hæmatoxylin–azur II–eosin)

Fig. 6. Electronmicrograph of œstrous ferret vulval skin. Fibroblast containing much rough-membraned endoplasmic reticulum in close contact with eosinophil; apposed cell membranes (*CM*); bundles of banded collagen fibrils. (× *c*. 6,700)

methods. Samples of vulval skin from one spayed and one œstrous animal were also prepared and sectioned for electronmicroscopy.

In the spayed animal (Figs. 1 and 2) the microscopical appearance of the vulval skin resembles that of skin from the rump. The collagenous fibres are dense and show little argyrophilia, the fibroblast cells consist of small nuclei only, with no visible cytoplasm, and there are no eosinophils. In early pro-œstrus the nuclei of some fibroblasts are slightly enlarged, a few having a small amount of basophilic cytoplasm; some eosinophils are present, but confined to the patches in which the fibroblasts are changed. In pro-œstrus (Fig. 3) all fibroblasts have basophilic cytoplasm, some only a small amount, but in others the obviously enlarged nuclei with prominent basophilic nucleoli are surrounded by much basophilic cytoplasm; many eosinophils are present, occasionally seen in contact with fibroblasts. In full œstrus (Figs. 4 and 5), when the vulval skin is enormously swollen and the amount of ground substance among the loosened and more argyrophilic fibres greatly increased, the fibroblasts are dramatically enlarged, with big nuclei and nucleoli and conspicuous negative Golgi images (demonstrable also positively by silver or osmic acid methods) in their strongly basophilic cytoplasm. Many fibroblasts are in contact with one or more eosinophil. Electronmicrographs of such associated cells show that the contact between the apposed cell membranes is indeed exceedingly close (Fig. 6). By contrast there were no such changes in the rump skin from pro-œstrous and œstrous animals.

Experimental histochemical studies of the changed fibroblasts in the ferret vulval skin, also in the modified connective tissue of œstrous, pregnant and post-partum animals of other species[4], indicate that these cells contain much ribonucleic acid. This has been confirmed by electronmicroscopy of ferret vulval skin, which shows that the cytoplasm of the enlarged fibroblasts contains an extensive rough membraned endoplasmic reticulum[6]. Some of the fibroblasts contain periodic acid–Schiff-positive substance in the cytoplasm. It appears that in all these various tissues an enlarged negative Golgi is visible in the fibroblast cytoplasm only when the cells have reached a considerable degree of enlargement.

The changes of eosinophils are more obviously numerical than morphological. Both microscopically and ultramicroscopically, however, they do not appear to contain much RNA. Some, but not all of them, are periodic acid–Schiff-positive, a few being brilliantly coloured, but the majority are a less intense shade.

The close contacts between eosinophils and fibroblasts appear to be much more frequent than to be expected from a random distribution. A mathematical test (which will be described in detail elsewhere) shows that the number of eosinophils in close proximity to fibroblasts is significantly in excess of the expected random distribution. This is so not only in the œstrous condition when many of the cells are in contact, but also in pro-œstrus when the eosinophils are near the fibroblasts but seldom actually touching.

The evidence for a definite association between eosinophils and enlarged fibroblasts in the modified connective tissues of œstrous and pregnant animals which have been examined thus seems fairly clear. Since these tissues are from various sites in the pelvic and perineal region of animals from a number of different species, it can be postulated that, in such structures closely concerned with the reproductive process and specifically sensitive to sex hormones, an association between active fibroblasts and eosinophils may be characteristic of mammals generally. The actual purpose of this association is at present less clear; but a theory can be advanced which fits both currently observed and already known facts about each type of cell.

The high RNA content of the fibroblasts is evidence that they are actively engaged in protein synthesis[7], while their enlarged Golgi suggest that they are actively secreting[8], probably this protein, which may be also partly related to the periodic acid–Schiff-positive substance (? mucopolysaccharide or glycophytohæmagglutinin exerts its mitogenic effect due to its ability to cause rejuvenation of the lymphocytes. Not all lymphocytes, however, are able to respond to the stimuli of phytohæmagglutinin, otherwise the percentage of blasts might be considerably greater. It would seem that not all the cells which develop nucleoli are destined to become blasts; but this may be due to deficiencies in the culture medium caused by the increased metabolism of the cells, rather than the inability of the cells to respond.

These results are in agreement with those reported by Cooper, Barkham and Hale[8], from their studies on the incorporation of thymidine into deoxyribonucleic acid (DNA). The increase in DNA synthesis they observed is probably due to the metabolism of the nucleoli-possessing type of cells, and later the blast cells. These cells show morphological features of active metabolism, namely, the presence of nucleoli, the increasing size of the nuclei and increasingly basophilic cytoplasm.

Our studies have also indicated that the appearance of blast formation coincides with the maximum uptake of amino-acid from the medium.

Phytohæmagglutinin therefore seems to act by rejuvenating the normal intermediate lymphocytes, probably the younger cells, causing a general increase in metabolism, particularly in respect to increased DNA synthesis, which ultimately results in the division of some cells.

One of us (M. W. E.) acknowledges a Medical Research Council Scholarship.

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INFILTRATION OF EOSINOPHILS INTO THE MODIFIED CONNECTIVE TISSUE OF ŒSTROUS AND PREGNANT ANIMALS

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THE accumulation of eosinophils in tissues in which they are not always present is usually regarded as pathological[1,2]. The purpose of this article is to describe the behaviour of eosinophils in tissues in which they are not always present but accumulate in association with the normal physiological conditions of œstrus and pregnancy.

Recent[3-5] and current investigations have shown that regionally specific changes occur in various pelvic and perineal connective tissues from a number of different species in both œstrus and pregnancy (also in diœstrus in animals such as the rat with very short cycles). The changes in œstrus are often, but not always, less marked than they are in the same tissues from pregnant animals. The evidence is in favour of the fibroblast cells being responsible for the characteristic changes in collagenous fibres and ground substance, as they are the only cells present in all the sites in all the species where such changes have been found; also the morphology of the fibroblasts becomes very different in œstrus or with the advance of pregnancy. It is fairly certain that these modifications are effected by endogenous hormones.

In many of these tissues, particularly those with the most markedly altered fibroblasts, eosinophils are distributed throughout the tissue, having presumably entered from the blood, as they are often seen in and surrounding sectioned small blood vessels. Eosinophils are absent or rarely seen in the same tissues from anœstrous or spayed animals, or in tissues from diœstrous animals with long cycles (for example, ewe).

Eosinophil infiltration has been seen in œstrus in the broad uterine ligament (ewe), uterus (ewe, rat, mouse, ferret), cervix (rat), vagina (rat), vulval skin (ewe, ferret). Eosinophils are also present in the uterus of diœstrous rats and mice and persistent œstrous rats.

Eosinophils have been seen in the following tissues from animals in late pregnancy or early post-partum involution: sacrosciatic ligament (ewe), broad ligament (ewe, cow, sow, rat, ferret), intramuscular connective tissue and tendon of origin of ischiococcygeus muscle (ewe, cow, sow), symphyseal ligament (mouse, hedgehog), sacro-iliac joint (ewe), retractor clitoridis muscle (ewe), uterus (ewe, rat, mouse, hedgehog), cervix (rat, hedgehog), vagina (ferret, rat, hedgehog), median canal (phalanger), vulval skin (ewe, rat). Involutionary stages for all these different structures have not been investigated, but in those which have (for example, broad ligament and sacro-iliac joint of ewe, symphyseal ligament and uterus of mouse), there are generally more eosinophils than in the same tissues from animals in late pregnancy.

Eosinophils have not been found in all the tissues in which fibroblast changes have been seen, and in the tissues in which they are present there is a considerable variation in the number of eosinophils. The differences appear to be related to the degree of change in the fibroblasts. In places in which eosinophils are numerous they are occasionally (for example, vulval skin of pregnant ewe, uterus of œstrous rat) or frequently (œstrous ferret vulval skin) seen in close contact with fibroblasts.

Since the grossly swollen vulval skin of the œstrous ferret represents the most extreme change in connective tissue of all the structures listed here, it has been made the subject of a special study. Altogether 10 ferrets were available (kindly supplied by Tasman Vaccine Laboratories, Lower Hutt) in several sexual states, including spayed, very early pro-œstrus, pro-œstrus and œstrus. Sections of vulval and rump skin were prepared by usual histological methods and stained with hæmatoxylin–azur II–eosin or periodic acid–Schiff or silver impregnation

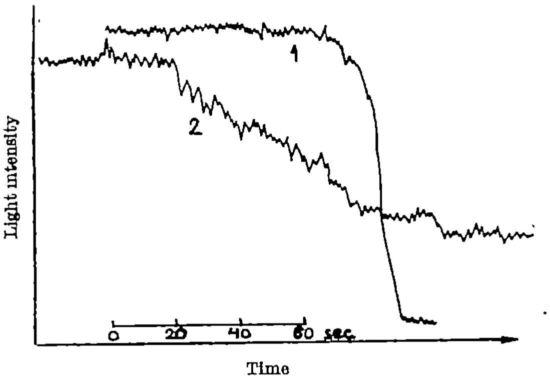

Fig. 1. Luminescence drop induced by substitution of *R* radicals for RO_2 in the oxidation of ethylbenzene (1) and methyloleate (2) at 60°. The rate of initiation is 7·5 × 10^{-7} mole $l.^{-1}$ $sec.^{-1}$. For ethylbenzene the drop is more sharp, as the reaction of this hydrocarbon with oxygen occurs more easily than in the case of methyloleate radicals

remained constant for a long period of time, a drop in the luminescence, caused by disappearance of RO_2, was observed (Fig. 1).

The higher the oxidation-rate and the lower the initial concentration of oxidation, the earlier the drop occurs. On the other hand, the shape of the drop itself should depend on kinetic parameters of the reaction. This was the basis for development of chemiluminescence methods for quantitative studies of oxidation reactions[4]. The correlation of results obtained by these and other methods is given in Table 1.

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Vibrationally Excited Products of Bimolecular Exchange Reactions

Many observations of gaseous bimolecular exchange reactions in which vibrationally excited products appear have been interpreted[1–3] in terms of a simple model developed by F. T. Smith[4]. Smith's treatment, based on the kinematics of a collinear collision, predicts that the upper limit to the fraction of energy of reaction which may appear as product vibration is $\sin^2\beta$, where β is the angle between the skewed axes of the potential energy surface on which a sliding mass point represents the progress of the collision. Observed vibrational energies in excess of the amount calculated in this way have usually been interpreted as contravening Smith's model. It is the purpose of the present communication to point out that when the Smith treatment is generalized by lifting the restriction that collisions be collinear, it becomes apparent that the fraction being calculated is more nearly a lower limit than an upper. As a result certain changes in the interpretation of the experimental evidence are required.

Suppose the reaction being considered is $A + BC \rightarrow AB(\text{excited}) + C$. Let the masses of A, B, and C be m_1, M and m_2 respectively; let the AB distance be r_1, the BC distance r_2, and the angle between r_1 and r_2 be α. Smith's calculation in effect replaces the steeply rising portion of the potential surface at small r_1 and r_2 for $\alpha = \pi$ with a vertical plane normal to the r_2 direction. The component of momentum of the sliding mass point which is parallel to r_2 is supposed to be reflected by this plane; this leads to the result quoted here. Smith suggests that to the extent that reflexion of the mass point is not specular, the amount of energy appearing in product vibration will be less than that calculated in this way.

For a fixed value of α (not necessarily π) the kinetic energy of the system is[4]:

$$T = \tfrac{1}{2}(\mu_1 - \rho\mu_3^2 r_2^2 \sin^2\alpha)\dot{r}_1^2 + \tfrac{1}{2}(\mu_2 - \rho\mu_3^2 r_1^2 \sin^2\alpha)\dot{r}_2^2$$
$$+ (-\mu_3 \cos\alpha + \rho\mu_3^2 r_1 r_2 \sin^2\alpha)\dot{r}_1\dot{r}_2$$

in which $\mu_1 = m_1(m_2 + M)/(m_1 + m_2 + M)$

$$\mu_2 = m_2(m_1 + M)/(m_1 + m_2 + M)$$
$$\mu_3 = m_1 m_2/(m_1 + m_2 + M), \text{ and}$$
$$\rho^{-1} = \mu_1 r_1^2 + \mu_2 r_2^2 - 2\mu_3 r_1 r_2 \cos\alpha$$

In general, no two-variable potential surface can be constructed on which the motion of a sliding mass point represents the collision, unless r_1 and r_2 are constants. This is an approximation which may be made in the present case, since interest centres on what happens at the point on the surface where the mass point is supposed to be reflected. With this identification of r_1 and r_2, one may proceed with the standard method[5] of potential surface construction and arrive at:

$$\sin(\tfrac{1}{2}\pi - \beta) = \frac{-\mu_3 \cos\alpha + \rho\mu_3^2 r_1 r_2 \sin^2\alpha}{(\mu_1 - \rho\mu_3^2 r_2^2 \sin^2\alpha)^{1/2}(\mu_2 - \rho\mu_3^2 r_1^2 \sin^2\alpha)^{1/2}}$$

The fraction of energy converted to vibration is then:

$$\sin^2\beta = (\mu_1\mu_2 - \mu_3^2)/(\mu_1\mu_2 + x)$$

in which:

$$x = \rho\mu_3^2 \sin^2\alpha(\rho\mu_3^2 r_1^2 r_2^2 \sin^2\alpha - \mu_1 r_1^2 - \mu_2 r_2^2)$$

When α is 0 or π, x is 0 and $\sin^2\beta = 1 - \mu_3^2/\mu_1\mu_2$, which is precisely Smith's result. One discovers by algebraic manipulation (making use of the fact that ρ^{-1} is the instantaneous moment of inertia of A, B and C about a perpendicular axis through their centre of mass, and therefore always positive) that $x \leqq 0$ for $0 \leqq \alpha \leqq \pi$ and any choice of rs and μs. Thus collinear collisions lead to a smaller fraction of energy converted to vibration than do any other type. Furthermore, since β varies from less than to greater than $\pi/2$ as α changes from π to 0, there is some intermediate value of α (much more probable geometrically than the collinear value $\alpha = \pi$) for which the potential surface axes form a right angle. For the Smith model this configuration corresponds to all the energy of reaction appearing as vibration; in the generalized model one then says that the upper limit for the fraction of energy converted to vibration is always unity, as is suggested by the experimental evidence, and by theoretical arguments adduced by J. C. Polanyi[6].

The observations concerning these reactions must therefore be reinterpreted as follows. Appearance of vibrational energy in excess of the amount calculated from the one-dimensional Smith model is always to be expected if the reflexion approximation for the motion of the representative mass point is a realistic one. Appearance of vibrational energy less than the amount calculated may mean that the reflexion approximation is a poor one, or that not all the heat of reaction is released as kinetic energy during the approach of the reactants, or that the observations have been made after some vibrational relaxation of the observed species has occurred.

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BIOCHEMISTRY

Structure of the Carbohydrate Moiety of Orosomucoid

Orosomucoid isolated from serum has been the subject of many investigations in recent years[1–4]. Popenoe[5] isolated an α_1-glycoprotein from the urine of patients with the nephrotic syndrome and showed it to be immunochemically identical to serum orosomucoid. More recently the glycoprotein has been isolated from the same source by means of cellulose ion-exchangers[6]. This glycoprotein was shown also to be identical to serum orosomucoid by immunoelectrophoresis and gel-diffusion techniques, and was found to be homogeneous by physical and chemical techniques[7].

The present investigations were concerned with the chemical structure of the carbohydrate portion of the latter glycoprotein by means of periodate oxidation.

Oxidation of the urinary orosomucoid in 0·04 *M* sodium metaperiodate (15 molar excess) at ambient temperature is complete after 10 days. Eighteen moles of formaldehyde and 36 moles of formic acid are liberated rapidly. During further slow oxidation an additional 54 moles of formic acid are liberated, but no additional formaldehyde. The final consumption of periodate is 190 moles per mole of orosomucoid (mol. wt. 46,500). The ratio formaldehyde/formic acid/periodate consumed after oxidation for 15 hr. is 2 : 4 : 9. Titratable non-dialysable acid increased from 18 equiv./mole of orosomucoid to 28 equiv./mole during the oxidation. The material contains no *O*-acetyl groups as determined by the method of Lipmann and Tuttle[8].

The urinary orosomucoid sample contained 18 *N*-acetyl neuraminic acid (NANA) residues per mole. When it was treated with 36 moles of sodium metaperiodate, 18 moles of formaldehyde and 18 moles of formic acid were liberated after 10 hr.

The carbohydrate residues remaining and fragments formed during the oxidation were investigated chromatographically after oxidation for 1 hr. and after total oxidation. The oxidized samples were reduced with sodium borohydride, hydrolysed (3 *N* H_2SO_4 for 4 hr.) and the amino-acids were removed by use of 'Deacidite *FF*' (carbonate).

Fucose and NANA were destroyed during the first hour of oxidation. After total oxidation appreciable amounts of galactose and mannose remained unoxidized, these substances being present in nearly equal quantities before and after oxidation, while the *N*-acetyl glucosamine residues appeared largely resistant.

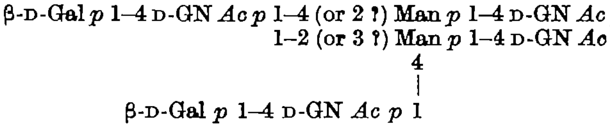
A spot migrating between galactose and mannose (butanol/ethanol/water, 5 : 1 : 4) was present in the hydrolysate of oxidized orosomucoid, and was shown to be glucose by oxidation to gluconic acid with the specific enzyme glucose oxidase. The absence of glucose in the hydrolysate of native urinary orosomucoid is consistent with the fact that it has not been reported by previous workers. One explanation for its release after oxidation is that its neighbouring unit(s) is oxidized, causing a weakening of an acid-resistant linkage, but the possibility of it arising by epimerization from mannose has not been excluded.

The fragments of the oxidized sugars contain, *inter alia*, appreciable quantities of glycerol and glycolaldehyde. The glycerol concentration appears to diminish during the slow stage of oxidation, indicating that oxidation of the activated carbon-5 hydrogen follows a cleavage between carbons-3 and -4, giving then additional formic acid with resultant loss of glycerol. Ionophoresis in molybdate buffer[9] showed the presence of a trace of threitol but no erythritol.

The amino-acid composition of the protein moiety remains qualitatively unchanged during the oxidation as shown by hydrolysis (5 *N* HCl for 17 hr.) and two-dimensional chromatography.

From these observations the following conclusions are indicated. *N*-acetyl glucosamine must be linked at position 3 or 4 or branched. The production of large amounts of glycerol and formic acid favours a 1–6 linkage for some of the galactose and/or mannose. The absence of erythritol proves that no mannose is linked at positions 1 and 4 alone, while the presence of a trace of threitol shows that only a small amount of galactose is linked at 1 and 4. Some of the mannose as well as galactose must be substituted at 1 and 3 or branched to account for that which resists oxidation.

In a recent paper, Eylar and Jeanloz[10] proposed the following structure for an octasaccharide obtained by mild hydrolysis of serum orosomucoid:



$$\begin{array}{rcl} \beta\text{-D-Gal}\,p\ 1\text{–}4\ \text{D-GN}\,Ac\,p\ 1\text{–}4\ (\text{or}\ 2\ ?) & \text{Man}\,p & 1\text{–}4\ \text{D-GN}\,Ac\,p \\ 1\text{–}2\ (\text{or}\ 3\ ?) & \text{Man}\,p & 1\text{–}4\ \text{D-GN}\,Ac \\ & 4 & \\ & | & \\ \beta\text{-D-Gal}\,p\ 1\text{–}4\ \text{D-GN}\,Ac\,p & 1 & \end{array}$$

(GN *Ac* is *N*-acetyl glucosamine)

The above findings are in accord with this structure first from the point of view of resistance of hexosamine units, and secondly, that only part of the mannose is susceptible to periodate oxidation.

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Recovery of Microbial Polysaccharide B-1459 with a Quaternary Ammonium Compound

A new cyclic process has been developed for isolating a microbial polysaccharide from fermented broth. The usual method has been by addition of a water-miscible solvent in which the polysaccharide is insoluble, for example, methanol or ethanol[1,2]. Because of the high viscosity of most microbial polymers in water solutions, it is difficult to obtain concentrations greater than 1·5 per cent in the fermented broth; consequently, large amounts of solvent are required to isolate and purify the polysaccharide.

In the new process a polyanion polysaccharide was isolated from fermented broth with a quaternary ammonium compound, and the quaternary ammonium compound recovered and re-used in a cyclic procedure, thereby decreasing the amount of solvent required for isolation. The polysaccharide isolated was synthesized by the bacterium *Xanthomonas campestris NRRL B*-1459 by fermentation of a medium containing glucose, organic nitrogen, and inorganic salts. At the completion of fermentation, the broth had a viscosity of 7,000 centipoises (determined by means of a Brookfield viscometer); (mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned) and a polysaccharide content of about 1·5 per cent.

Tests showed that the quaternary ammonium compound would be most effective for recovery of polysaccharide in dilute broth in the presence of an electrolyte, such as potassium chloride. Dilution of the broth also facilitated removal of cells and insoluble materials.

Percentages of potassium chloride and quaternary ammonium compound stated in the recovery procedure are based on the weight of undiluted broth.

The broth was diluted to a viscosity of 450 cp. by addition of two parts by weight of water, and the cells removed by centrifugation. Two per cent potassium chloride was added to the centrifugate and the polysaccharide precipitated with 2·75 per cent (v/w) of 'Arquad 16–50', an industrial grade quaternary ammonium compound, which consists primarily of cetyltrimethylammonium chloride. Electrolyte concentration was critical since addition of 1 per cent potassium chloride gave a voluminous gel-like precipitate; whereas with 3 per cent potassium chloride, no precipitate formed.

A fibrous, slowly settling, precipitate formed and was separated from the broth by centrifugation at 13,000*g*. The weight of the cake was about 8 per cent of the original broth weight. The centrifuged liquid contained about 2 per cent of the quaternary ammonium compound used, as assayed by the method of Colichman[3].

To remove the quaternary ammonium compound from the polysaccharide, the cake was agitated with four times its weight of methanol containing 0·05 per cent potassium chloride to prevent gelling in the methanol. The cake filtered easily on a vacuum filter. A total of three washes was made, after which the water-soluble polysaccharide was vaccum dried.

The combined filtrates from the methanol washes were concentrated under vacuum to recover methanol in the distillate and 'Arquad 16–50' in the concentrate. Distillate from the concentration contained 97 per cent methanol. Concentrate from the evaporation was assayed for the quaternary ammonium compound by determining the efficacy of precipitation on polysaccharide broth samples. The concentrate contained 80 per cent of the original quaternary ammonium compound. Consequently, 20 per cent of the original amount of fresh quaternary ammonium compound was added to the recovered concentrate for precipitation of polysaccharide from broth in the second cycle. A total of six cycles was made in this manner. Recovery of quaternary ammonium compound after each cycle was 80 per cent.

Table 1. Weight and Viscosity of Water Solutions of Polysaccharide recovered with Re-cycled Quaternary Ammonium Compound

Cycle No.	Polysaccharide (gm./100 gm. broth)	Viscosity (cp. at 25° C.) concentration 2 per cent	1 per cent	½ per cent
1	1·56	8,640	2,330	300
2	1·55	9,960	2,270	260
3	1·60	8,580	2,140	260
4	1·57	8,820	2,300	275
5	1·42	9,460	2,350	265
6	1·58	9,080	2,450	285

The weight of polysaccharide recovered from each cycle was uniform, except for an unexplained lower recovery on the fifth cycle; and viscosities of water solutions of the polymer showed little variation (Table 1). These viscosities compare favourably with those of polysaccharides recovered by methanol precipitation.

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Urea Cycle and Cellular Deoxyribonucleic Acid Content

While preparing a manuscript on the metabolism of Amphibia to appear in a forthcoming book[1], I was struck by an interesting relationship between the occurrence of the ornithine-urea cycle[2] in the liver of vertebrates and the deoxyribonucleic acid (DNA) content of their somatic cells.

Vendrely[3] has tabulated the values of the DNA content per somatic cell for 73 species of vertebrates. Values ranged, for example, from 0·94 × 10^{-12} gm. DNA per somatic cell in the shad, *Eucinostomus gula*, to 168 × 10^{-12} gm. in the amphibian, *Amphiuma* sp. With several exceptions (wood turtle, snapping turtle, alligator, water snake, mouse, and the lamprey eel, all of which exhibited values of 5·0 ± 0·1 × 10^{-12} gm.) the urea cycle occurs in, and only in, those vertebrates[4,5] containing more than 5·0 × 10^{-12} gm. DNA per nucleus of somatic cell. That is to say, animals examined, the livers of which are capable of effecting a synthesis, *de novo*, of urea, that is, from carbon dioxide and ammonia, generally possessed nuclear DNA values above a cut-off value of 5·0 × 10^{-12} gm.; animals incapable of effecting this synthesis generally had values below the cut-off value (Table 1). Because of the difficulties in determining precise values for the DNA content per cell, it is not certain whether or not there is an overlap of cellular DNA content and the

Table 1

Deoxyribonucleic acid (10^{-12} gm./somatic cell)	Organisms (adult)	Urea cycle
> 5·0	Amphibia Dipnoi* Elasmobranchii Mammalia	Present
	Chelonia	
5·0	Agnatha	Absent ?
	Squamata	
< 5·0	Aves Osteichthyes Invertebrata	Absent

* Evidence for occurrence of urea cycle is presumptive.

occurrence or non-occurrence of the urea cycle in certain species of Agnatha, Squamata, and Chelonia. It is of further interest to note that invertebrates, none of which is known to possess a functioning urea cycle, have values below the cut-off value. It has been suggested elsewhere[4] that there has occurred a deletion of some of the urea cycle enzymes during the course of vertebrate evolution. In this regard, Mirsky and Ris[6], whose data make up a large portion of the values tabulated by Vendrely[3], suggested that in the course of vertebrate evolution there were decreases in the DNA content per cell. The data are consistent with the idea that there has been first a general upwards trend in the level of cellular DNA followed by a general decreasing trend during the course of animal evolution. In the evolutionary line leading from amphibian stock through the Cotylosauria (stem reptiles) the reduction probably did not fall to the cut-off value until after the appearance of the progenitors of turtles and mammals, for all species of these investigated possess an active urea cycle, while this is not the case with birds, lizards, snakes, and alligators (crocodiles).

It is offered as a working hypothesis that the reduction in DNA during vertebrate evolution involved at certain points the loss of information for synthesis of one or more enzymes of the urea cycle.

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Mammalian Phosphoprotein Phosphatase

THE demonstration by Harris[1] and Barth *et al.*[2,3] of an enzyme in frog egg releasing phosphate from an endogenous phosphoprotein substrate was followed by the observation[4] that mammalian tissue preparations split the phosphate bonds of exogenous phosphoproteins such as casein and phosvitin, at acid *p*H, at rates of about 60 μmoles phosphoprotein phosphorus/gm. wet wt. tissue/hr. Both these enzymic activities were attributed to 'phosphoprotein phosphatases'. Studies on preparations from rat liver and spleen[4,5] described a Mg^{2+} and thiol-activated enzyme, labile to heat and salt fractionation, and active on casein and phosvitin but not on simple phosphate esters, thus distinguishing it from acid phosphomonoesterase. It was presumed to function by the splitting of the phosphoseryl bonds of phosphoproteins without accompanying proteolysis[4,5,21].

More recently, reports from a number of laboratories have described the extensive purification of a phosphoprotein phosphatase from rat, mouse and ox liver and spleen[6-16]. Like the earlier described enzymes, this preparation was active at acid *p*H, but in other respects it resembled them only slightly. Thus it was stable to the routine use of prolonged acid autolysis, salt fractionation, temperatures up to 70°, and dialysis, during purification; these procedures were all reported to partly or wholly inactivate the unstable enzyme[4,5]. It was activated by thiols and Fe^{2+}, but not by Mg^{2+}, and was inhibited by molybdate. But the major distinction lay in the specificity of the enzyme. Removing virtually all the phosphate of casein and phosvitin, it was also active with, *inter alia*, fructose diphosphate, acetyl phosphate, adenosine mono- and tri-phosphate, phosphoramidate, pyrophosphate and phenyl phosphate, splitting phosphate from some of these substrates at rates many times in excess of that for phosphoproteinase activity.

The low specificity of the enzyme suggests its original designation as 'phosphoprotein phosphatase' as fortuitous; indeed, Singer and Fruton[11] originally studied it as a phosphoramidase. It would appear to represent nothing else than a non-specific acid phosphatase of the type of β-glycerophosphatase.

I have examined, and purified 100-fold, the phosphoprotein phosphatase of guinea pig, rat and ox brain[17,18]. This enzyme, which represents the sole phosphoproteinase of these tissues, had in common with those earlier described an acid *p*H optimum, but differed from the stable liver and spleen enzyme[6-16] in being very unstable, losing activity on dialysis, salt and acetone fractionation, freezing and thawing and at *p*H's outside the range 5·0–7·5. It was activated by Mg^{2+} and Mn^{2+}, but not by thiols, and was inhibited by 0·1 m*M* Fe^{2+}. The enzyme was highly specific, releasing only a small percentage of the phosphate present in casein and phosvitin, but[19] quantitatively active with an endogenous phosphoprotein fraction from a variety of mammalian tissues. It showed no activity with β-glycerophosphate, phosphorylserine, fructose diphosphate, adenosine mono- and triphosphate, phosphoramidate, pyrophosphate or phenyl phosphate. It was present only in the heavy mitochondrial and soluble fractions from brain.

Thus the cerebral enzyme, in contrast to the stable spleen and liver enzyme, appears to represent a genuine phosphoprotein phosphatase, resembling in this the enzymes of frog and chick egg[1-3,20] and the unstable liver and spleen preparations of Feinstein and Volk[4] and Roche, Thoai and Pin[5]. Preliminary evidence suggests that the phosphoprotein phospha-

Table 1. COMPARISON OF THE PROPERTIES OF PHOSPHOPROTEIN PHOSPHATASE AS REPORTED BY VARIOUS AUTHORS

Enzyme from	Liver, spleen	Liver, spleen	Brain
References	4, 5	6–16	17–19
*p*H optimum	5–6	5–6	5–6
Stability	+ –	+ +	–
Activation			
Mg^{2+}	+	–	+
Thiols	+	+	–
Fe^{2+}		+	Inhibition
Inhibition			
Molybdate		+	–
Substrates			
Casein, phosvitin	+	+ +	+
Ovalbumin	–	–	–
β-Glycerophosphate	–	–	–
AMP		+	–
ATP	–	+ + +	–
Pyrophosphate	–	+ +	–
Phenyl phosphate	–	+ + +	–
Phosphoramidate		+ +	–
Fructose diphosphate		+	–
Phosphoserine	–	–	–
Localization		Lysosomal + soluble	Mitochondrial + soluble

tases of kidney, intestinal mucosa, and human red cells, are also of this type[19].

Some of these differences are set out in Table 1. They point to the conclusion that mammalian tissue contains at least two classes of enzymes capable of splitting phosphoproteins, one being stable and non-specific, and present primarily in liver and spleen, and the other more widely distributed, labile, and highly specific. It is suggested that it is this latter enzyme which plays a part in the rapid *in vivo* metabolism of endogenous phosphoproteins.

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'Soluble' Cysteine Desulphurase of Rat Liver as an Adaptive Enzyme

IN a previous communication from this laboratory, results were presented which show that the enzymatic formation of hydrogen sulphide from L-cysteine in the liver of the rat occurs through two pathways[1]. During the course of purification of the pyridoxal phosphate-dependent enzyme present in the non-particulate fraction of the liver (which we referred to as 'soluble' desulphurase), it has been observed that the purified enzyme which catalyses the formation of hydrogen sulphide and ammonia from L-cysteine is also responsible for the formation of ammonia from some other substrates and particularly from DL(+)-*allo*-cystathionine; the ratio of specific activities of the enzymatic preparations towards cystathionine and towards cysteine remained the same even after an approximately 300-fold purification. We concluded[1] that the soluble cysteine desulphurase was probably identical with the cystathionase-homoserine deaminase crystallized by Matsuo and Greenberg[3]; but, from the results of some inhibition experiments, we suggested that the mechanism of action of cystathionase appeared to consist of the deamination of substrates leading to the labilization of γ-substituents rather than the reverse mechanism proposed by Matsuo and Greenberg. Recently, Goswami *et al.*[4] observed that the L-cysteine desulphydrase activity in an homogenate of chick liver (where the two pathways demonstrated in rat liver are also present[5]) was increased if L-cysteine or L-histidine were injected to the animals for 4 days, while other amino-acids (DL-methionine, L-arginine, L-glutamic acid) were not effective.

In the investigation described here we studied the variations of the levels of soluble cysteine desulphurase and of some deaminases in rat liver under the influence of injections of various amino-acids, some of which (L-methionine, DL-homoserine) are related to cystathionine and cysteine while the others (L-alanine, DL-tryptophan) are without any apparent relation to these substrates.

We used Wistar male rats from the Centre de Sélection des Animaux de Laboratoire du C.N.R.S. with mean body-weight of 80 gm. The L-amino-acids were injected intraperitoneally into the rats in doses of 3·3 m.moles/kgm. according to Lin and Knox[6], the DL-amino-acids in doses of 6·6 m.moles/kgm. The animals were killed 4 hr. after the injection; the preparation of the supernatant fraction of the liver and the conditions of determination of formation of hydrogen sulphide have been described earlier[7], the only difference being that in the present experiments the final concentration of L-cysteine was 10^{-1} *M* instead of 10^{-2} *M*; pyridoxal phosphate was always added to the preparations of liver. The determination of the level of enzymatic deamination of various substrates by the supernatant was carried out according to a process analogous to the method employed by Matsuo and Greenberg[3]; in these experiments also pyridoxal phosphate was always added.

Table 1 presents the results obtained after injections of L-methionine. It can be observed that this treatment increases all the enzymatic activities, the increases being significant, in our experimental conditions, in the cases of desulphuration of L-cysteine and of deamination of DL-homoserine.

Table 1. RESPONSE OF ENZYME-LEVELS TO L-METHIONINE

	Desulphuration of L-cysteine	Deamination of L-cysteine	Deamination of DL(+)-*allo*-cystathionine	Deamination of DL-homoserine
Control	102 ± 4·5* (6)	7·5 ± 0 65 (8)	50·1 ± 8·8 (8)	9·8 ± 0·6 (7)
Injected	148 ± 11 (8)	8·1 ± 0·5 (8)	58·1 ± 2 7 (9)	13·5 ± 1·2 (7)
	$P < 0{\cdot}01$	$0\ 3 < P < 0{\cdot}5$	$P = 0{\cdot}1$	$P = 0{\cdot}02$

The activities are expressed, for the desulphuration, as μgm. hydrogen sulphide (mean of the group measured) formed in 30 min by 1 ml. of the supernatant, and for the deaminations as μgm. ammonia (mean of the group measured) formed in 15 min. by 0·1 ml of the supernatant. Figures in parentheses are number of animals.
* *S.E.*

Table 2 shows the response of some enzymatic activities to injections of DL-homoserine. After this treatment the level of cysteine desulphuration was significantly increased, the same type of results were obtained in the case of deamination of DL-homoserine. In these experiments, too, the deamination of cysteine was also increased, but not in a significant manner.

Table 2. RESPONSE OF ENZYME-LEVELS TO DL-HOMOSERINE

	Desulphuration of L-cysteine	Deamination of L-cysteine	Deamination of DL-homoserine
Control	66·5 ± 5·5* (15)	5·7 ± 0·65 (12)	9·5 ± 0·8 (11)
Injected	97 ± 3 (13)	7·1 ± 0·6 (12)	12·4 ± 0·9 (8)
	$P < 0{\cdot}01$	$0\ 1 < P < 0\ 2$	$0{\cdot}02 < P < 0\ 05$

* *S.E.*

It is noteworthy that after injections of L-alanine and of DL-tryptophan the level of cysteine desulphuration in the liver remained unchanged; the actions of L-methionine and of DL-homoserine thus appear to be specific. The injection of DL(+)-*allo*-cystathionine was not effected, due to the poor solubility of this substance. In experiments in which L-cysteine was administered, we observed no modifications of the level of desulphuration although L-cysteine is a substrate for the enzyme; even after administration of L-cysteine for 4 days according to Goswami *et al.*[4], the level of desulphuration remained unchanged; this fact is perhaps due to other reactions of L-cysteine and especially to rapid oxidation of this substance in the organism of the rat. Perhaps these results also show that cysteine is not one of the best substrates for the enzyme investigated.

From these results it appears that the soluble cysteine desulphurase of rat liver is an adaptative enzyme the level of which is controlled by the amount of some amino-acids related to the substrate. It has also been observed that this enzyme is regulated by hormonal influences: the level of the soluble desulphurase is significantly decreased in the liver of thyroxine-treated rats and increased in the liver of thyroidectomized rats[5]; in addition, the level of the enzyme, which is not modified after adrenalectomy, is nevertheless increased after injection of hydrocortisone[5].

The fact that the administration of L-methionine increases the level of the soluble desulphurase and increases also the levels of deamination of DL(+)-*allo*-cystathionine and of L-cysteine and, moreover, the fact that in the liver of DL-homoserine-treated rat the deamination of DL-homoserine and the desulphuration of L-cysteine are both significantly increased, strongly suggests that all these reactions are catalysed by the same enzyme.

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Vitamin K_1 in Chloroplasts

THERE has been considerable speculation concerning the role of vitamin K in photosynthesis. Arnon[1] has shown that several water-soluble naphthoquinones stimulate photosynthetic phosphorylation, although vitamin K_1 is inactive as a stimulant in these reactions. Dam[2] has shown that material which shows the biological activity of vitamin K is contained in chloroplasts and is localized in the green parts of plants. On the other hand, vitamin K_1 has not been isolated from chloroplasts and has not been detected previously by chemical assay in extracts from chloroplasts[3]. In our laboratory we have not previously been able to observe vitamin K_1 in extracts from chloroplasts even though we could find the vitamin in extracts from whole leaves. We have now found chemical evidence for vitamin K_1 in extracts from spinach chloroplasts. The compound has been purified by silicic acid column chromatography and has shown absorption maxima at 269, 261, 249, 242 mμ in alcoholic solution. After treatment with potassium borohydride the absorption decreases in the 260–270 mμ region and increases to a new maximum at 244 mμ with an isosbestic point at 254 mμ. This material also gives a positive Irrevere–Sullivan test[4] as well as a positive Dam–Karar test[5] for vitamin K.

The compound from spinach chloroplasts shows an R_F identical to vitamin K_1 from alfalfa when chromatographed on silicone-impregnated paper[6] and chromatography reveals only one quinone-positive spot.

The amount of material obtained after chromatography on the silicic acid column corresponds to 0·01 mole of vitamin K_1 per mole of total chlorophyll. This value agrees well with the value reported by Dam[7] for other tissues.

We believe that two factors are of primary importance in the estimation of K_1 in spinach chloroplasts. First, the compound is difficult to extract and is often not removed even when plastoquinone is extracted. We find that extensive extraction is necessary with a mixture of polar and nonpolar solvents. For this extraction we use a mixture of chloroplasts having a chlorophyll content of approximately 1·73 mgm. chlorophyll/ml.; 0·2 *M* potassium phosphate buffer, *p*H = 6·5, a solution of 1 : 1 propanol to heptane and water in the v/v ratios of 1 0; 1·2; 3·0; 2·5. The mixture is then shaken on a reciprocal shaker for 3 hr., separated by means of a separatory funnel, and the aqueous phase re-extracted several times with petroleum ether until only a pale proteinaceous residue of the chloroplasts remains. Second, the borohydride reduction assay, when used to determine K_1, must be used with care, since K_1 is readily autooxidizable and the spectral changes associated with reduction of the quinone may not be observed.

Chloroplasts for these studies were prepared by differential centrifugation of a homogenate prepared by grinding spinach leaves in 0·5 *M* sucrose in a Waring blender at half the maximum speed for 1 min. as previously described[8].

We have checked our chloroplast preparations under the electron microscope and find no gross contamination with either small particles or fibrous material. There is also no significant amount of coenzyme Q in the preparation, indicating that there is no significant mitochondrial contamination.

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Occurrence of Arachidonic and Related Acids in the Protozoon *Ochromonas danica*

IT is generally accepted that arachidonic acid is only found in animal lipids. However, similar fatty acids have been reported from several micro-organisms[1–3]. It is therefore of interest to search for the occurrence of arachidonic acid in the lipids of organisms close to the origin of flora and fauna. The composition of fatty acids of the protozoon *Ochromonas danica* has been investigated by us.

This phytoflagellate can be cultivated under photosynthetic conditions and in the dark. The sample used for detailed analysis came from cultures grown in light for 7–10 days on a synthetic medium which did not contain any lipid[4]. The cells were collected by centrifugation; their lipids were extracted by chloroform–methanol, saponified, and the acids esterified[5].

Table 1 gives results of gas-liquid chromatography of the methyl esters[6], but several other acids, some of them not yet identified, were detected in small amounts. The samples did not contain conjugated or *trans* double bonds but alkaline isomerization confirmed the presence of high unsaturation. The tentative identifications by gas-liquid chromatography retention times were confirmed by isolation of the unsaturated fatty esters and determination of their structure.

Isolation proceeded by the following steps: low temperature crystallization removed saturated and oleic acids; liquid–liquid chromatography[7] of the unsaturated fraction yielded essentially two peaks consisting of two and three components; preparative gas-liquid chromatography of 30–40 mgm. samples separated these peak fractions into the individual components, except linolenic and γ-linolenic esters, which were not separated from each other on a preparative scale.

The positions of the double bonds were determined by ozonolysis and subsequent hydrogenation to aldehydes and aldehyde-esters[8]. These products were identified by gas-liquid chromatography and/or thin-layer chromatography[9] of the 2,4-dinitrophenylhydrazones.

Methyl oleate was isolated from the more saturated fraction of the initial crystallization and its structure was verified by the above procedures.

Table 1. MAJOR FATTY ACIDS OF *Ochromonas danica* GROWN IN LIGHT AND CHEMICALLY DEFINED MEDIUM (percentage composition)

Myristic	15	Linolenic	2
Palmitic	10	γ-Linolenic	10
Stearic	3	Arachidic	1
Oleic	8	8,11,14-Eicosatrienoic	5
Linoleic	16	Arachidonic	11
		Docosapentaenoic*	2–4

* Probably 4,7,10,13,16-docosapentaenoic acid.

Methyl docosapentaenoate was isolated from another sample of *O. danica* lipids by preparative gas-liquid chromatography of the total esters. Ozonization-hydrogenation showed it to be 4, 7, 10, 13, 16-docosapentaenoate. Gas-liquid chromatography indicated the occurrence of this, or a very similar, acid in the sample described in Table 1.

As with other micro-organisms, the fatty acid composition of *O. danica* varies with the culturing conditions. On defined medium under light the organism produced linolenic and γ-linolenic acids at a ratio of about 1 : 4, and this was not drastically affected when the cultures were grown in the dark. In contrast, their ratio was found reversed when *O. danica* had been grown on a complex medium[10]. However, arachidonic acid represented 10–15 per cent of the total fatty acids at all conditions so far tested.

The discovery of arachidonic together with linoleic, γ-linolenic and 8, 11, 14-eicosatrienoic acids in *O. danica* is of great interest since, in the rat, these latter acids are precursors of arachidonic acid[11]. The occurrence of arachidonic acid in micro-organisms related to *O. danica* is likely. The finding may open a microbiological approach to the study of arachidonic acid metabolism and function.

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PHYSIOLOGY

Œstrous and Ovarian Response of the Ewe to a Horse Anterior Pituitary Extract

MULTIPLE-OVULATION in the ewe can be readily induced by a single injection of pregnant mare serum gonadotrophin given on the twelfth–fourteenth day of the œstrous cycle[1]. However, little is known of the ovarian response of the cyclic ewe to other gonadotrophins.

In recent work[2], carried out in the autumns of 1960 and 1961, in which large numbers of fertilized eggs were required, merino ewes were treated with a horse anterior pituitary extract prepared from frozen anterior pituitaries. The frozen pituitaries were finely ground and then homogenized in a Waring blender with a 2 per cent solution of sodium chloride. The homogenate was centrifuged at 1,200 r.p.m. for 20 min., the residue discarded, and the supernatant precipitated with 4 vol. of ethyl alcohol. The precipitate was dried over phosphorus pentoxide and stored at room temperature. The extract was prepared for injection by suspending it in a finely ground state in sterile water at a concentration of 6 mgm./ml. Each ewe received a total of 100 mgm. of the extract administered as 6, equal, 12-hourly subcutaneous injections, commencing on the twelfth day after service by a vasectomized ram. During and after treatment the ewes were run with entire merino rams.

In the autumn of 1960, 50 ewes were treated with the extract. Eighteen of these were treated again in the autumn of 1961. Of the 50 treated in 1960, 49 were served within 96 hr. of the final injection of horse anterior pituitary extract, 31 (63 per cent) of these within 36 hr. of the cessation of treatment.

Table 1. RESPONSES TO HORSE ANTERIOR PITUITARY EXTRACT

Date of treatment	No. ewes		No.* corpora lutea		Eggs recovered		Eggs fertilized	
	Treated	Served	Total	Mean	Total	Percentage of eggs shed	Total	Percentage of eggs recovered
1960	50	49	583	11·9 ± 1·1	472	81·0	325	68·9
1960	18†	18	250	13·9 ± 1·9	205	82·0	136	66·3
1961	18†	12	117	9·8 ± 3·0	83	70·2	61	73·5

* Total and mean numbers of corpora lutea in only those ewes which were served.
† Ewes treated in both years.

Laparotomies performed for the purpose of egg recovery, 48–80 hr. after the onset of œstrus, revealed that 45 of the 49 ewes had ovulated, shedding a total of 583 eggs (Table 1). The 4 ewes which were served, but failed to ovulate, showed a marked follicular response to treatment. The ovaries of these ewes contained a total of 67 (mean of 16·7 ± 2·4) unruptured Graafian follicles greater than 5 mm. in diameter, while the 45 ewes which ovulated had only 84 (mean of 1·9 ± 2·1) unruptured follicles. 472 eggs were recovered from ewes which had ovulated, and 325 of these eggs showed normal cleavage and were considered to have been fertilized.

Of the 18 ewes treated with 100 mgm. horse anterior pituitary extract in 1960 and again in 1961, 18 and 12 respectively, were served within 96 hr. of the final injection of horse anterior pituitary extract (Table 1). Laparotomies showed that in both years, all ewes served had ovulated. Although significantly fewer ($\chi^2 = 5{\cdot}00$; $P < 0{\cdot}05$) of the ewes were served following a second treatment no significant difference could be found between the mean numbers of corpora lutea in those ewes which were served following the first and second treatments ($t = 1{\cdot}82$; $P > 0{\cdot}30$). Similarly the second stimulation by horse anterior pituitary extract did not appear to have any real effect on either the proportion of eggs recovered or the proportion of recovered eggs found to have been fertilized.

Similar horse pituitary extracts have been used to induce multiple-ovulation in the rabbit[3], and in this species a refractory condition, as illustrated by the ovulatory response to a second treatment, develops rapidly[3,4]. The results of the study reported here show that a similar refractory condition was not evident in the ewe when 12 months elapsed between successive treatments with a horse anterior pituitary preparation.

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Plasma Fibrinolytic Activity in the Immature Animal

FOLLOWING the demonstration that hyaline membrane formation in adults[1] is related to upset of fibrinolysis and that pulmonary hyaline in new-born infants consists predominantly of fibrin[2], some of the recent work in this field has been directed to the investigation of possible abnormalities of fibrinolytic mechanisms in the lungs of these children. Lieberman[3], for example, has shown that extracts of lung contain a specific inhibitor of plasminogen activator in many of the children who have died from this condition and presents evidence that the placenta may release such inhibitors when undergoing degenerative changes. Briefly, the mechanism in the normal individual is that plasminogen activator, a tissue enzyme, converts plasminogen to plasmin, the active fibrinolytic enzyme of the body. An alternative hypothesis is that hyaline membrane fibrin has its origin in plasma fibrinogen which, because of circulatory disturbances, enters the alveoli as a transudate[4].

Many enzyme systems do not appear to be active late in fœtal life or after birth, and in prematurely born infants there may be an even greater delay[5,6]. If plasmin belonged to this group of enzymes, then the high incidence of hyaline membrane disease in prematurely born infants might, in part at least, be due to this delay in appearance of fibrinolytic activity in the plasma. This communication describes an experimental investigation of blood plasmin activity in the first five days of life. New-born kittens and rats were used and the method of plasmin assay used was a modification of that of Bidwell[7].

The blood was obtained by cardiac puncture and was immediately mixed with 3·8 per cent sodium citrate solution (nine parts of blood, one part of citrate). The plasma was separated by centrifugation.

Two sets of tubes were prepared, each containing 2·4 ml. of veronal–acetate-buffered saline *p*H 7·4, 0·1 ml. of plasma was added to all the tubes, which were then placed in a water-bath maintained at 37° C. After exactly 5 min., 0·1 of a thrombin solution (20 u./ml.) was added and the mixture stirred vigorously with a roughened cylindrical glass rod. The first set of tubes was removed from the water-bath, the fibrin clots wound on to the glass rod and transferred to test-tubes containing 6 ml. of isotonic saline which had been standing for about 30 min. in melting ice. The clot was then washed with two further lots of ice-cold saline, each washing taking 15 min. The fibrin clots in the second set of tubes were treated in a similar manner after 2 hr. incubation at 37° C. The last saline washings were poured away and 1·0 ml. of 0·1 *N* sodium hydroxide added down the rod. The test-tube was then placed in a boiling water-bath for 2 min., cooled to room temperature and 3·0 ml. of 12·5 per cent w/v sodium carbonate were added. 0·5 ml. of 0·01 *M* copper sulphate solution was then pipetted into the tubes, the mixture stirred with the glass rod and to this was added 0·5 ml. of Folin and Ciocalteau's reagent (diluted 1 in 3).

After 30 min. the density of the colour produced was read in a photoelectric colorimeter, using a red filter, against a blank prepared with 1·0 ml. of 0·1 *N* sodium hydroxide and the rest of the reagents as described. The results were expressed as the percentage lysis after 2 hr. of the fibrin clot which formed at zero time.

Table 1. PLASMIN-LEVELS IN THE INFANT RAT AND KITTEN COMPARED WITH THE ADULT RAT AND CAT (EXPRESSED AS PERCENTAGE LYSIS OF FIBRIN CLOT AFTER 2 HR.)

	Rat			Cat		
Age (days)	Mean plasmin level (per cent)	No. of observations	*S.D.*	Mean plasmin level (per cent)	No. of observations	*S.D.*
1	11·3	8	1·8	4 5	4	3·0
3	5·2	4	12·5	13·3	3	1·8
4	17·0	3	4·1	50·6	8	9·2
5	52·3	3	4 5	37·5	4	6·0
adult	61 5	7	11·7	46·2	4	6·2

Table 1 shows that low plasmin-levels are present in early post-natal life in both the rat and the cat. In the cat, plasma fibrinolytic activity comparable with that of the adult is attained in 4 days, while this does not occur in the rat until the age of 5 days. The difference in time at which the enzyme appears in the two animals may be attributed to the less-mature state of the rat at the time of birth compared with the kitten. These findings suggest that in these two animals plasmin belongs to the group of enzymes which appear to be inactive until after birth.

At the present time we are conducting a more extensive investigation of fibrinolytic activity in the blood of premature and full-term laboratory animals and human infants.

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Effects of Stilbœstrol and Thiouracil on the Electrocardiograms of the Sheep

THE use of stilbœstrol and thiouracil in enhancing the fattening efficiency of livestock has emphasized the importance of undertaking the present investigation on the physiological effects of these drugs on sheep.

Table 1. EFFECT OF FEEDING STILBŒSTROL AND THIOURACIL ON THE ELECTROCARDIOGRAM OF SHEEP. (Mean value of three leads)

Group	Heart rate (beats/min.)	Intervals (sec.)			Potentials (mV.)				
		PR	*QRS*	*QT*	*P*	*Q*	*R*	*S**	*T*
Control *M*[1]	78 0	0·124	0 057	0·317	0·100	−0·330	0·220	−0·100	−0 290
±*S.D.*	8 5	0 001	0 020	0·084	0·030	0·110	0 120	—	0 100
Stilbœstrol *M*	75 0	0 127	0·059	0·322	0·110	−0·320	0·260	—	−0·710
±*S.D.*	11 2	0·001	0·021	0·036	0·030	0 080	0·140	—	0 290
Thiouracil *M*	65·0	0·134	0·062	0·335	0·075	−0·400	0·190	—	−0 660
±*S.D.*	12 8	0 001	0 023	0·038	0 030	0·100	0·120	—	0·220

*Figures are not sufficient.

1 *M* represents the average of four sheep with ±*S.D.*, standard deviation.

Electrocardiographic tracings were recorded fortnightly for 6 months on 12 adult sheep, divided into three identical groups of four each. They were fed daily on a ration consisting of groundnut cake (4 oz.), barley (12 oz.), salt (1 oz.) and oat hay *ad lib.* A capsule of 'Adoxiline' (Glaxo) was given as vitamin A and D supplement once a week. The first group served as control, in the second 20 mgm. stilbœstrol (M. and B.) in pellet form was implanted under the skin, and 1 gm. of thiouracil (B.D.H.) per 100 lb. live weight was supplemented daily in the third group. For electrocardiographic recording, the method described by Mullick[1] was followed.

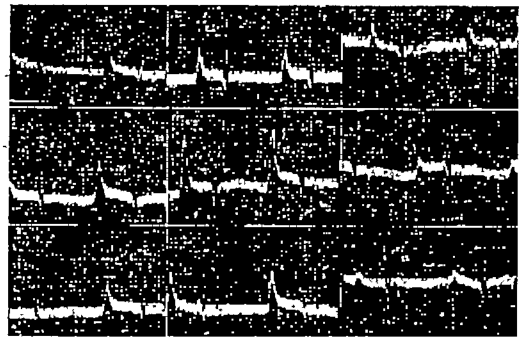

Fig. 1. 1, Control group; 2, stilbœstrol group; 3, thiouracil group

Table 1 and Fig. 1 show the summary of the electrocardiographic records.

Normal electrocardiogram: all the *P* waves were upright. Slight variation was recorded in the form of *QRS*. In all the tracings, *Q* and *R* waves were prominent, whereas very few *S* waves were noted. All *T* waves were inverted.

Electrocardiogram following stilbœstrol implantation: The heart-rate was slow and the interval and potential figures were higher. The significant change in the potential of the *T* waves indicated greater force of the heart output in each beat, whereas in other waves the changes were not appreciable. The absence of *S* waves was conspicuous.

Electrocardiogram following thiouracil feeding: The same trend of change in the heart-rate, intervals and potentials was noted as before but to a smaller extent.

It appears that the pharmacological action of these drugs may affect the normal physiological function of the cardiac cycle, and hence requires consideration in their administration.

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Effect of Phenylalanine Diet on Learning in the Rat

The subject of phenylketonuria, an inborn error of metabolism, has been recently reviewed[1]. This defect in the homozygous individual is characterized by the lack of active phenylalanine hydroxylase and severe mental retardation. The heterozygous carrier of one defective gene is able to oxidize phenylalanine to tyrosine and is reported to appear normal. However, a slower rate of phenylalanine oxidation and slightly elevated plasma-levels of phenylalanine have been observed in these individuals. This communication reports the effect on the rate of learning of a dietary-induced heterozygous state in the rat.

Pregnant Holtzman rats were divided into two groups, one of which received an adequate purified diet (*P*–1), and the other, the *P*–1 diet supplemented with 1 per cent DL-phenylalanine (*P*–2). At four weeks of age the young were placed on the respective diets of the mothers. There was no significant difference in weight between groups *P*–1 and *P*–2 during the experimental period. At 16 weeks of age both groups were subjected to seven sessions of 160 trials each on the conditioned-avoidance procedure[2]. Caged rats were placed in a situation where failure to turn a small wheel, on presentation of a warning sound of seven seconds duration, produced a mild but aversive 0·6 m.amp. electroshock. The avoidance trials occurred after variable time-intervals which averaged 60 sec. in length. On completion of the test, blood was drawn from the tail for the determination of phenylalanine, and urine was collected under toluene for the determination of keto-acids.

The phenylalanine concentration in deproteinized blood was determined microbiologically with the phenylalanine-requiring *E. coli* mutant 83–5 (kindly supplied by Bernard Davis). A significantly higher ($t = 1\cdot83$, $P < 0\cdot1$) phenylalanine-level was observed in the experimental group (61 ± 5 μgm./ml.) than in the control group (50 ± 3 μgm./ml.). Total urinary keto-acids was estimated by a modification of the method of Penrose and Quastel[3]. The average amount of keto-acids excreted in the urine by the control group was 2·2 ± 0·34 mgm./24 hr., which is not significantly different from the amount excreted by the experimental group, 2·5 ± 0·35 mgm./24 hr. Figs. 1 and 2 graphically depict the marked difference between groups *P*–1 and *P*–2 on the conditioned-avoidance test. The control group developed the conditioned-avoidance behaviour quite rapidly. The phenylalanine-fed rats failed to develop the appropriate behaviour and their avoidance failure was both widely distributed and erratic from day to day.

Forty-five days following the first test on the conditioned-avoidance procedure the rats were re-run for another 7 sessions. Twenty days prior to the second test, both groups were fed the *P*–1 diet. At the end of the test the average concentration of blood phenylalanine in the experimental group had fallen to 52 ± 2 μgm./ml. Fig. 3 shows the improvement in performance of 4 of the animals of this group. By the fourth session their performance curves were down within the limits shown by the control rats (Fig. 4).

Our results are in accord with the findings recently reported by Yuwiler and Louttit[4]. It is apparent that a high dietary intake of phenylalanine may be correlated with the failure to develop

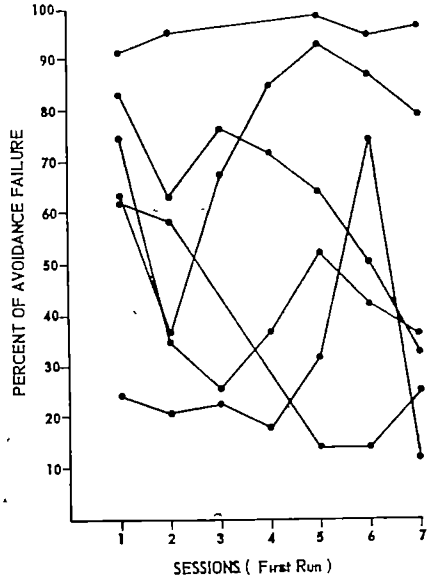

FIG 1 SIX RATS ON PHENYLALANINE DIET (P-2)

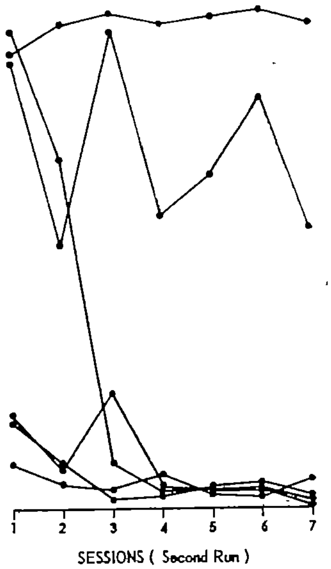

FIG 3 P-2 RATS ON CONTROL DIET 20 DAYS PRIOR TO 2nd RUN

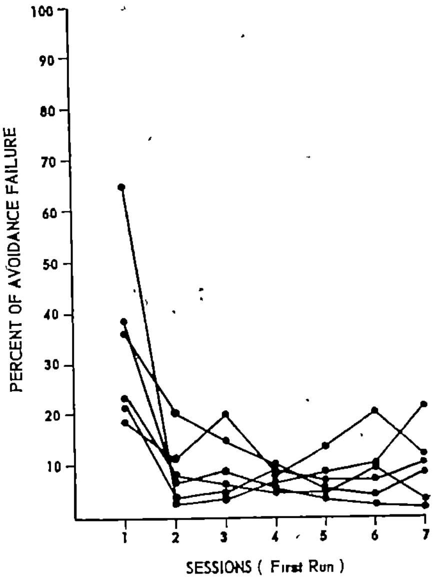

FIG 2 SIX RATS ON CONTROL DIET (P-1)

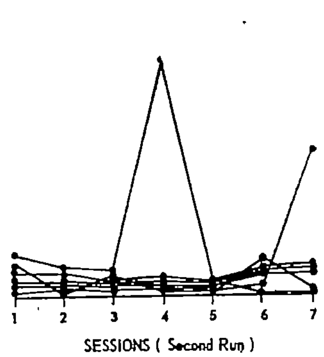

FIG 4 P-1 RATS

the conditioned-avoidance behaviour in the rat.

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HÆMATOLOGY

α-Chain of Rabbit Hæmoglobin

HUMAN hæmoglobin, the structure of which has recently been determined[1], consists of two pairs of homologous amino-acid chains—the α- and β-chains. The work reported here was concerned with the species differences between the α-chains of rabbit and human hæmoglobins.

Rabbit hæmoglobin (prepared by Drabkin's method[2]) consists of two electrophoretically distinct components (Müller, C. J., private communication), which differ in the presence or absence of bound glutathione. This is supported by our finding that oxidation of rabbit hæmoglobin with performic acid splits off glutathione. During the acetone–hydrochloric acid treatment used to remove hæm from hæmoglobin[3], glutathione is also split off, and the resulting rabbit globin thus represents a single protein. After the α- and β-chains of rabbit globin had been separated by counter-current distribution[4], the α-chain was denatured with urea and split by trypsin. The resulting α-'core', a large insoluble peptide, was precipitated at pH 6·4, oxidized with performic acid, and split by chymotrypsin. The soluble tryptic peptides and chymotryptic core peptides were separated by column chromatography over 'Dowex 1X2' (ref. 5), purified by paper chromatography, and quantitatively analysed[6]. In Table 1 the rabbit peptides are compared with the corresponding human and horse[7] peptides with regard to amino-acid composition.

It follows from these results that the peptides of rabbit, horse, and man are quite similar but in most cases not identical. The α-chains of all three species are of the same length—141 amino-acids. However, no more than 114 amino-acids in human and rabbit α-chains can be identical; that is, one must assume

Table 1. AMINO-ACID COMPOSITION OF PEPTIDES FROM THE α-CHAIN OF HÆMOGLOBINS

	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Leu	Tyr	Phe	Try	Ileu	Cys
1 *R*	1			1		1		1		1	1		1					
M	1			1		1		1		1	1		1					
H	1			1		1				2	1		1					
2 *R*	1			1	1												1	
M	1			1	1						1							
H	1			1	1						1							
3 *R*	1				1		1			1						1		
M	1								1	2						1		
H	1					1				2						1		
4 *R*		1	1			1	3		4	2	1			1			1	
M		1	1				3		3	4	1		1	1				
H		1	1				3		4	3	1		1	1				
5 *R*	1				2			1	1			1	1		2			
M	1				2	1			1			1	1		2			
H	1				2				1	1		1	1		2			
6 *R*	1	2		1	2	1	2	1	1					1	3		1	
M	1	2		1	1	2	1	1	1	1	1		1	1	2			
H	1	2		1	1	2	1	1	1	1	1		1	1	2			
7 *R*	1	1							1	1								
M	1	1							2									
H	1	1							1	1								
8 *R*	1																	
M	1																	
H	1																	
9 *R*	1				1	1	1			1	1		1					
M				2	1					2	1		1					
H				1	1				1	1	1		2					
10 *R*	1	3		3	1	2		1	2	3	1		5					
M	1	3		4		2		1		5	2	1	3					
H	1	3		4		2		1	2	3	1		5					
11 *R*			1										1					
M			1										1					
H			1										1					
12 *R*	1			2				1			2				1			
M	1			2				1			2				1			
H	1			2				1			2				1			
13 *R*		1			1	1					1		5					1
M		1			1	1					1		5					1
H		1			1	2							5					1
14 *R*		2		1		1	1	1		1					1			
M		1					1	1		3			1		1			
H		1		2				1		1	1		1					
15 *R*	1	2		1	2	1		2		3	2		1		1			
M	1	1		1	1	3		1		3	2		2		1			
H	1	1		1	1	3		1		3	2		2		1			
16 *R*																		
M	1				2	1					1		1					
H																		
17 *R*			1											1				
M			1											1				
H			1											1				

R, rabbit, *M*, man, *H*, horse.

The peptides are listed in their order in the chain, beginning with the N-terminal peptide No. 1. Peptides 13–16 form the so-called 'core'.

that at least 27 positions in the chains are occupied by differing amino-acids. For example, in peptide 2, lysine, asparagine, and threonine are common to all species, but valine appears to have been replaced by isoleucine in the rabbit. Of particular interest are the peptides 9 and 10, which contain seven and twenty-two residues, respectively. In man this region occurs as a single tryptic peptide with twenty-nine residues, of which only one, the C-terminal residue, is lysine. In the rabbit an additional lysine stands in place of an asparagine twenty-two residues further, and since trypsin cleaves peptide bonds at the carboxyl end of each lysine or arginine, one obtains two tryptic peptides instead of one. It should be noted that the same variant—asparagine replaced by lysine at this locus—occurs in the pathological human hæmoglobin Hb *G* (ref. 8).

The twenty-seven or more exchanges in the rabbit α-chain are not confined to a small region but are distributed over the whole chain, that is, in eleven of the seventeen peptides. Nevertheless, the structurally critical amino-acids—cysteine in peptide 13, the hæm-stabilizing phenylalanines of peptide 6, the accumulation of basic residues in peptides 7 and 8, and the histidines of peptides 7 and 10, which bind the hæm group—remain unaffected by exchanges in all three species.

The α- and β-chains of hæmoglobin are thought to have evolved from a single-chain myoglobin-like precursor, and to have become differentiated from each other as well as from myoglobin itself by successive mutations giving rise to exchanges of amino-acids. These mutations are clearly illustrated by the differences described here between α-chains from various species. Thus, in peptide 7 a glycine residue in man appears as alanine in the horse and rabbit, and the human β-chain also has alanine at this locus[1]. Presumably alanine represents the more primitive condition, and the mutation alanine to glycine occurred here in the α-chain at a stage of evolution later than that represented by the horse and rabbit. The α-chains from different species resemble each other more nearly than do different chains from the same species: that is, horse and rabbit α-chains share about 126 and 114 residues, respectively, with human α-chain, whereas human α- and β-chains have only 66 residues in common. Rabbit and human α-chains are thus evolutionary more distant than horse and human α-chains.

Finally, studies of protein mutations and species variations have made it clear that the same primary amino-acid transitions appear again and again, that is, any one amino-acid can mutate directly into only a few other amino-acids. For example, the transition isoleucine–valine, which was mentioned here in connexion with rabbit peptide 2, has also been observed as a species variant of insulin[9] and hypertensin[10,11] and arises as a nitrite-induced mutant of tobacco mosaic virus[12]. Such a recurrence of certain primary mutations is to be expected from considerations based on the genetic 'code', by which the nucleotide sequence of DNA determines the amino-acid sequence of proteins. For example, if a certain amino-acid were determined by a given nucleotide triplet, then this amino-acid would be able to mutate directly into at most nine other amino-acids (there are four different nucleotides, and any one of three sites in the triplet might thus mutate into any one of three other nucleotides). In practice, the number of possible transitions may be less, since not all triplets may be genetically meaningful. From the peptide compositions reported in this article, the differences between rabbit and human α-chains provide at least 27 such transitions, most of which are probably direct and the exact identification of all of which will require sequence analysis. It is thus clear that species variants in protein structure will provide important evidence concerning the genetic code.

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Failure to elicit a Delayed Hypersensitivity Reaction against Blood Group Substance from Erythrocytes

IN some recent work[1], we have explored the antigenicity in guinea pigs of the secreted human blood group substances A (ref. 2) and Le^a (ref. 3). There was a delayed hypersensitivity response to A and Le^a, and to other human blood group substances, showing a very marked degree of cross-reactivity, irrespective of the immunizing antigen. This cross-reaction and other evidence were taken to indicate that the polypeptide part of blood group mucopolysaccharides was the entity chiefly concerned in producing and eliciting delayed hypersensitivity to these substances. The reaction of A and Le^a substance with circulating antibody was much more specific than was the delayed hypersensitivity reaction. The small degree of cross-reactivity of circulating antibody could be attributed to a small amount of Le^a activity in A substance. The more precise nature of the circulating antibody was explained in terms of a response to the specific polysaccharide entity of blood group substances. Thus, it was postulated that the secreted blood group substance macromolecule was capable of evoking separate immunological responses to its two main chemical components, the polypeptide and the polysaccharide.

Blood group substance obtained from human group A erythrocytes, unlike secreted blood group substance, is a glycolipid without any appreciable content of amino-acids; in a recently reported sample[4], the sum of sphingosine and hexosamine nitrogen represented 98·8 per cent of the total nitrogen. Through the kindness of Dr. J. Kościelak, who prepared this material, we were able to test the immunological activity of such erythrocyte-derived blood group A substance. We tested guinea pigs for evidence of delayed hypersensitivity as well as circulating antibody. Each of a group of 8 guinea pigs was immunized by the intracutaneous injection of purified human A substance[2], obtained by Prof. W. T. J. Morgan from ovarian cyst fluid. The antigen was emulsified in saline with an equal volume of Freund's complete adjuvant (Difco). Four of the animals received 10 μgm., the other 4 were given 50 μgm. A substance. Ten days later, the guinea pigs were tested for delayed hypersensitivity by intracutaneous injection of 0·1 ml. test doses of antigens. The reactions were read 24 hr. later. The immunizing A substance, at a test dose of 10 μgm., gave delayed skin reactions with diameters ranging from 8 to 20 mm. A test dose of 1 μgm. gave a 5–6 mm. reaction. Two samples of the erythrocyte-derived A substance were similarly tested in the same guinea pigs at the same time, in doses of 1, 10 and 100 μgm. Reactions were negative throughout. A sample of erythrocyte-derived B substance was also tested. At a dose of 100 μgm., this gave some slight immediate, but no true delayed, responses ; similar reactions to B substance were found in control animals which had been immunized with adjuvant only.

Table 1. Cutaneous anaphylaxis in guinea pigs immunized with ovarian cyst blood group substance (A 401), and tested with this, with two samples of erythrocyte-derived A substance, and with erythrocyte-derived B substance

	Test substances (10 μgm.)			
	A 401	A 223	A 235	B
Animals immunized A 401				
No. 1	14*	11	14	0
2	12	9	16	0
3	20	10	10	3
4	20	11	15	3
5	10	4	8	0
6	16	6	4	0
Adjuvant control				
No 7	0	0	0	0
8	0	0	0	0

* Reactions measured by diameter in mm. of area coloured by intravenous Evans blue at site of challenge injection.

Circulating antibody was tested for in six other guinea pigs which had been immunized by the subcutaneous injection of 2 doses of 100 μgm. of the purified human A substance (A 401), derived from ovarian cyst fluid, as in the previous experiment. The animals were tested by the direct cutaneous anaphylaxis method using Evans blue, as described in a previous publication[1]. Results are given in Table 1. All animals reacted with the immunizing antigen. They also reacted with the erythrocyte-derived A substances—strongly in the case of animals 1–4, more weakly in Nos. 5 and 6. There was no reaction to B substance in 4 animals and a very weak, probably non-specific one, in the other 2. No reaction to any of the test substances occurred in control guinea pigs injected with complete adjuvant only.

We may conclude that circulating antibody induced against secreted blood group substance is capable of giving cross-reaction with a substance of similar group specificity, but obtained from erythrocytes. This contrasts with the findings with respect to delayed hypersensitivity, where no such cross-reaction was seen. These results are in agreement with the concept derived from our earlier work[1]. Delayed hypersensitivity, being chiefly attributable to the polypeptide moiety of the blood group substance macromolecule, cannot be elicited with erythrocyte-derived A substance, since it does not possess this component. Cross-reaction occurs, however, with the circulating antibody, since this depends chiefly on the group-specific polysaccharides, which secreted substance shares with erythrocyte-derived material.

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IMMUNOLOGY

Acceptance of Tumour Homografts by Thymectomized Mice

Recent experiments in guinea pigs[1], rabbits[2] and mice[3,4] indicate that the thymus plays an important part in the development of immunological competence. Although the observations that patients with acquired agammaglobulinæmia often have a benign thymoma[5,6] suggested a role for the thymus in immunological reactivity it was only very recent investigations which led to consideration of the thymus in immunological development. Glick *et al.*[7] and Mueller *et al.*[8,9] showed that removal of the bursa of Fabricius, a lymphoid organ developing as an outpouching of the posterior gut and an organ homologous to the thymus in several ways, interfered with the ultimate development of full immunological competence. Mueller *et al.*[9] showed further that prevention of development of this lymphoid organ by treatment of chick embryos with progestational hormones, for example, 19-*nor*testosterone, provided potent inhibition of the development of immunological capacity.

In the examination of the inhibiting effect of neonatal thymectomy on the development of the capacity to reject skin homografts it was observed that significant prolongation of skin graft survival was achieved only in strains of mice isogenic at the *H*-2 histocompatibility locus but homologous with respect to other 'weaker' histocompatibility genes as described by Counce *et al.*[10]. In the present communication it is established that even the *H*-2 histocompatibility difference may be overcome by thymectomy performed in the neonatal period when a mammary adenocarcinoma is used as the homotransplanted tissue.

In one group of experiments mice of the $Z(C_3H)$ strain were thymectomized, sham-thymectomized or splenectomized at 0–24 hr. of age. Thymectomy was performed by gentle suction of both thymic lobes through a longitudinal thoracic opening in the midline and traversing the manubrium sterni. 1–2, 22 or 50 days after surgery mice were subjected to the subcutaneous transplantation of a piece of a mammary adenocarcinoma spontaneously arising in a breeder female mouse of the *A* strain. Incidence of 'takes' as well as progressive growth of the transplanted tumours resulting in death of the host were determined in the different groups.

In a second set of experiments groups of 35 to 40-day-old $Z(C_3H)$ mice were prepared as follows: (*a*) thymectomized, (*b*) splenectomized, (*c*) thymectomized and splenectomized, (*d*) sham-thymectomized, (*e*) non-operated controls. Twelve or 55 days after surgery mice of all groups were transplanted subcutaneously with a bit of homologous tumour tissue of the same kind as before.

The results of the first experiment were as follows (Table 1): (1) In the group grafted 1–2 days after the operation, 62 per cent of the sham thymectomized controls and 100 per cent of the thymectomized animals were growing the tumour at the time of weaning (30 days of age). (2) In the group grafted at 22 days, 5 per cent of the sham-operated controls, 8 per cent of the splenectomized and 87 per cent of the thymectomized mice accepted the tumour. (3) In the group grafted at 50 days, none of either the sham-operated controls or the splenectomized mice accepted the tumour, whereas 57 per cent of the thymectomized animals grew it successfully. In all groups mice accepting the tumour homograft were killed by the progressive growth of the malignant tissue. In the second set of experiments in which thymectomy, splenectomy or both operations were performed in 35- to 40-day-old mice the results showed that the tumour homografts were rejected in all instances.

Table 1. Incidence of Successful Transplantation of an *A* Strain Mammary Adenocarcinoma in $Z(C_3H)$ Mice splenectomized or thymectomized at Birth (0–24 hr. after Delivery)

Group	Days between operation and grafting 1–2	22	50
Sham thymectomized	13/21*	1/20*	0/10*
Splenectomized	—	1/13	0/10
Thymectomized	11/11	13/15	4/7

* No. of mice accepting tumour/No. of animals grafted.

Although in the group of mice operated at birth and grafted 1–2 days after surgery a high percentage of the sham-operated controls accepted the homologous tumour (62 per cent), due in all probability to the immunological immaturity of the hosts at that age (1–2 days)[11,12], the incidence of successful takes in thymectomized mice receiving similar tumour transplants was significantly higher (100 per cent) the difference being statistically significant ($P < 0{\cdot}01$). On the other hand, it is interesting to note that the thymectomized mice were killed faster by the progressive growth of the tumour (47 ± 1·6 days) than the sham-operated controls (63 ± 4·0 days). The results of this experiment as well as those in which grafting was performed at 22 and 50 days following surgery demonstrate that thymectomy in new-born mice allows the acceptance and growth of tumour tissue originally developed in a mouse differing at the *H*-2 histocompatibility locus. In contrast, neither thymectomy in young adult mice nor splenectomy at birth or in older animals was capable of affecting the development of the usual homograft reaction. Previous experiments had shown that thymectomy performed in neonatal mice does not lead to an immunological depression of sufficient degree to overcome the strong histo-incompatibility conditioned by a difference at the *H*-2 locus[3,4]. In those experiments skin homografts were used to test the immunological capacity of thymectomized mice, whereas in the present experiments a spontaneous mammary adenocarcinoma was utilized. The different results might perhaps be explained either on the basis of a difference in the antigenicity of the tissues grafted, the tumours being less antigenic than skin, or on the basis of greater growth potential of neoplastic as compared with normal tissues. These observations add strong support to the concept that the thymus plays an important part in transplantation reactions and tumour immunity in early life.

It is postulated that the lymphoid tissue of the gut and the thymus gland in particular are important sources of origin of immunological competent cells during late gestational and early neonatal life. The thymus, indeed, may represent the 'fertile crescent' of greatest importance as a centre of origin and centrifugal distribution of cells possessing immunological competence in neonatal mammals.

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Induction of Partial Acquired Tolerance to a Carcinogen

GREEN[1] postulated *inter alia* in his immune theory of cancer, that the chemical induction of cancer might produce a specialized immune response in which antibody attack on the protein-bound carcinogen in the cells masked or deleted the tumour-specific antigens.

In this investigation an attempt has been made to induce acquired tolerance to a chemical carcinogen, and to note its effect on skin tumour induction in adult life.

Wistar rat embryos were inoculated by injection with 0·1 mgm. 20-methylcholanthrene (20-MC) in the last week of intra-uterine life. Another small group of rats were given 1 mgm. of the carcinogen by intramuscular injection within 3 hr. of birth. They were challenged by bi-weekly painting with 0·5 per cent 20-methylcholanthrene to the shaved abdomen, starting at the sixth week and continuing until tumours developed or until death.

Table 1 shows the time of appearance of skin tumours. At 52 weeks all the controls bore tumours,

Table 1

No. of surviving rats	Dose	Skin appearance of tumours (weeks)
12	20-MC 0·1 mgm. intra-fœtal	52, 55, 57, 58, 62, 77, 77, 78 (mean 64)
7	1·0 mgm. 3 hr. after birth	56, 61, 66, 72 (mean 64)
12	Controls	36, 39, 41, 47, 47, 52, 52, 52, 52 (mean 46)

but only one of the experimental animals. All surviving rats eventually developed carcinomata on the painted area, but the average time of appearance was 17 weeks later in the pretreated group, and this suggests a state of partial acquired tolerance.

A second experiment was designed to increase this tolerance by giving a series of follow-up injections during the neonatal period. Five groups of rats were given an intra-uterine injection of 0·5 mgm. 20-methylcholanthrene followed by a varying number of subcutaneous injections during the first four weeks of life.

Table 2

No. of rats surviving	Fœtal injection 20-MC (mgm.)	Postnatal dose of 20-MC (mgm.)	No. with sarcomas	Time to development of last tumour (weeks)
7	0·5	7·5	7	46
10	0·5	6	10	36
4	0·5	4·5	4	49
6	0·5	4	6	60
9	0·5	3	8	35

They were all challenged by painting the unshaven abdomen bi-weekly with a 2 per cent suspension of 20-methylcholanthrene in 'Trex', from the age of six weeks until tumours appeared or until death intervened.

All the rats developed sarcomas at the site of subcutaneous injection, and only one rat survived long enough to develop a skin tumour on the painted area. The postnatal injection of carcinogen did not boost acquired tolerance, but appears to have broken it down.

In a third experiment rats in two groups were each injected with 1·0 mgm. of 20-methylcholanthrene, the first group within 3 hr. of birth, the second group at four weeks of age. They were painted as before with 2 per cent 20-methylcholanthrene in 'Trex' during adult life.

All the rats injected at four weeks developed sarcomas by fifty weeks, but none of the group receiving the same dose immediately after birth had developed any sarcomas by seventy-eight weeks, though four of these seven had skin tumours.

Although immune responses have been shown to occur in the fœtuses of several mammals by Silverstein[2], Uhr[3], and Kalmutz[4], who attribute acquired tolerance as described by Dixon and Maurer[5], to an immunological paralysis similar to that demonstrated by Felton[6] in adult mice given large doses of pneumococcal polysaccharide, it appears that a single intrafœtal or early neonatal injection of 20-methylcholanthrene in rats can induce a partial suppression of antibody formation to the protein-carcinogen complex as shown by the delay in the appearance of skin tumours when the animal is painted with the carcinogen. When, however, intrafœtal injection is followed by subcutaneous injection of the weanlings, this non-response is broken down and the animals behave like the control group.

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BIOLOGY

Preservation of Bull and Human Spermatozoa by Freezing in Liquid Nitrogen Vapour

My work on factors involved in low-temperature preservation has favoured the design of a simple and efficient freezing method for the preservation of spermatozoa of the bull and the human. I have used this method successfully for more than three years and find it as good as, or superior to, present methods, including those requiring expensive automatic freezing equipment. It uses liquid nitrogen and is an improvement over 'dry ice' methods.

Briefly, this work with spermatozoa has revealed the following as to low-temperature factors.

(1) *Glycerolation temperature.* Freeze–thaw survival of bull spermatozoa decreases as temperature of glycerolation increases from 5° to 21° to 37° C. (ref. 1), while human spermatozoa are independent of such a glycerolation temperature–freezability relationship[2].

(2) *Rate of cooling above freezing.* Egg yolk (lecithin) protects bull spermatozoa from temperature shock while human spermatozoa show little or no susceptibility to temperature shock[2,3] naturally.

(3) *Rate of cooling below freezing.* Average rates of 16°–25° C./min. result in post-thaw motilities at least as high as the best of present methods, better than those realized with rates of 1° and 2° C./min.

(4) *Final temperature.* Cooling from −77° to −196° C. and rewarming from −196° to −77° C. has no effect on post-thaw motilities of frozen samples.

(5) *Time at final temperature.* During frozen-storage for one year at −77° C. there is a post-thaw percentage motility loss of 26 for bull spermatozoa and 10 for human spermatozoa, while at −196° C. the loss is only 4 and 0 per cent respectively for bull and man[2].

(6) *Rate of rewarming.* Both bull and human spermatozoa show no differences in post-thaw motility when rewarmed from −77 or −196° C. at rates of from 1°–60° C./min.[2].

The essential steps in the method as devised for spermatozoa from man and from both dairy and beef (Santa Gertrudis) bulls are:

(1) *Pretreatment of semen.* Bull semen is diluted with a suitable extender[4]. Human semen is not diluted[5].

(2) *Glycerolation.* Glycerol is added to bull semen at 0° or 5° C. in stepwise fashion to a final concentration by volume of 7 per cent[4]. Human semen is glycerolated (stepwise) at room temperature to a final concentration of 10 per cent by volume.

(3) *Packaging.* Following glycerolation, 1 ml. volumes of semen are placed in labelled glass ampoules (Kimball 2020 *s* 28 or equivalent) the tips of which are sealed by flame (*HS*-1 sealer, Popper and Sons, New York) and clipped on a fibreglass or metal cane.

(4) *Freezing.* Canes with ampoules are suspended from a 'Styrofoam' cover over the surface of liquid nitrogen in half (or less) filled Linde Co. (Long Island, New York) *LNR*-*25B* liquid nitrogen container. The nitrogen vapour temperature above the liquid is −172° to −194° C. Average rate of cooling to −75° C. is ± 25° C./min. for one cane, slower with more canes in boxes, etc. The *LNR*-*25B* container is best for combined freezing and storing with liquid nitrogen.

(5) *Storing.* After as little as 5–10 min., canes in vapour can be quickly transferred to a storage canister which hangs partially immersed in liquid nitrogen of the same container.

(6) *Thawing.* Frozen-stored semen is rewarmed by dropping ampoules into a water-bath at either 0°, 5°, 21° or 37° C., whichever is best suited to the investigator's needs.

I have used the basic steps in this liquid nitrogen vapour method to preserve cells other than spermatozoa. These include ascites tumours, sarcomas and three parakeet tumours. I suggest that this method will prove applicable to the preservation of many other cells. However, I must stress the necessity for evaluating each cell type from each animal on the basis of those factors mentioned here before following the technique exactly.

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The Placenta as a Homotransplant

There has been increasing interest during the past decade in the immunological aspects of pregnancy. Since the placenta is of fœtal origin, it has been postulated that the former is antigenically different from the mother and thus representes a homotransplant in the uterus[1,2]. Recently it has been shown[3] that human syncytiotrophoblast cells can bind a γ-globulin, possibly an antibody, which is present in *postpartum* serum. The remarkable findings of Douglas *et al.*[4] that relatively large numbers of such trophoblastic cells enter the maternal circulation during normal human gestation has added impetus to speculations concerning the mechanism by which the mother is able to tolerate presumably foreign tissue. That the rabbit fœtus is antigenic for the rabbit was clearly demonstrated by Woodruff[5], but to our knowledge the only report concerning the capacity of placenta itself to immunize the mother is that of Hašková[6]. She found that F_1 placentæ, although less antigenic than F_1 fœtal tissues, readily stimulated transplantation immunity in mice of the paternal but not the maternal strain. In this work, however, we have shown that the F_1 murine placenta contains antigenic determinants which are capable of inducing homograft immunity in mice isogenic with the mother. Douglas and Dancis have reached similar conclusions[7]. Our investigations, utilizing the entire placenta, do not necessarily indicate, however, that these determinants occur in the portion of the placenta which is in direct contact with maternal tissues or circulation.

C3H virgin female mice were mated with *C57* male mice. At 15–17 days of gestation, laparotomy was performed under 'Nembutal' anæsthesia and blood was removed from the fœtal heart with a fine Pasteur pipette rinsed with heparin. The corresponding placenta was stripped off the uterine wall, trimmed of visible fœtal membranes, minced with scissors and passed through a stainless steel sieve into physiological saline. The cell suspension was centrifuged at low speed, washed once with 5 ml. saline and after resuspension in saline, a cell count was performed using 1 per cent acetic acid as the diluent. A suspension of F_1 adult spleen cells was prepared in a similar manner. Groups of 12 virgin female *C3H* mice 6–8 weeks old were injected intraperitoneally with 0·1 ml. of either placenta, adult spleen or fœtal blood cells three times during a 9-day period. Three days after the last immunizing injection, all the mice were grafted on their backs with *C57* female skin, each graft measuring approximately 1 cm.^{2}. The plaster bandages were removed at six days, the animals were killed and the entire graft removed and fixed in Carnoy's formalin. One section was taken from each graft for histological study. The criteria used for evaluating graft survival were those of Billingham *et al.*[8].

Table 1 summarizes the results of two experiments. In experiment 1, it can be seen that previous injection of the placental cell suspension immunized the recipients for an accelerated homograft rejection. Thus, none of 10 non-immunized animals showed severe epithelial damage at 6 days, in contrast to the severe or moderate reaction of all of 7 animals previously injected with a placental cell suspension. As expected, all animals previously immunized with spleen cells showed either severe or complete epithelial destruction. No attempt had been made to use equal amounts of placenta and spleen for immunization. Fœtal blood cells in the small amounts injected exhibited virtually no antigenicity. Twelve isograft controls not shown in Table 1 showed absence of epithelial damage. Thus, a suspension containing F_1 (♀ *C3H* × ♂ *C57*) placental cells can induce transplantation immunity to *C57* skin in virgin *C3H* mice. These results are not necessarily in conflict with those of Hašková[6], in which a different strain combination of mice was utilized and only one injection of placenta was used for immunization.

A second experiment was carried out to confirm this conclusion and also to test the possibility that the antigenicity of the placental cell suspensions was due solely to the presence of contaminating fœtal blood cells. As shown in Table 1, the antigenicity of placental cell suspensions was confirmed. Four of 7 placental-injected mice showed severe epithelial damage in contrast to none of 6 control animals. Both the suspension of fœtal blood cells and the

Table 1

Exp.	Cell suspension injected	No. of nucleated cells per mouse			No. of mice grafted	Epithelial damage		
		Day 1	Day 5	Day 9		Total or severe	Moderate	Mild or none
1	None	0	0	0	10	0	3	7
	Placenta	$3·5 \times 10^5$	$1·1 \times 10^6$	$1·2 \times 10^6$	7	4	3	0
	Spleen	3×10^7	$2·5 \times 10^7$	$1·5 \times 10^7$	7	7	0	0
	Fœtal blood	4×10^5	$1·5 \times 10^4$	3×10^6	7	0	3	4
2	None	0	0	0	6	0	2	4
	Placenta	$3·5 \times 10^6$	$1·6 \times 10^5$	7×10^4	7	4	2	1
	Diluted placenta	5×10^4	$6·5 \times 10^4$	$3·5 \times 10^4$	8	2	4	2
	Fœtal blood	5×10^4	$6·5 \times 10^4$	$3·5 \times 10^4$	8	1	3	4

placental suspension diluted to contain the same number of nucleated cells appeared to show slight antigenicity. Of these two, the placental suspension appeared more antigenic, although the difference may not be significant. It would appear unlikely, however, that the antigenicity of the placental cell suspension could be entirely accounted for on the basis of fœtal blood cells, since the latter represents only a small fraction of the nucleated cells in the placental cell suspension (the vast majority are either placental cells or maternal blood cells). Nevertheless, the second part of this experiment, the attempt to define the cells which caused the antigenicity of the placental suspension, should be interpreted with caution, because of the lack of sensitivity of the assay system used. Since the dose-response curve in homo-transplantation immunity is rather flat[9], this system is particularly insensitive for the assay of antigen.

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Twilight Transitions and Biological Rhythmicity

IN laboratory investigations of biological rhythmicity, changes in ambient illumination are usually brought about by turning lamps on and off[1]. The responses of deer mice to artificial bright–dim light cycles with simulated twilight transitions are at present being examined in this laboratory.

Mice are particularly resistant to the imposition of unnatural periodicities. Previous investigations led to the conclusion that activity cycles deviating by more than 3 hr. from 24 cannot be imposed on them by on–off light régimes[2]. The results recorded here indicate that utilization of simulated twilight transitions allows a greater degree of control over periodicity.

Enclosures[3] and recording techniques[3,4] are described elsewhere. Enclosures were housed individually in isolated light-proof and sound-proof cabinets in an air-conditioned dark-room (20°–22° C.). Animals were 1-ft. distant from the source of light; results do not depend on the presence or absence of nesting quarters or shade.

Five individuals have been examined so far. Detailed results (Figs. 1c–e and 2) are presented for a mature *Peromyscus maniculatus* female which gave the most clear-cut response. Activity was assessed from records of the time of wheel-running, instantaneous values of rate and direction of running, and revolutions per 10-min. intervals.

Transitions between bright daylight and minimum light of night last for several hours, depending on latitude and season. Light intensity roughly halves

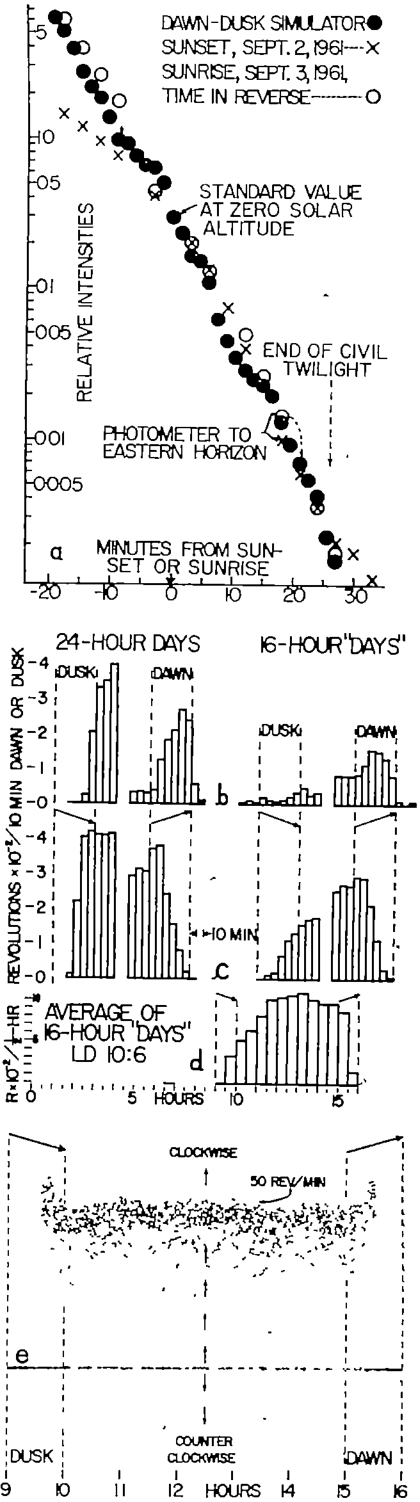

Fig. 1. *a*, Simulated twilight changes compared with those of local sunset and sunrise. The limbs of the sunrise curve (open circles) have been reflected about the ordinate of zero solar altitude to facilitate comparisons. *b*, Revolutions run per 10-min. interval during dawn and dusk by the most poorly synchronized mouse. The plots extend 30 min. before and after the 60-min. twilight transitions. *c*, Same as Fig. 1*b* but for the best synchronized animal. *d*, Revolutions run per 30-min. intervals by the female on a 16-hr. (L : D 10 : 6) periodicity plotted on a 16-hr. grid. *e*, Reconstruction of 'Speedomax *G*' record (points at 4-sec. intervals) of instantaneous speed and direction of wheel-running during a 16-hr. period (L : D 10 : 6)

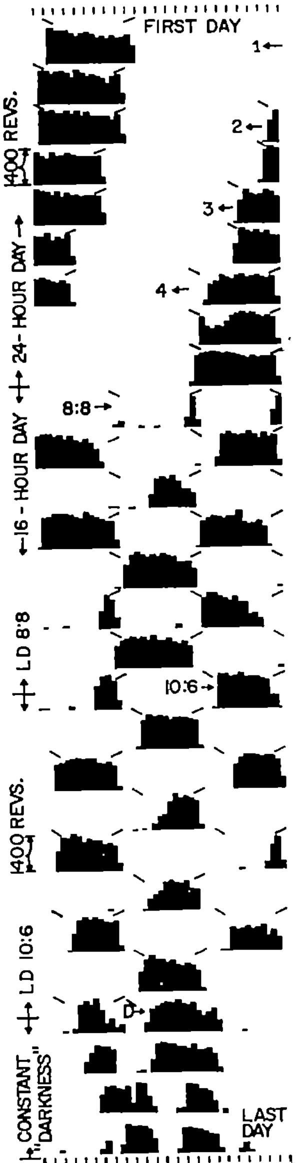

Fig. 2. Revolutions run per 30-min. intervals by the female for the 28-day period covered by the experiment, plotted on a 24-hr. grid. Dawn and dusk periods are indicated as in Fig. 1. Changes in programme are marked

(or doubles) every 3 min. during twilight. Design of the dawn–dusk simulator was guided by determinations of local sunset and sunrise transitions. The curves of Fig. 1*a* are in arbitrary units of intensity relative to a value of 0·3 at zero solar altitude. As a first approximation, only the relative changes in intensity occurring in the hour before (and after) the end (and beginning) of local civil twilight have been simulated. The intensities encompassed range from about 1/2,500th that of bright sunlight to about 1/25th that of the full moon[5].

Changes in light intensity were effected by continuously varying the d.c. voltage applied to 4 General Electric No. 44 incandescent bulbs through the range 1–6·5 V. The light intensity changes[4] by a factor of 5,000, roughly from 0·0008 to 3·96 foot-candles. Deer mice are very active at the lower intensity, which approximates conditions on a clear moonless night[6].

The dawn–dusk simulator consists of a motor-driven 15-ohm rheostat (Ohmite No. 0146) provided with travel-limiting microswitches tripped by cams. A reversible motor rotates the rheostat contact-brush through 180° in a 1-hr. period. The rheostat is connected in series with 8 bulbs in parallel, 4 per enclosure. The relative intensity changes are plotted (solid circles) in Fig. 1*a*. The midpoint of the rheostat movement is set to coincide with the time of zero solar altitude for comparative purposes.

Among the advantages of this method are: (1) occurrence of short-term variations of intensity caused by fluctuations in coil-to-contact-brush resistance and stepwise shorting of coils; (2) progressive reddening of the light at low intensities. The former simulates shading produced by varying cloud cover and micro-topographical features; the latter gives an effect similar to the progressive colour change of direct sunlight caused by scattering of short wavelengths at low solar altitudes.

After adapting the animal for 22 days to a 24-hr. bright-to-dim light-cycle (L : D 16 : 8), the effects of phase shifts were tested. Beginning of dusk was advanced 1, 2, 3 and 4 hr. on the first, third, fifth and seventh days, a cumulative shift of 10 hr. These alterations of régime were followed closely (Fig. 2, first 8 days). A 16-hr. periodicity (L : D 8 : 8) was then instituted. The new periodicity also was followed (Figs. 1*d* and 2). However, whereas the animal customarily ran throughout the entire nocturnal period on a 24-hr. day, in the course of the next 22 16-hr. cycles it failed to sustain continuous nocturnal running on several occasions, even when the relative length of the nocturnal period was decreased (Fig. 2, L : D 10 : 6).

After 22 16-hr. cycles, a constant nocturnal régime was initiated by presenting a dusk immediately following the last dawn. The animal responded with a 10-hr. phase-shift, commencing to run again and completing a second activity period. Although apparently returning to a circadian rhythm, it continued to show two closely spaced major activity periods in each subsequent cycle (Fig. 2, last 4 days).

The response to dawn (Fig. 1*c*) was practically identical on 16- and 24-hr. cycles—a peak of running and unerring cessation before 'daylight'. Dawn was a potent terminator of running; light following dawn was equally potent as an inhibitor (Fig. 1*d*). The ratio of wheel rotations per unit time from beginning of dusk to end of dawn, to those from end of dawn to beginning of dusk (D–L/L–D) was 1396/1 for 24-hr. cycles, 1161/1 for 16-hr. cycles. The sharpness of the 16-hr. periodicity is striking (Fig. 1*d*). Stepwise increments in revolutions during the hours following dusk primarily reflect more running, not faster speeds.

Fig. 2*e* shows a representative record of instantaneous speed and direction of running. The 'dawn response' involves a spurt of faster running, not simply more intense running. But a spurt of faster running also occurs during dusk. Thus, both twilight transitions appear to affect deer mice. Although dusk is sometimes 'ignored', the dawn changes are usually compelling—behaviour which is appropriate to nocturnal species. In this connexion, peaks of rodent activity also have been observed in the wild during dawn and dusk[7].

Dawn had a potent effect on even the most poorly synchronized subject. Fig. 1*b* shows the dawn and dusk responses of this animal (a mature male) to the same régime. It ran only 45 per cent as much during 24-hr. cycles (D–L/L–D = 63) and 37 per cent as much during 16-hr. cycles (D–L/L–D = 3·5) as did the female. Often it was not running at the beginning of dawn, so that its subsequent spurt of running was more accentuated.

These findings suggest the importance of using light régimes in the laboratory that are more nearly representative of natural changes than are simple on–off cycles.

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New Records of *Gobius forsteri*

INCREASING use of the aqualung by zoologists has led to the exploration of sublittoral areas the topography of which had previously excluded investigation by the conventional methods of trawling and dredging. One of the more sensational discoveries on British coasts has been that of the leopard spotted goby by Mr. G. R. Forster of the Plymouth Laboratory. This was a species new to the British fauna, and regarded by Corbin[1] as also new to science, being described in *Nature* under the name of *Gobius forsteri* (Teleostei-Percomorphi). In addition to southern Cornwall and Devon, records were given for St. Helier (Channel Islands) and Santander (Spain). Recent work[2] has suggested that the goby may be identical with *Gobius thori* De Buen[3], a species founded on one specimen from the Aegean Sea collected as long ago as 1910. Further examples from the Mediterranean area are needed to settle this question, but in the meantime it is of interest to record *G. forsteri* from two new localities, extending the known northern limit of distribution by more than 250 miles. These are Dale, Pembrokeshire, and Port Erin, Isle of Man.

At the former, Prof. E. W. Knight-Jones, diving in August 1960, found that *G. forsteri* was "quite common immediately to the south of Dale Point and West Blockhouse Point, near the mouth of Milford Haven, in deep gullies just below the zone of *Laminaria hyperborea*". In the Irish Sea, the species was not revealed until July 11, 1961, when one of us (R. G. H.) discovered the goby on the inner side of a ruined breakwater which extends for some distance across the mouth of Port Erin Bay. At a depth of about 30 ft. (9·2 m), the fishes were seen in crevices of the concrete breakwater blocks or between these and a bottom of muddy sand, at the lower limit of the *Laminaria* zone. Since then, the species has been seen on the opposite side of the bay, below Bradda Head, and there is an unconfirmed record from Perwick Bay, Port St. Mary.

The finding of this hitherto unrecorded fish within a few hundred yards of the Marine Biological Station, Port Erin, directs attention to an ignorance of the bottom fauna of rocky inshore waters which only diving and, to some extent, trapping can remedy. These records provide another indication of how closely *G. forsteri* is tied to its own special habitat of rock crevices and gullies. In the course of field work on Manx gobies from 1958 to 1960, Port Erin Bay and the sea outside the ruined breakwater have been the scene of frequent trawling with a variety of gear—otter trawl, bottom young-fish trawl, and Agassiz trawl—all usually employed with a stramin cover on the cod end to retain small fishes. Within the angle of the breakwater, the bottom has been extensively *D*-netted by Naylor[4], and a fish trap was set in this area in late 1958. Apart from this recent work, the neighbourhood of Port Erin has been studied more or less intensively by marine zoologists since W. A. Herdman and the Liverpool Marine Biological Committee opened their first laboratory there in 1892. None of this work has ever suggested the presence of *G. forsteri* in the area, and the species even escaped notice during a submarine algal survey[5].

The extent to which this goby penetrates the Atlantic boreal region[6] is yet to be determined. Evidence from duration of breeding season is available only for captive specimens in the Plymouth Aquarium, where spawning took place during May–July[1]. This period is rather short for a lusitanian and possibly Mediterranean teleost[7], and suggests that the species may reach its northern limit at the British Isles like *Gobius cobitis* Pallas (= *G. capito* C. and V.)[8] and *G. paganellus* L.[9]. A shortening in duration of breeding season with approach to the northern boundary of the breeding range has been demonstrated in a number of other fishes with similar distribution, including the gobiids *G. paganellus* and *Pomatoschistus flavescens* (Fabr.)[10]. However, *G. niger* L. extends as far north as Trondheimfjord[11] in Norway, and *G. forsteri* could possibly follow suit.

The solution to this problem obviously lies in further exploration of sublittoral areas on rocky coasts of Scotland and Scandinavia. Since the fish in question is readily identified, a search of this type would provide an exercise of scientific interest for a group of amateur divers or local branches of the British Sub-Aqua Club. One of us (P. J. M.) would be pleased to give more details to anyone willing to help in such a project, or to identify any gobies collected by divers, and thanks Prof. E. W. Knight-Jones, Drs. Joanna M. Kain and N. S. Jones, and Mr. D. Eggleston for specimens and information concerning *G. forsteri*.

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Dispersal of Coffee Rust *Hemileia vastatrix* B. et Br.

RAYNER[1] has recently stated that uredospores of *Hemileia vastatrix* can be dislodged from pustules by sudden mechanical shock. In this he is in agreement with Nutman, Roberts, and Bock[2], and with Bock[3].

The question is not, however, whether spores can be shaken into the air, but of their subsequent fate. Can such spores cause infection at a distance or not? In other words, is *H. vastatrix* air-borne as are the cereal rusts, or is it distributed over short distances only, by water-splash? Moreover, which of these methods of dispersal is effective in building up seasonal epiphytotics? Rayner[4–6] has supported the former view, and in particular has suggested that the winds of the south-west monsoon carried the disease from Africa (its country of origin) to Asia. Bock[3], on the other hand, using the rotating-rod spore traps of Harrington, Gill and Warr[7], has shown that under field conditions, at wind-speeds of up to 4·9 m.p.h. inside the plantation, spores dislodged by mechanical shock do not rise above the level of the pustules from which they are detached, but fall rapidly to the ground. The data published by Burdekin[8] support Bock's conclusions.

A characteristic of the air-borne rusts, as has been pointed out by Gaumann[9], is that a single spore can cause infection. We have transferred nearly 2,000 counted groups of uredospores of *H. vastatrix* to droplets of water on leaf-disks. Of a total of nearly one thousand 1-spore and 2-spore transfers less than 0·2 per cent resulted in infections, and even with the 10-spore and 20-spore groups only 1·1 per cent of infections were recorded. Groups of 150 spores, however, gave more than 90 per cent of infections. These results are in agreement with those of Bock[3], who, using different techniques, demonstrated that an average concentration of spores on the leaf surface of 15–30 spores per cm.^{2} is necessary for infection.

Bock[3] has also shown, on the basis of 450-hr. trapping in a heavily infected plantation, that if all the spores which became air-borne during a 24 hr. period were concentrated on the lower surfaces of the leaves, this would represent an average of only 0·12 spores per cm.^{2}, too low a number to cause infection. On the other hand, a single shower of 0·8 in. resulted in a concentration of 25 spores per cm.^{2}. He also found that a linear relationship held between the number of spores dispersed on the lower surfaces of leaves, and the amount of rainfall (in excess of 0·3 in.) causing the dispersal.

Rayner[1] has stated that the lesions, because they are confined to the lower surfaces of leaves, would be too infrequently hit by water-splash for this to be the predominant manner of dispersal. We have shown, by flash-photography, that upward splash is entirely adequate for this purpose. In one particular instance, when the leaf bearing the pustule was protected from drops falling from above, precipitation amounting to 0·6 in., completely removed all loose spores from a heavily-encrusted pustule by upward splash from neighbouring leaves alone. The concentration of the spore-suspension falling from the tips of leaves during a rain storm often very greatly exceeds that necessary for infection.

Additional evidence in favour of dispersal by water has been provided by Bock[10] in a study of the development of rust epiphytotics during which the lesions on more than 8,960,000 leaves were counted and recorded more than 5 rust cycles, or two and a half years. He concluded that his data were completely explicable if water-dispersal were effective, but not if spore movement were by air.

The long-range dispersal of *H. vastatrix* has been cited by Rayner[6] as evidence favouring dispersal by air, although Butler[11] considered that movement from Africa to Asia was probably by infected plant material. In fact Rayner[6] has himself given one instance of the introduction of the disease into the New World by this means: fortunately this was detected in time and suppressed. Coffee in South America is still free from the disease—a circumstance inexplicable if long-range dispersal by air were effective.

Full accounts of the work on inoculum-potential and splash-distribution of *H. vastatrix* will appear elsewhere.

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Embedding Large Slices of Plant Tissue for Electron Microscopy

THE most suitable impregnating resins for ultra-thin sectioning of plant tissue penetrate very slowly to the centre of large masses of cells. This has restricted the range of problems which can be investigated. The following simple technique, an adaptation of that suggested by Luft[1], was evolved to embed and section successfully pieces of plant tissue, both meristematic and vacuolated, up to 1 mm.^{3}.

Shell 'Epikote Resin 812', which is slightly less viscous than Ciba 'Araldite resin *M*' but otherwise possesses all the virtues of the latter, was used exclusively. Luft's method was followed closely, and the most successful balance of hardeners for meristematic tissue was found to be four parts of the methyl nadic anhydride mixture to six parts of the dodecenyl succinic anhydride mixture. Equal parts of the two hardener mixtures are preferred for slightly harder tissue, for example, vacuolated cells of the root tip of broad bean.

The tissue is isolated, fixed and stained in the conventional way. Potassium permanganate, formaldehyde and osmium tetroxide were all used successfully as fixatives, and no differences in the penetration of the resin were noticed. The tissue is dehydrated through an ascending alcohol series and after the 100 per cent ethanol stage is given two ½-hr. washes in 100 per cent propylene oxide. The blocks of tissue are then transferred to a fresh 10 ml. of propylene oxide, and the complete resin mixture, plus accelerator, is added slowly in 1–2 ml. amounts. The mixture is swirled vigorously between each addition of the resin so that all the resin dissolves. The proportion of resin to solvent is built up slowly over a period of at least a week. The denser the tissue is, the longer this infiltration should be. Finally, the tissue blocks are removed from the resin/solvent mixture, drained on smooth filter paper and allowed to sink through freshly made and well-mixed resin in '00' gelatin capsules. No vacuum treatment is necessary. The blocks are then baked at 35° C. overnight. This lowers the viscosity of the resin, allows the tissue blocks to sink and bubbles to escape. After 12 hr. at this temperature the tissue blocks can be re-orientated and baked for a further two days at 45° C., after which they are ready for sectioning. Silver sections can be used unsupported on 100-mesh hexagonal support grids for low-magnification work and on 200-mesh or 400-mesh for high-magnification work. Millonig's[2] method of section staining to enhance the contrast of osmium tetroxide-fixed material was used successfully, but no section staining is necessary with potassium permanganate fixation.

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ENTOMOLOGY

Sterilization of the Codling Moth by Gamma-Irradiation

Work is in progress in British Columbia to determine if the codling moth, *Carpocapsa* (= *Cydia*) *pomonella* (L.), can be eradicated by the sterile male technique, which was used so successfully against the screw-worm, *Callitroga hominivorax* (Cqrl.), in Curaçao[1].

When male pupæ, within 1 day of adult emergence, were exposed to 40,000 rads of γ-radiation, from a cobalt-60 source, dominant lethals were induced in about 99 per cent of the sperm without affecting adult emergence, mating, or adult longevity. Dominant lethality was also induced in 99 per cent of the sperm, without undesirable side effects, when 12–24-hr.-old male moths were subjected to the same dosage. Exposure of male pupæ or adults to higher dosages of radiation reduced the frequency of mating. Dominant lethals were induced in at least 96 per cent of the sperm when mature male larvæ, or eggs that had completed about two-thirds of their development, were subjected to 9,300 rads. However, many of the irradiated insects died before they reached the adult stage, and the frequency of mating was reduced. The female codling moth was sterilized by a lower dosage of γ-radiation than the male. This was true whether the insect was exposed in the egg, larval, pupal, or adult stage, though the difference was most noticeable following pupal irradiation. The female was also more easily killed by γ-radiation than the male, the difference being particularly marked in the egg stage. In general, radiosensitivity of both sexes decreased as development progressed from the egg to the adult stage.

Sperms from irradiated males competed poorly with those from normal males; mating of a single normal female with a single irradiated male (exposed as a pupa to 40,000 rads), either before or after a mating with a normal male, did not prevent the laying of eggs that were mostly viable.

When irradiated male moths (pupæ exposed to 30,000 rads; this dosage induces dominant lethality in about 90 per cent of the sperm) were caged with an equal number of normal female moths, the sex ratio of the adult offspring was approximately 9 males : 1 female. The female offspring were completely sterile, and the males were mostly so, for when they were mated with normal female moths, less than 2 per cent of the eggs laid were viable. However, when irradiated male moths were caged with normal male and female moths, in the proportion of 10 : 1 : 1, the sex ratio of the adult offspring was 1 : 1 and both sexes were mostly fertile.

In laboratory experiments in which (*a*) 50 irradiated males, (*b*) 50 irradiated males and 50 irradiated females, or (*c*) 50 irradiated females (both sexes exposed as pupæ to 30,000 rads), were added to cages containing 5 normal male and 5 normal female moths, the deposition of viable eggs was reduced 98 per cent in (*a*), 66 per cent in (*b*), and 60 per cent in (*c*).

In orchard experiments (cages over dwarf apple trees) in which both irradiated males and females (exposed as pupæ to 40,000 rads) were caged with normal male and female moths, in the proportion of (*a*) 10 : 10 : 1 : 1, and (*b*) 20 : 20 : 1 : 1, the number of adults in the F_1 generation in (*a*) remained about the same as the number of normal adults present in the parent generation, whereas the number of F_1 adults in (*b*) was reduced to one-sixth of the number of normal adults present in the parent generation. However, when irradiated males alone were caged with normal male and female moths, in the proportion of (*c*) 10 : 1 : 1, and (*d*) 20 : 1 : 1, the number of adults in the F_1 generation was reduced in (*c*) to about one-third, and in (*d*) to about one-tenth of the number of normal adults in the parent generation.

These results indicate that the sterile male technique may be a promising method for the control of the codling moth. The method will be tested in the field.

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A Survey Method of Trypanosome Infections in *Glossina*

The incidence of trypanosome infections in wild populations of tsetse flies (*Glossina morsitans* West (Muscidae: Diptera)), varies from one locality to another, and seasonal changes also occur. The method hitherto used to determine infection-rates has been the dissection of samples of live flies as described by Lloyd and Johnson[1]. This has the disadvantage of requiring the establishment of a team of microscopists in the bush, so that examination of simultaneously collected samples over wide areas is difficult. The recognition of trypanosomes in the mouthparts of preserved wild *Glossina* is not possible because regurgitation of stomach contents, which may occur when the insect is placed alive in the preservative, renders many of them opaque.

Table 1. COMPARISON OF TRYPANOSOME INFECTION-RATES OBTAINED FROM PRESERVED HEADS AND BY DISSECTION OF LIVE *Glossina*

Area	Dissection of live flies		Preserved heads	
	No. examined	Infected (per cent)	No. examined	Infected (per cent)
A	250	10·8 ± 1·9	250	10·4 ± 1·9
B	359	18·4 ± 2·0	357	15·7 ± 1·9
C	132	26·5 ± 3·8	78	26·9 ± 5·0
	741	17·3 ± 1·4	685	15·0 ± 1·4

We have therefore developed a method in which Survey assistants are issued with small bottles containing methyl alcohol into which the heads of flies, severed with a small pair of scissors, are placed and then sent to the central laboratory for dissection and staining with Giemsa. There is no regurgitation, and trypanosomes can easily be seen and, in most cases, are identifiable (Figs. 1 and 2).

Flies must be lightly held before severing the head to avoid forcing gut contents into the mouthparts. It is also desirable to retain alive, in separate tubes, those flies captured while feeding, preserving the heads only when the mouthparts are free of blood. The method does not permit the recognition of immature infections of the gut nor, of course, of polymorphic trypanosomes in the salivary glands. However, infections of these types are comparatively rare, and for the purposes of investigations relating to the epidemiology of cattle trypanosomiasis the incidence of mature infections other than of *Trypanosoma congolense* and *T. vivax* is seldom important.

Preliminary tests were carried out in three widely separated areas in which flies were alternately either preserved as described or were dissected. The results are given in Table 1. In the live dissection figures infections of the gut only are omitted. There were 23 from all three areas, or 3·1 per cent.

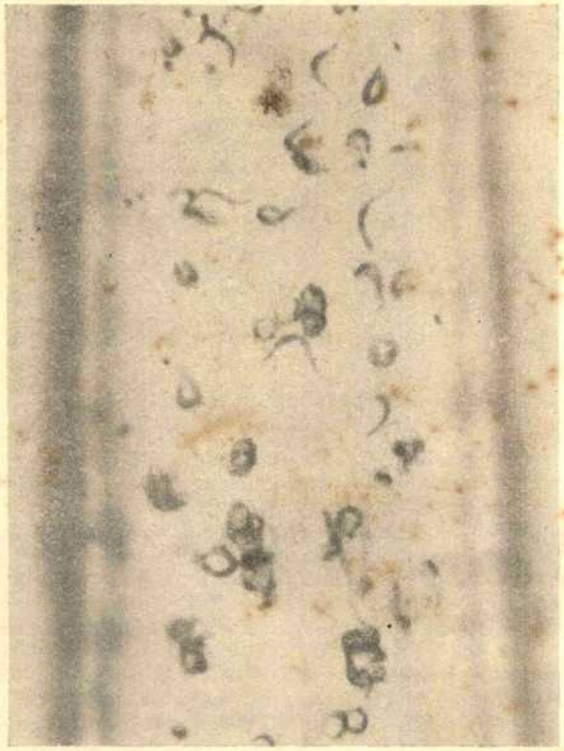

Fig. 1. Diffuse infection of *T. congolense* in labrum of *G. morsitans* preserved in the field. (× 1,200)

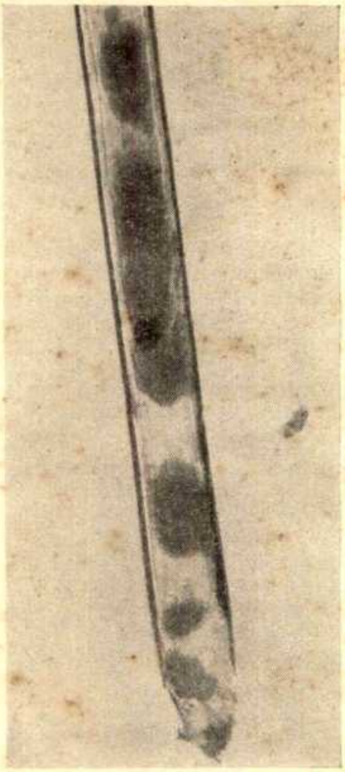

Fig. 2. Dense colonies of *T. congolense* near tip of labrum of *G. morsitans* preserved in the field. (× 180)

A feature of a trial examination of mouthparts of whole preserved flies was the difference in proportion of mouthparts with or without regurgitated blood in male and female flies captured off a bait ox. Of 556 males 40 per cent had regurgitated blood in the mouthparts, but of 150 females 70 per cent were obscured in this manner. This was to have been expected, if, as is generally accepted, male flies come to a bait in part under the influence of stimuli other than hunger, while females attack only to feed.

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Flight of Grasshoppers in the Laboratory

OBSERVATIONS on mass flights of the migratory grasshopper, *Melanoplus bilituratus* (Walker) = (*M. mexicanus mexicanus* (Sauss.)), in 1938–40, indicated that large populations had moved 165–575 miles usually in a downwind direction[1]. During these prolonged flights the insects travelled 15–30 miles a day at an average speed of about 10–12 m.p.h. The wind during such flights was between 2 and 10 m.p.h.

The flight time of adults of *Camnula pellucida* (Scudder), *M. bilituratus* and *M. bivittatus* (Say) was measured in a laboratory wind tunnel by suspending them in an air stream on a pendulum-like suspension bar similar to that used by Krogh and Weis-Fogh[2]. The wind speed could be adjusted from 2·2 to 15·4 m.p.h. Flight time was taken as that interval during which the grasshoppers were flying into the wind in a normal flight posture[2] while keeping the suspension bar in a vertical position. Excessive changes in the wing-beat frequency or wing-beat amplitude, occurring at the beginning and at the end of a flight period, caused the suspension bar to deviate from the vertical position. Since changes in such flight characteristics are vital to 'lift'[3], these intervals of 'interrupted' flight were excluded from the data on duration of flight.

It was assumed that so long as the suspension bar remained in a vertical position the flying speed of the grasshopper was equal to the wind speed. In preliminary tests this was shown to be about 5 m.p.h. Air stream velocities greater than 8 m.p.h. interrupted flight and velocities less than 3 m.p.h. failed to maintain the insects in flight.

The duration of continuous flight of well-fed, 1- to 3-week old, laboratory-reared adults, under known conditions of temperature and humidity, is shown in Table 1.

Table 1

	Max. (min.)	Av. (min.)	Temp. (° F.)	R.H. (per cent)	No. of tests
C. pellucida, males	40	27	97–104	10–30	51
C. pellucida, females	180	158	97–104	10–30	48
M. bilituratus, males	163	36	90–106	10–30	36
M. bilituratus, females	134	50	90–106	10–30	42
M. bivittatus, males	40	17	96–105	10–30	45
M. bivittatus, females	70	43	96–105	10–30	51

The best flight was shown by sexually mature adults aged 7–20 days, excluding gravid females. Two- and three-day old *Melanoplus* adults showed a tendency to maintain sustained flight in only 4 out of 41 tests. They usually merely fluttered feebly or extended their tegmina in a 'planing' fashion and rarely assumed the proper flight position. Individuals older than three weeks would fly for a minute or two, flutter asynchronously, and then 'plane'. In more than 90 tests with month-old specimens of *M. bilituratus* and *M. bivittatus*, none would fly continuously for more than a minute. This would tend to substantiate field observations in which ". . . the more mature grasshoppers were continually dropping out (of a swarm) . . ."[1].

Prolonged flight of *C. pellucida* did not occur at temperatures below 95° F. The optimum temperature lay between 100° and 104° F. *M. bilituratus* flew at temperatures as low as 88° F.; but the best prolonged flights were made at about 96° F. *M. bivittatus* were reluctant to start flying. Often their flight was erratic, clumsy, or sporadic, but was maintained by some individuals at about 100° C. Such observations correspond very closely with observations made during natural flights in the field. Parker[4] and Parker *et al.*[1] recorded temperatures of 90°–98° F. during days of high flight activity of '*mexicanus*'. Furthermore, such flights ". . . do not depend upon high temperature alone but are greatly influenced by wind". This was also borne out in laboratory tests.

Increased humidity did not induce any change in the insects' ability to fly. At low humidities (approximately 10 per cent relative humidity) and high temperatures (about 110° F.) *M. bivittatus* became excessively active, ceased flying, kicked violently and went into heat torpor. Increasing the relative humidity to 50 per cent prevented hyperactivity and heat torpor, but it did not improve its flight capacity. The flight behaviour of *M. bilituratus* and *C. pellucida*, at temperatures of 110° F., was similar to that of *M. bivittatus*.

The present laboratory tests indicated that *C. pellucida* females could remain in flight for the longest period of time. They were followed closely by *M. bilituratus*. *C. pellucida* males and both sexes of *M. bivittatus* were relatively poor flyers, especially the latter, which may be regarded as being near sedentary insects. It was also shown that if grasshoppers flew for 3 hr. each day at a speed of 5 m.p.h. and assisted by a wind of some 5–8 m.p.h., they could readily travel distances of 30 miles. These observations point up the non-swarming behaviour of *M. bivittatus*, which is probably a result of the comparative shortness of the wings in relation to the body-weight. They also show the good flight potential of *C. pellucida*, even though this species has never been reported to migrate great distances.

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MICROBIOLOGY

Chemotaxis in *Myxococcus*

We have been studying the formation of fruiting bodies by the myxobacterium *Myxococcus xanthus*. One area of concern was the mechanism by which vegetative rods accumulate to form the aggregates which later give rise to mature fruiting bodies. Bonner[1] has suggested that chemotaxis may be involved. Lev[2] and Jennings[3] have described a compound in mature fruiting bodies which can induce fruiting in cells growing on a medium in which fruiting does not ordinarily occur, but it is not clear that their substance attracts vegetative cells.

We have worked out a technique which gives fairly convincing evidence for chemotaxis in *Myxococcus*. It is similar in principle to methods[4] which have been used to detect chemotaxis in the slime amœbæ (*Acrasiales*), a group in which this phenomenon has been adequately dealt with in the literature[5].

Our method is as follows. A culture of strain *FB* of *Myxococcus xanthus* is grown in *C* broth (20 gm. of 'Difco Casitone', 1 gm. of magnesium sulphate, 1·36 gm. of potassium dihydrogen phosphate and 10 gm. of Merck soluble starch per litre of distilled water) with aeration at 30° C. until the concentration is about 3×10^8 cells/ml. About 0·01 ml. of the log phase culture is placed on the surface of a plate of *F* agar (0·8 gm. of 'Difco' nutrient broth powder, 3 gm. of sodium chloride and 15 gm. of 'Difco' powdered agar per litre). The plate is incubated at 30° C. for 24 hr. Then an autoclaved sheet of No. 300 *P. T.* cellulose dialysing membrane (Brosites Machine Corp., Inc., New York) is placed over the surface of the agar and smoothed with a glass rod. A second 0·01 ml. drop of log phase strain *FB* is pipetted on to the membrane above the original spot, and the plate is incubated for three days more.

Growth of the bacteria is more luxuriant below the membrane than above it, but the fruiting bodies which form below the membrane are distorted and flattened. Most of the fruiting bodies which form

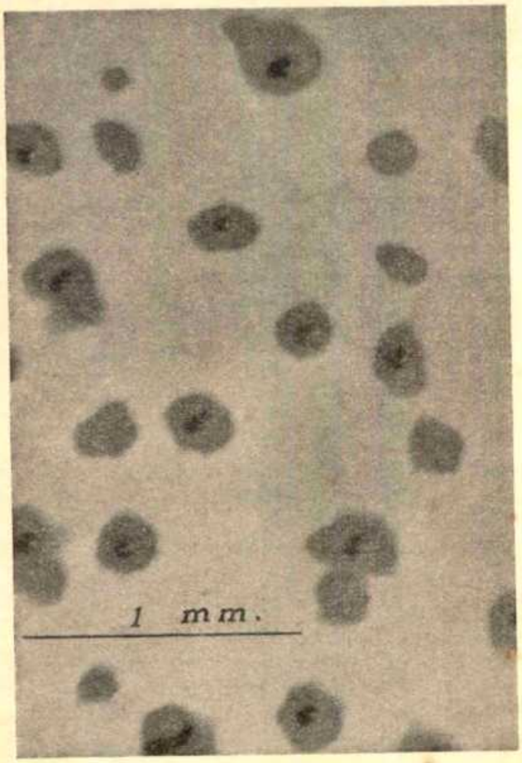

Fig. 1. Fruiting bodies of *Myxococcus xanthus* strain *FB* above and below a dialysing membrane. A drop of a 1:20 dilution of Loeffler's alkaline methylene blue stain was run over the membrane to stain the fruiting bodies lying on top

above the membrane lie directly over those below (Fig. 1). That this is not due to elasticotaxis[6] is suggested by control plates which have glass beads (of about the size of fruiting bodies) below the membrane and bacteria above it. There seems to be no relationship between the positions of the fruiting bodies which form on the membranes under these conditions and the glass beads below.

Our interpretation of these results is that centres of aggregation lying below the membrane excrete a compound capable of passing through the membrane and of initiating the formation of an aggregate (which later becomes a fruiting body) on top of the membrane. The movement of vegetative cells on top of the membrane is oriented towards points where this compound is most concentrated.

We thank Dr. M. Dworkin for a culture of strain *FB*. The senior author was assisted by training grant 2*E*–144, and the junior author was the recipient of research grant *E*–3221(*C*3), both from the U.S. Public Health Service.

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Ions and the Germination of Spores of *Bacillus cereus T*

DETAILED experimental analysis of the two major types of aerobic bacterial spores, namely, strains of *Bacillus megaterium* which respond germinatively to the presence of glucose or an L-alanine–inosine mixture[1], have been completed[2,3]. Both organisms exhibited a non-specific requirement for ions, either inorganic or organic, for germination. In solutions of appropriate salts, the exogenous requirements for the above-mentioned organic compounds could be varied, and even eliminated altogether. The over-riding factor in the germination of these two types is ions. We proposed that many ions are intrinsically germinative for these organisms, that the organic adjuvants (glucose, L-alanine, inosine) are dependent on salts for maximal germinative powers, and that they augment the germinative capacity of ions[3]. This situation we call 'ionic germination'.

Ionic germination studies have now been extended to another species, *Bacillus cereus*, strain *T* (formerly *terminalis*), also one of the 'L-alanine–inosine' type. Methods of cultivation, production, and preparation of suspensions of spores, and the optical density method of measuring germination are those commonly used[2,4]. Verification of germination was obtained by microscopic determination of the loss of refractility, acquired darkening, stainability, and by the loss of heat resistance of the spores. Preliminary results indicated that suitably washed spores did not germinate when the L-alanine–inosine mixture was made up in deionized water, but did so promptly when the organic compounds were added to *tris* (10^{-1} *M*) or phosphate ($3 \cdot 3 \times 10^{-2}$ *M*) buffer. A 'titration' experiment in $3 \cdot 3 \times 10^{-2}$ *M* phosphate buffer revealed that a mixture containing as little as 10^{-5} *M* L-alanine and 2×10^{-4} *M* inosine elicited germination of the entire spore population (about 10^{8}/ml.) in 20 min. at 40° C. Table 1 shows that, in phosphate buffer, *B. cereus T* germinated rapidly and completely in the presence of inosine only, but not in the presence of L-alanine only. Germination in 10^{-1} *M tris* buffer was considerably inferior to that in phosphate buffer. Thus, L-alanine is unnecessary, and inosine is essential, in phosphate buffer. The germination obtained in deionized water by the higher concentrations of L-alanine and inosine combined we suspect is due, in part at least, to ionic impurities introduced with these organic compounds. High concentrations of L-alanine, for example, 5×10^{-2} *M*, elicited germination in phosphate buffer, but it was incomplete and clearly inferior to inosine at equivalent concentrations. (After these experiments were completed, there appeared a report[5] of the germination of a strain of *B. cereus* by a high concentration ($1 \cdot 7 \times 10^{-1}$ *M*) of L-alanine in *tris* buffer.) The by-passing of the L-alanine 'requirement' for germination in an appropriate ionic environment resembles the corresponding situation in *B. megaterium* Texas[2,3].

The phosphate buffer contained at least three ionic species, namely, Na^+, K^+, and $PO_4^{\equiv}$. Experiments appropriate for the objective revealed the unexpected fact that sodium ion is critical for the germination of this strain of *B. cereus*. An extension of the testing (Table 2) permits the following conclusions: (*a*) Significant germination did not occur in the absence of added Na^+. A possible exception was the weak activity of the chemically similar Li^+. (However, Na^+ as an impurity is a possibility.) (*b*) The germinative activity of Na^+ depends on the accompanying anions.

Table 1. GERMINATION OF SPORES OF *Bacillus cereus T**

Suspension fluid	Optical density reduction (per cent)								
	L-alanine			Inosine			L-alanine + inosine		
	None	10^{-5} *M*	10^{-4} *M*	None	2×10^{-4} *M*	2×10^{-3} *M*	None	10^{-5} *M* + 2×10^{-4} *M*	10^{-4} *M* + 2×10^{-3} *M*
Deionized water	0	0	0	0	0	3	0	4	50
Deionized water containing phosphate buffer, $3\cdot3\times10^{-2}$ *M*, *p*H 8·0	2	1	0	2	2	58	2	62	65

* In 30 min. at 40° C. Values of about 60 per cent represent germination of all the spores (about 10^{8} per ml.) in the suspension.

Table 2. GERMINATIVE ACTIVITIES OF VARIOUS SALTS FOR SPORES OF *Bacillus cereus T*

Salt and concentration ($\times10^{-2}$ *M*)		Optical density reduction (per cent)*: No alanine or inosine	Optical density reduction (per cent)*: L-alanine, 10^{-5} *M* and inosine, 2×10^{-4} *M*
None	–	0	4
NaCl	4		4
KCl	4		1
LiCl	4		11
$MgCl_2$	2		2
$CaCl_2$	2		8
$SrCl_2$	2		4
$BaCl_2$	2		3
KF	4	2	4
NaF	4	3	60
KCl	4		2
NaCl	4		5
KBr	4	1	8
NaBr	4		11
KI	4	0	14
NaI	4		8
KNO_3	4	1	3
$NaNO_3$	4		9
K_2SO_4	4	0	1
Na_2SO_4	4		24
$KHCO_3+K_2CO_3$, *p*H 9 2	4	0	3
$NaHCO_3$, *p*H 9 2	4		12
Na_2CO_3, *p*H 11 2	4		12
$KH_2PO_4+K_2HPO_4$	4	1	3
$KH_2PO_4+Na_2HPO_4$	4	2	62
Na_2HPO_4, *p*H 9 1	4	1	58
NaH_2PO_4, *p*H 4 6	4		7
K formate	4	1	3
K acetate	4	1	6
Na acetate	3	4	72
K propionate	4	1	4
Na propionate	4		70
K butyrate	4	1	2
K citrate	4	0	2
Na citrate	4		64
K malonate	4	1	2
K succinate	4	1	3
Na succinate	4	1	71
K ethylenediamine tetra-acetate	4	0	1
Na ethylenediamine tetra-acetate	4		13
K dipicolinate	4	0	4
Na dipicolinate	4	3	55
NH_4 phosphate	4	1	3
NH_4 nitrate	4	0	2
NH_4 propionate	4	0	5

* In 30 min. at 40° C.; salt solutions prepared in deionized water.

Table 3. BY-PASSING OF L-ALANINE OR INOSINE IN THE GERMINATION OF SPORES OF *Bacillus cereus T*

Salt (4×10^{-2} *M*)	Optical density reduction (per cent)*: Addition			
	None	L-alanine, 5×10^{-5} *M*	Inosine, 2×10^{-3} *M*	L-alanine, 10^{-5} *M* and inosine, 2×10^{-4} *M*
None	0	3	5	3
NaF	3	52	54	57
Na_2HPO_4	1	57	71	55
Na acetate	8	47	73	71
Na succinate	1	40	64	63
Na dipicolinate	8	25	64	54

* In 30 min. at 40° C.: salt solutions prepared in deionized water

These may be inorganic or organic, and they are non-specific, but not all anions are compatible with Na^+ in germination. (*c*) In general, the dependence on the ionic environment is similar to that obtained with the strains of *B. megaterium*, especially the Texas strain[3]. However, important differences are noted for *B. cereus T*, for example, the inactivity of the alkaline earth chlorides, nitrate ion, ammonium salts, or the potassium salts of fluoride, fatty acids, and others. The most active compound tested was sodium acetate (4×10^{-2} *M*), which germinated the entire spore population of *B. cereus T* in less than 10 min. The germinatively active salts, even the most chemically dissimilar ones, by-passed the need for either L-alanine or inosine, but not both (Table 3).

Not enough evidence is at hand yet to gain an insight to the function of the ions in spore germination. However, ionic requirements for germination are exhibited by all the spores tested thus far, and their responses to various ions are different. Obviously, any theory relative to the mechanism of germination has to take into account this phenomenon, and in our hands data obtained on the role of organic adjuvants without due regard to an optimal ionic environment were misleading. Our working hypothesis is that various ions are the primary germinative agents, and non-ionic compounds, such as sugars, amino-acids, and ribosides, which are more or less interchangeable, augment the germinative activities of the ions. A more extended discussion is given elsewhere[3]. This work was supported in part by grants from the Office of Naval Research, the National Institutes of Health, and the National Science Foundation.

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VIROLOGY

Effect of Œstrogenic Hormones on Agglutinability of Chicken Erythrocytes by Arthropod-borne Viruses

IN an earlier paper[1] from this laboratory it was shown that cock erythrocytes are agglutinated by diphasic tick-borne encephalitis virus, whereas hen erythrocytes usually are not agglutinated.

Further studies have revealed that this sex difference in the agglutinability of chicken erythrocytes is not limited to the tick-borne encephalitis virus but seems to be a phenomenon common to all arthropod-borne group *A* and *B* viruses. The difference can be demonstrated with sucrose-acetone extracted mouse brain antigens[2] as well as with tissue culture antigens prepared in an inhibitor-free maintenance medium[3]. When the agglutinability of erythrocyte samples was examined with these tissue culture hæmagglutinins at room temperature, the red

Table 1. HÆMAGGLUTINATION TITRES OF TISSUE CULTURE ANTIGENS TESTED WITH COCK ERYTHROCYTES AT ROOM TEMPERATURE

Virus	Strain	Optimal *p*H	Titre
Group *A*			
Eastern equine encephalomyelitis	Prototype	6·0	64*
Semliki forest	Prototype	6·0	16
Sindbis	*AR* 339	6·0	128
Western equine encephalomyelitis	McMillan	6·0	64
Group *B*			
Yellow fever	17 *D*	6·2	128
Uganda *S*	Prototype	6·2	32
Japanese *B*	Nakayama	6·4	64
Diphasic tick-borne meningoencephalitis	Belyanchikov	6·4	128
West Nile	Egypt 101	6·6	256

* Reciprocal of highest dilution showing hæmagglutination, per 0·5 ml.

blood cells of all the hens tested were found to be inagglutinable, whereas cells from different cocks were always agglutinated and to approximately the same titre (Table 1). If the mouse brain or tissue culture antigens are treated with protamine sulphate, however, such antigens are, for some as yet unknown reason, also capable of agglutinating hen erythrocytes to some extent, the sex difference then being less marked.

Since red blood cells of newly hatched chicks are equally agglutinable irrespective of sex, it is evident that females must lose the reactivity of their erythrocytes at some time in their lives. This time was found to coincide with the attainment of sexual maturity at the age of 4–5 months when the hens started to lay.

From the above observation it was inferred that female sex hormones may play some part in this disappearance of agglutinability. Experiments were therefore performed to test the effect of various sex hormones on the agglutinability of chicken erythrocytes. Cockerels weighing about 0·5 kgm. were given three intramuscular injections at 24-hr. intervals of one of the following hormones: œstradiol benzoate, 5 mgm.; diethylstilbœstrol, 5 mgm.; progesterone, 25 mgm.; testosterone propionate, 25 mgm.; each group consisting of three fowls. These amounts of the hormones were dissolved in 1 ml. of peanut oil. Erythrocyte samples were taken from a wing vein 1, 3, 5, 7, and 9 days after the last hormone injection. The agglutinability of the erythrocyte samples was determined with tissue culture antigens prepared from nine group *A* or *B* arbor viruses (Table 1). The tests were carried out at six different *p*H's ranging from 5·8 to 6·8 at room temperature according to the technique described by Clarke and Casals[2].

It appeared that neither progesterone nor testosterone propionate had any effect on the agglutinability of the erythrocytes. On the other hand, both œstradiol benzoate and diethylstilbœstrol resulted in complete or almost complete disappearance of the agglutinability. With the strongest hæmagglutinins, traces of hæmagglutination were sometimes seen. This change in agglutinability was transitory and lasted 5–7 days after hormone administration was discontinued, the agglutinability then gradually reappearing. With a single dose of 25 mgm. of œstradiol benzoate or diethylstilbœstrol, the same effect on agglutinability could be produced in adult cocks, too.

In adsorption experiments with tick-borne encephalitis virus carried out following methods described in an earlier paper[4], there was a clear correlation between agglutinability of the erythrocytes and their capacity for adsorption of the virus. Again, cells which were not agglutinable did not adsorb virus either.

It is known from the work of Entenman *et al.*[5] that the œstrogens produce a hyperlipæmia in the plasma of chickens, the levels of all the lipid classes of the plasma being increased. A possible explanation for the effect of œstrogenic hormones on agglutinability is that virus receptors on the surface of red cells may become coated with some substance from the hyperlipæmic plasma. Another possibility is that the œstrogens may reduce the number of arbor virus receptors on chicken erythrocytes. Investigations to ascertain the mechanism of the phenomenon described here are in progress.

In this connexion it is interesting that a similar sex difference in agglutinability was also observed with turkey erythrocytes during the period of egg-laying. Goose erythrocytes, on the other hand, which were introduced by Porterfield[6] for hæmagglutination studies with arbor viruses, do not exhibit this sex difference.

The hormone preparations used were supplied by Lääketehdas Orion Oy and Oy Medica Ab, Pharmaceutical Manufacturers, Helsinki. This work was aided by a grant from the Sigrid Jusélius Foundation.

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Some Characteristics of a Virus occurring in Poplars

DURING the past few years the Plant Protection Service of the Netherlands and the Netherlands General Inspection Board of Arboriculture (N.A.K.B.) have paid much attention to a virus disease of poplars resembling Canadian poplar mosaic described by Atanasoff[1], and poplar mosaic mentioned by Klinkowski[2]. The N.A.K.B. tries to eliminate the disease by certifying only non-affected trees and by destroying affected stocks.

The disease is known to attack all commercial cultivars of the section Aigeiros Duby (*Populus canadensis* Moench). These are, arranged in decreasing order of conspicuousness of symptoms, *P. deltoides* Marsh and *P. canadensis* Moench cv. *robusta, heidemij, gelrica, serotina, marilandica*. Poplars of the section *Leucoides Spach* also show symptoms.

In June, light green spots emerging from the smaller veins of the spring leaves develop into distinct star-like patches. On *robusta* and *gelrica* in particular the spots soon become yellow. By late summer and autumn the symptoms have progressed as far as the older summer leaves. Seriously affected trees show symptoms extending far into the top leaves.

From fresh poplar leaves a virus was transmitted by the sap to various herbaceous hosts. Cowpea (*Vigna sinensis* Endl. var. Giant Black Eye) and *Petunia hybrida* Vilm. were among those found to be susceptible to the virus, cowpea in particular proving to be a good test plant. The inoculated primary leaves, after incubation for about 10 days, develop red local lesions which sometimes extend along the veins. After more than 20 days, systemic symptoms appear on the younger leaves, vein-clearing being followed by numerous red spots, especially near the veins.

On *Petunia hybrida* the symptoms are not very clear, semi-necrotic rings occasionally appearing in mature inoculated leaves about 25 days after inoculation. However, the virus could also be demonstrated to be present in inoculated leaves which had failed to develop symptoms.

Compared with other methods of mechanical transmission a modification of the leaf contact method[3] proved to be the most efficient. Poplar leaves were folded several times, cut with a blade to obtain an exposed surface, and dipped in a phosphate buffer solution (*p*H 7·5). Inoculation was then performed by wiping the exposed surfaces over the test leaves which had been dusted with 'Carborundum'. The inoculated leaves were washed immediately after inoculation.

Juice pressed from affected cowpea plants was diluted 1/10 in 0·01 *M* phosphate buffer *p*H 7, whereupon the temperature inactivation point and the dilution endpoint of this crude virus solution was determined[4]. No infectivity could be demonstrated after 10 min. heating at 54° C. nor after dilution to 1/10,000.

Virus preparations observed under the electron microscope revealed the presence of elongated rod-shaped particles. These uniform particles were found not only in a partly purified virus suspension derived from diseased poplar leaves but also in specimens prepared by the dip method[5] from poplar, cowpea and petunia leaves showing symptoms. Provisional measurements of 250 virus particles suggested an average length of about 735 mμ.

Other means are also being used in an attempt to identify the virus.

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PSYCHOLOGY

Absolute Threshold for Electric Shock

For some time it has been suspected that the most relevant parameter when using electric shock as a stimulus is the applied wattage. Forbes and Bernstein[1], Gilmer[2], Hill *et al.*[3], Campbell and Teghtsoonian[4], Green[5], all found evidence to support this contention. Although constant current and constant voltage outputs have long been in use, a device capable of supplying a constant power output compensating for the changes in impedance found in any biological circuit has not hitherto been available.

To obtain a constant power output it is necessary to monitor continuously the instantaneous product of voltage and current, to compare this with a reference, and use the resultant error signal to control the output of the generator. This is achieved by continuously comparing the load current with a function proportional to the reciprocal of the load voltage. This function is derived by width-modulation, followed by integration and smoothing, of pulses generated by a phantastron triggered at 2·5 kc./sec. The error signal is arranged to control the generator output by way of a high-gain d.-c. amplifier. (The apparatus was designed by Mr. B. Corbett and supplied by Messrs. System Research Ltd.)

In the present experiment the three types of output, constant current, current voltage and constant power, were all used in conjunction with three sizes of electrode, 0·075, 0·15 and 0·3 in. diameter, giving nine conditions in all. On each trial a rectangular d.-c. stimulus of 1 sec. duration was applied to the ball of the thumb and index finger of the left hand. Thresholds were determined for all nine conditions using standard psycho-physical procedures.

A total of some 12,000 trials, including training series, were needed to obtain reliable thresholds. The experimenter acted as his own subject for this first experiment.

The results were quite clear cut. The threshold in terms of power remained close to 50 mW. regardless of the area of contact. The current threshold increased from 225 μamp. with the smallest electrodes to 458 μamp. with the largest electrodes, while the threshold in terms of voltage fell from 220 to 101 V. Current density, voltage density and all other indices also failed to provide a stable measure of the threshold.

Clearly, then, power is the single best measure of the shock threshold, since a 16-fold increase in the area of contact does not materially alter this index, but does affect other indices substantially and systematically.

The results of a second experiment make it necessary to qualify this general statement. A further three thresholds were determined for the three types of stimulus, using electrode jelly to improve contact with the medium-sized electrodes. Under these conditions the threshold dropped to 18 mW. This suggests that the crucial factor is the power dissipated in the subcutaneous tissues where the receptors actually lie. This view is supported by the casual observation that there would appear to be a simple, direct relationship between the thickness of a subject's skin and his threshold. A fine, delicate skin, such as many women possess, seems to go with a low threshold, while the man who works with his hands and has a coarse, heavy skin has a far higher threshold.

Since an experimenter being his own subject invites the classic bias of stimulus error, it was thought advisable to repeat the main part of the experiment with a completely naïve subject. The three types of stimulus were used with the largest and smallest pairs of electrodes, without electrode jelly.

Under constant power conditions the threshold was close to 32 mW. with both large and small electrodes. The current threshold increased substantially with the larger electrodes, while the voltage threshold dropped proportionally even more. This result therefore confirms the main finding that under constant power conditions the threshold in mW. is independent of the area of contact.

From a practical point of view two conclusions appear to be worth considering.

(1) As a negative reinforcer, where the experimental animals run over a grid, it is obviously of little use to control either the voltage or the current, as this leaves the wattage to vary with the area of contact. In these circumstances it would therefore pay to standardize shock as a negative reinforcer in terms of wattage. Since also the stimulus is felt only on

the make and break there is a strong argument in favour of using a.c. rather than d.c. stimulation. This would make it impossible for the animal to control the frequency of the shock by freezing on the grid.

(2) As an agent in shock 'therapy' the implications are more serious. The occasional unfortunate demise of a patient who is receiving the 'therapy' suggests that there are times when the stimulus may have exceeded its intended effect. If the wattage were controlled rather than the voltage or current it is possible that some of these mishaps might be avoided.

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Effect of Strain and Group Size on the Response of Mice to 'Seconal' Anæsthesia

The uniformity of first crosses of inbred strains as compared with the parental stocks has now been demonstrated on a number of occasions and the literature on the subject has been extensively reviewed by Lerner[1] and by Biggers, McLaren and Michie[2]. The work of Michie and McLaren[3,4] is of particular interest here for, as well as demonstrating the effect of heterosis on variation, they used as their metameter barbiturate sleeping time in mice. The work reported here was an investigation of the interaction between this genetic influence on variation and an aspect of the social environment, group size.

Indications that the numbers of animals caged together before an experiment influence variation have been provided almost entirely by the work of Chance. He showed, for example, that in gonadotrophin assays in rats the lowest variance was recorded in animals maintained in groups of two before the experiment and that solitary animals were most variable[5]. Chance also found in a preliminary survey that mice kept six in a cage were more variable than mice two per cage as judged by their response to 'Nembutal'.

The experiments to be described here were carried out as follows: Inbred mice of the *CE* and *CBA* strains were supplied to us when they were approximately five weeks old. After arrival they were kept in groups of 30–50 for one week to allow acclimatization and were then set up in the experimental groups of 1, 2 and 8 per cage. They were housed under reversed lighting conditions and at constant temperature. The animals were anæsthetized three days after setting up the experiment and again a week later; thus in part 1 of the tests the animals had been in their number groups for three days and in part 2 for ten days. The anæsthetic used was quinalbarbitone sodium ('Seconal'), a solution of which was prepared by grinding up proprietary tablets in distilled water. The injection route was intraperitoneal and the dose was graded for body-weight in order to give a mean anæsthetic time of approximately 20 min. For the purpose of the experiment a mouse was assumed to be anæsthetized if it failed to give a righting reflex when placed on its back. Two mouse rolling machines were constructed so that 24 animals could be examined at one time. The animals were examined at 2-min. intervals from the time of injection until, on recovery, they gave a righting reflex on three successive trials. After this they were removed from the machine.

The results are shown in Table 1: as each strain was tested twice, the figure given is the weighted mean of two within day, within strain, within number group variances. Michie and McLaren used a logarithmic transformation on their anæsthetic times, but such a treatment was not consistent with the observed distribution of our data and therefore the actual anæsthetic times in minutes were used to calculate the variances shown.

Table 1

Strain	Part 1			Part 2		
	No. per cage					
	1	2	8	1	2	8
	Variance					
CE	143	101	185	248	76	186
CBA	620	398	448	297	83	420
CBA♂ × *CE*♀F_1	52	50	130	78	68	88
CE♂ × *CBA*♀F_1	93	91	119	74	77	203

Three points emerge from these results. First, that there is an effect ascribable to genetic homeostasis; it is clear that the variance of the F_1 hybrids is considerably less than that of the parental stocks (cf. Michie and McLaren[4]). Secondly, the strains themselves are different in their response, the *CBA* strain being more variable than the *CE* strain. The *CE* × *CBA* hybrid is more variable than the reciprocal cross and shows, as does the maternal strain, a high variation in groups of eight animals that have been together for ten days. This would indicate that some maternal effect is involved. Thirdly, the 2-per-cage group is less variable than are the solitary animals or groups of 8-per-cage. This effect is most marked at ten days and in the pure lines.

It has frequently been observed[6,7] that isolated mice become aggressive and the high variation shown by this number group may be connected with the behavioural disturbance involved in isolation. Grant and Chance[8] have shown that rank orders are only demonstrable in male laboratory rats when the numbers caged together are less than six. Uhrich[9] showed rank orders in groups of laboratory mice (3–6 per cage) and if the same numerical limits apply to this species as to the rat it is possible that the low variation shown by the 2-per-cage group as compared with the 8-per-cage group is a reflexion of the stable social situation of the former. If so, this effect, which reduces the variation in the response as much as does the heterozygosity of the F_1 hybrid, might be called by parallel nomenclature 'social homeostasis'.

I thank the Universities Federation for Animal Welfare for the junior research fellowship which enabled me to undertake this work and for financial assistance.

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FORTHCOMING EVENTS

Monday, July 2—Friday, July 13

Imperial College of Science and Technology, Department of Chemical Engineering and Chemical Technology (at Imperial College, London, S.W.7)—Summer School in "Health Physics (Radiation Protection)".

Monday, July 2—Thursday, July 5

Second Congress of the International Federation of Societies of Cosmetic Chemists (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1).

Tuesday, July 3

Royal Society of Medicine (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Annual Meeting.

Wednesday, July 4

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W.C.2), at 9.30 a.m.—Symposium on "A.C. Railway Electrification".

Thursday, July 5—Friday, July 6

Institute of Physics and the Physical Society, Spectroscopy Group (in collaboration with the British Non-Ferrous Metals Research Association and the British Iron and Steel Research Association, at the Spa Hotel, Buxton, Derbyshire)—Symposium on "Spectroscopy in the Metallurgical Industry".

Monday, July 9—Thursday, July 12

British Institution of Radio Engineers; Institute of Physics and the Physical Society; and the University of Birmingham (in the Electrical Engineering Department, The University, Birmingham)—Symposium on "Sonar Systems".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer in Biochemistry—The Registrar, University College of Wales, Aberystwyth (July 5).

Assistant Lecturer (with a degree in a biological subject) in Zoology, to teach biology and human anatomy and physiology to "O" level and zoology to "A" level and degree level—The Principal, Wigan and District Mining and Technical College, Wigan (July 5).

Lecturer (with experience in the use of digital computers and in numerical analysis) in the Computing Laboratory—The Secretary of University Court, The University, Glasgow (July 6).

Assistant Lecturer, Grade B (graduate, preferably with experience in teaching, research or industry) in Chemistry—The Clerk to the Governors, Mid-Essex Technical College and School of Art, Market Road, Chelmsford, Essex (July 7).

Lecturer or Assistant Lecturer in Radioastronomy—The Registrar, The University, Manchester 13 (July 7).

Lecturer (with a good honours degree in pharmacy) in Pharmacy—The Registrar, The University, Manchester 13 (July 7).

Assistant Lecturer or Lecturer (honours graduate in either oceanography, physics or mathematics, and willing to spend several weeks each year at sea) in the Department of Oceanography—The Registrar, The University, Liverpool, quoting Ref. No. CV/101/N (July 9).

Lecturer or Assistant Lecturer in Numerical Analysis in the Department of Applied Mathematics—The Registrar, The University, Liverpool, quoting Ref. No. CV/112/T (July 9).

Lecturer or Assistant Lecturer in Biological Chemistry—The Registrar, The University, Manchester 13 (July 12).

Lecturer or Assistant Lecturer (man or woman graduate) in the Department of Chemistry—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (July 12).

Lecturer (Grade II) in Veterinary Pathology in the Clinical Laboratory of the Veterinary Medicine Department at Langford (Bristol 13 miles)—The Registrar and Secretary, The University, Bristol (July 13).

Assistant Lecturer (to teach through the medium of Welsh) in the Department of Philosophy; and an Assistant Lecturer in Psychology—The Registrar, University College of Wales, Aberystwyth (July 14).

Lecturer or Assistant Lecturer in Physics—The Registrar, The University, Sheffield (July 14).

Research Assistant (graduate in chemistry or geology) in Geology, to assist Dr. C. S. Exley in spectrographic work—The Professor of Geology, The University, Keele, Staffs (July 14).

Professor of Electronic Computation and Director of the Digital Computing Laboratory, School of Electrical Engineering, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (July 20).

Assistant Lecturer (with a degree in chemistry, agricultural chemistry or agriculture with a strong interest in chemistry) in Agricultural Chemistry—The Principal, Seale-Hayne Agricultural College, Newton Abbot, Devon (July 21).

Cytologist (honours graduate in botany or agricultural botany), for work involving some general routine examination of grasses, cereals and potatoes, but original research would be encouraged, particularly in connexion with the genecological studies of hill land species now in progress—The Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian (July 21).

Head (electrical engineer of high academic distinction, experience of research and teaching to Dip.Tech. or final degree standard, and preferably industrial experience) of the Electrical Engineering Department (Grade IV), Brighton College of Technology—The Director of Education, Brighton Education Committee, 54 Old Steine, Brighton, Sussex (July 23).

Chair of Agricultural Biology; and Chair of Agricultural Economics in the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 25).

Senior Lecturer in Chemistry at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 28).

Graduate Metallurgist, for fundamental research on deformation and fatigue in metals—The Registrar, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Laboratory Technician (Physics) (preferably with electronics experience), for work on a D.S.I.R. supported project dealing with extreme infra-red spectroscopy—The Registrar (PT), Queen Mary College (University of London), Mile End Road, London, E.1.

Phillips Petroleum Fellow in Polymer Science, to work in the general field of the polymerization of cyclic ethers—Prof. Geoffrey Gee, Department of Chemistry, The University, Manchester 13.

Research Assistant in Organic Chemistry, to work on some aspects of the chemistry of nucleic acids and their derivatives under the supervision of Dr. G. Shaw—The Registrar, Bradford Institute of Technology, Bradford 7.

Senior or Chief Technician (graduate or experienced technician with a special interest in training junior staff, advising research students and maintaining and devising apparatus) in the Department of Zoology—The Assistant Bursar, The University, Reading, Berks.

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